THE AUTOIMMUNE DISEASES

NOEL R. ROSE
IAN R. MACKAY

Fourth Edition

ELSEVIER
ACADEMIC
PRESS

11830 Westline Industrial Drive, St. Louis, MO 63146, USA
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA
84 Theobald's Road, London WC1X 8RR, U.K.

THE AUTOIMMUNE DISEASES, FOURTH EDITION
ISBN-13: 978-0-12-595961-2
ISBN-10: 0-12-595961-3

Notice

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our knowledge, changes in practice, treatment, and drug therapy may become necessary or appropriate. Readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of the practitioner, relying on their own experience and knowledge of the patient, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions. To the fullest extent of the law, neither the Publisher nor the Editors assume any liability for any injury and/or damage to persons or property arising out or related to any use of the material contained in this book.

ISBN-13: 978-0-12-595961-2
ISBN-10: 0-12-595961-3

Acquisitions Editor: *Margaret MacDonald*
Developmental Editor: *Victoria Lebedeva*
Publishing Services Manager: *Melissa Lastarria*
Project Manager: *Kelly E.M. Steinmann*
Marketing Manager: *Katherine Clevenger*

For all information on all Elsevier Academic Press Publications visit our Web site at www.books.elsevier.com

Printed in the United States of America.

Last digit is the print number: 9 8 7 6 5 4 3 2 1

Copyrighted Material

Contents

Section IV Epilogue

Contributors

Frank Alderuccio Department of Immunology, Monash University Medical School, Prahran, Victoria, Australia

Elaine L. Alexander Aeolus Pharmaceuticals, Inc., San Diego, California, USA

Gunnar V. Alm Department of Molecular Biosciences, Section of Veterinary Immunology and Virology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Irina Apostolou Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Elizabeth G. Araujo Department of Medicine, Section of Rheumatology, University of Chicago, Chicago, Illinois, USA

Jean-François Bach Immunologie Clinique Hôpital Necker, Paris, France

Jennifer M. Barker Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, Colorado, USA

Anthony Basten Centenary Institute of Cancer Medicine and Cell Biology, Faculty of Medicine, University of Sydney, Sydney, Australia, Centenary Institute, Newtown, New South Wales, Australia

Kenneth L. Baughman Advanced Heart Disease Section, Harvard Medical School and Brigham & Women's Hospital, Boston, Massachusetts, USA

David Bernhard Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University, Innsbruck, Austria

Corrado Betterle Chair of Clinical Immunology, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy

Carol M. Black Centre for Rheumatology, Royal Free and University College Medical School, London, UK

Steven Brass Harvard Medical School, Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA

Robert Brink Centenary Institute of Cancer Medicine and Cell Biology, Faculty of Medicine, University of Sydney, Sydney, Australia, Centenary Institute, Newtown, New South Wales, Australia

Robert A. Brodsky Oncology—Hematologic Malignancies Division, Johns Hopkins University, School of Medicine, Baltimore, Maryland USA

Camilla Buckley Neurosciences Group, Department of Clinical Neurology, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK

Gerd-Rüdiger Burmester Department of Rheumatology and Clinical Immunology, Charité Central Campus; Department of Transfusion Medicine, Charité Central Campus; Department of Gastroenterology, Infection Medicine and Rheumatology, Charité Campus Benjamin Franklin; Charité University Medicine Berlin, Humboldt University and Free University of Berlin, Berlin, Germany

Livia Casciola-Rosen Department of Dermatology, Johns Hopkins University School of Medicine; Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine; and Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Patrizio Caturegli The Johns Hopkins University, Department of Pathology, Baltimore, Maryland, USA

Lucienne Chatenoud Université René Descartes Paris, Paris, France

Marcus R. Clark Department of Medicine, Section of Rheumatology, University of Chicago, Chicago, Illinois, USA

Ross L. Coppel Department of Microbiology, Monash University, Clayton, Victoria, Australia

Joe Craft Section of Rheumatology, Department of Medicine, and Section of Immunobiology, Yale University School of Medicine, New Haven, Connecticut, USA

Ronald G. Crystal Division of Pulmonary and Critical Care Medicine and Department of Genetic Medicine, Weill Medical College of Cornell University, New York, New York, USA

Albert J. Czaja Division of Gastroenterology, Mayo Clinic, Rochester Minnesota, USA

Chella S. David Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

Anne Davidson Departments of Medicine and Microbiology, Columbia University Medical Center, New York, New York, USA

Peter J. Delves Department of Immunology and Molecular Pathology, Division of Infection and Immunity, University College London, London, UK

Christopher P. Denton Centre for Rheumatology, Royal Free and University College Medical School, London, UK

Betty Diamond Departments of Medicine and Microbiology, Columbia University Medical Center, New York, New York, USA

Luis A. Diaz Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Thomas Dörner Department of Rheumatology and Clinical Immunology, Charité Central Campus; Department of Transfusion Medicine, Charité Central Campus; Department of Gastroenterology, Infection Medicine and Rheumatology, Charité Campus Benjamin Franklin; Charité University Medicine Berlin, Humboldt University and Free University of Berlin, Berlin, Germany

George S. Eisenbarth Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, Colorado, USA

Paul Emery Academic Unit of Musculoskeletal Medicine, University of Leeds, Leeds, UK

Lorenzo Emmi Center for Research, Transfer and High Education, MCIDNENT, University of Florence, Florence, Italy

Andrew G. Engel Department of Neurology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, USA

Ronald J. Falk Division of Nephrology and Hypertension, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Martin J. Fritzler Faculty of Medicine, University of Calgary, Calgary, Canada

Brian Gelbman Division of Pulmonary and Critical Care Medicine, Weill Medical College of Cornell University, New York, New York, USA

M. Eric Gershwin Division of Rheumatology, Allergy, and Clinical Immunology, University of California at Davis, Davis, California, USA

Bruce C. Gilliland Department of Medicine, Division of Rheumatology and Department of Laboratory Medicine, University of Washington School of Medicine, Seattle, Washington, USA

Bruno Giometto Department of Neurosciences, University of Padova, Padova, Italy, and Department of Neurology, Azienda, Treviso, Italy

James W. Goding Department of Immunology and Department of Physiology, Monash University, Clayton, Victoria, Australia

Tom P. Gordon Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, Bedford Park, South Australia, Australia

Jörg J. Goronzy Department of Medicine, Kathleen B. and Mason I. Lowance Center for Human Immunology, Emory University School of Medicine, Atlanta, Georgia, USA

David Green New York University Medical School, New York, New York, USA

Matthew A. Gronski University Health Network, Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, University of Toronto, Canada

Luiza Guilherme Heart Institute (InCor), School of Medicine, University of São Paulo; Institute for Immunology Investigation, Millenium Institute University of São Paulo, School of Medicine, São Paulo, Brazil

David A. Hafler Harvard Medical School, Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA

Hideaki Hamano Department of Medicine, Gastroenterology, Shinshu University School of Medicine, Matsumoto, Japan

Reinhard Hohlfeld Institute for Clinical Neuroimmunology, Klinikum Grosshadern, University of Munich, Munich, Germany

Katherine Morland Hammitt Sjögren's Syndrome Foundation, Bethesda, Maryland, USA

Leonard C. Harrison Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville 3050, Victoria, Australia

Blair Henderson Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University, Innsbruck, Austria

Hans Hengartner Institute of Experimental Immunology, University Hospital, Zurich, Switzerland

Matthias von Herrath Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, San Diego, California, USA

Julio Hilario-Vargas Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Michael W. Jackson Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, Bedford Park, South Australia, Australia

J. Charles Jennette Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Richard Jones The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Division of Hematologic Malignancies, Baltimore, Maryland, USA

Jorge E. Kalil Heart Institute (InCor), School of Medicine, University of São Paulo; Institute for Immunology Investigation, Millenium Institute; and Clinical Immunology and Allergy, Department of Clinical Medicine, University of São Paulo, School of Medicine, São Paulo, Brazil

Olle Kämpe Departmentt of Medical Sciences, Uppsala University and University Hospital, Uppsala, Sweden

Insoo Kang Section of Rheumatology, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, USA

Simon Karpatkin New York University Medical School, New York, New York, USA

Shigeyuki Kawa Department of Medicine, Gastroenterology, Shinshu University School of Medicine, Matsumoto, Japan

Kendo Kiyosawa Department of Medicine, Gastroenterology, Shinshu University School of Medicine, Matsumoto, Japan

Michael Knoflach University Hospital for Neurology, Innsbruck, Austria

Karsten Kretschmer Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Leila I. Kump The Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

Parviz Lalezari The Biomedical Research Institute, and Departments of Medicine and Pathology, Montefiore Medical Center and Albert Einstein College of Medicine, New York, New York, USA

Robert G. Lahita Professor of Medicine Mount Sinai Medical School, New York, New York. Chairman of Medicine and Vice President, Liberty Health System, Jersey City Medical Center, Jersey City, New York, USA

Grace A. Levy-Clarke The Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

Ning Li Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Zhuqing Li The Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

Judith L. Luborsky Departments of Pharmacology and Obstetrics and Gynecology, Rush University Medical Center, Chicago, Illinois, USA

Zhi Liu Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Claudio Lunardi Department of Clinical and Experimental Medicine, University Hospital, Verona, Italy

Knut E. A. Lundin Institute of Immunology and Department of Medicine, Rikshospitalet University Hospital, Oslo, Norway

Michael P. T. Lunn National Hospital for Neurology and Neurosurgery, London, UK

Livia Lustig Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

Charles R. Mackay The Garvan Institute of Medical Research, Department of Arthritis and Inflammation, Darlinghurst, New South Wales, Australia

Fabienne Mackay The Garvan Institute of Medical Research, Department of Arthritis and Inflammation, Darlinghurst, New South Wales, Australia

Ian R. Mackay Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia

Sarah Mackie Academic Unit of Musculoskeletal Medicine, University of Leeds, Leeds, UK

Michael G. McHeyzer-Williams The Scripps Research Institute, Department of Immunology, La Jolla, California, USA

Kevin J. Maloy Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Menelaos N. Manoussakis Department of Pathophysiology, School of Medicine, National University of Athens, Athens, Greece

Michael P. Manns Department of Gastroenterology, Hepatology and Endocrinology, Medizinische Hochschule, Hannover, Hanover, Germany

Emanual Maverakis Department of Dermatology, University of California Davis, Sacramento, California, USA

Lloyd F. Mayer Mount Sinai School of Medicine, New York, New York, USA

Agnes Mayr Hospital Bruneck, Bruneck, Italy

Ian G. McFarlane Institute of Liver Studies, King's College Hospital, London, UK

Giorgina Mieli-Vergani Institute of Liver Studies, King's College Hospital, London, UK

Frederick W. Miller Environmental Autoimmunity Group, Office of Clinical Research, National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, Maryland, USA

Øyvind Molberg Institute of Rikshospitalet University Hospital, Oslo, Norway

Muriel Moser Laboratoire de Physiologie Animale, Institut de Biologie et Médecine Moléculaires, Université de Bruxelles, Gosselies, Belgium

Kamal D. Moudgil Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, USA

Haralambos M. Moutsopoulos Department of Pathophysiology, School of Medicine, National University of Athens, Athens, Greece

Gerald T. Nepom Benaroya Research Institute at Virginia Mason, Seattle, Washington, USA

Robert B. Nussenblatt The Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

Pamela S. Ohashi University Health Network, Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, University of Toronto, Canada

Ron Palmon Mount Sinai School of Medicine, New York, New York, USA

Pärt Peterson Department of Molecular Pathology, Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia

Michelle Petri Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Fiona Powrie Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Barrak M. Pressler College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, and Division of Nephrology and Hypertension, Department of Medicine, University of North Carolina at Chapel Hill. Chapel Hill, North Carolina, USA

Gloria A. Preston Division of Nephrology and Hypertension, Department of Medicine, University of North Carolina at Chapel Hill. Chapel Hill, North Carolina, USA

Antonio Puccetti Department of Clinical and Experimental Medicine, University Hospital, Verona, Italy

Mark Quinn Academic Unit of Musculoskeletal Medicine, University of Leeds, Great George Street, Leeds, UK

Simon Read Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Bart O. Roep Division of Autoimmunity, Leiden University Medical Center, Department of Immunohematology and Blood Transfusion, Leiden, The Netherlands

Sergio Romagnami Center for Research, Transfer and High Education, MCIDNENT, University of Florence, Florence, Italy

Lars Rönnblom Department of Medical Sciences, Section of Rheumatology, Uppsala University, Uppsala, Sweden

Noel R. Rose Center for Autoimmune Disease Research Department of Pathology and W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins University Medical Institutions, Baltimore, Maryland, USA

Antony Rosen Department of Medicine, Johns Hopkins University School of Medicine and Department of Dermatology, Johns Hopkins University School of Medicine Baltimore, Maryland, USA

Robert A. S. Roubey Division of Rheumatology and Immunology, University of North Carolina, Chapel Hill, North Carolina, USA

Jean-Marie R. Saint-Remy Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

Pere Santamaria Department of Microbiology and Infectious Diseases, and Julia McFarlane Diabetes Research Centre, The University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada

Carlo Selmi Division of Rheumatology, Allergy, and Clinical Immunology, University of California at Davis, Davis, CA. Division of Internal Medicine, San Paolo School of Medicine, University of Milan, Milan, Italy

Eli E. Sercarz Division of Immune Regulation, Torrey Pines Institute for Molecular Studies, San Diego, California, USA

Kazim A. Sheikh Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Yehuda Shoenfeld Department of Medicine "B" and Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel, Sackler

Faculty of Medicine, Tel Aviv University, Incumbent of the Chair of Laura-Schwartz-Kipp for Research of Autoimmune Diseases, Tel Aviv University, Tel Aviv, Israel

Joachim Sieper Department of Rheumatology and Clinical Immunology, Charité Central Campus; Department of Transfusion Medicine, Charité Central Campus; Department of Gastroenterology, Infection Medicine and Rheumatology, Charité Campus Benjamin Franklin; Charité University Medicine Berlin, Humboldt University and Free University of Berlin, Berlin, Germany

Arthur M. Silverstein Institute of the History of Medicine, John Hopkins Medical School, Baltimore, Maryland, USA

Ludvig M. Sollid Institute of Immunology, Rikshospitalet University Hospital, Oslo, Norway

Wolfram Sterry Department of Dermatology and Allergy, Charité University of Berlin, Campus Mitte, Berlin, Germany

Veena Taneja Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

Stuart Tangye The Centenary Institute of Cancer Medicine and Cell Biology, Newtown, New South Wales, Australia

Moshe Tishler Department of Medicine "B", Asaf-Harofe Medical Center, Zrifin, Israel, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Yaron Tomer Division of Endocrinology, Diabetes, and Bone Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA

Ban-Hock Toh Department of Immunology, Monash University Medical School, Prahran, Victoria, Australia

George C. Tsokos Department of Cellular Injury, Walter Reed Institute of Research, Silver Spring, Maryland, USA

Kenneth S. K. Tung Department of Pathology, University of Virginia, Charlottesville, Virginia, USA

Shey-Cherng Tzou The Johns Hopkins University, Department of Pathology, Baltimore, Maryland, USA

Raivo Uibo Department of Immunology, University of Tartu, Biomedicum, Tartu, Estonia

David L. Vaux Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research IG Royal Parade, Parkville 3050, Victoria, Australia

Diego Vergani Institute of Liver Studies, King's College Hospital, London, UK

Marika Vianello Department of Neurosciences, University of Padova, Padova, and Department of Neurology, Azienda, Treviso, Italy

Angela Vincent Neurosciences Group, Department of Clinical Neurology, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK

Roberta Vitaliani Department of Neurosciences, University of Padova, Padova, and Department of Neurology, Azienda, Treviso, Italy

Ulrich H. Von Andrian Center for Blood Research, Department of Pathology, Harvard Medical School, Boston, Massachusetts, USA

Harald Von Boehmer Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Mindi R. Walker Benaroya Research Institute at Virginia Mason, Seattle, Washington, USA

Sally A. Waterman Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, Bedford Park, South Australia, Australia

Howard L. Weiner Harvard Medical School, Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA

Cornelia M. Weyand Department of Medicine, Kathleen B. and Mason I. Lowance Center for Human Immunology, Emory University School of Medicine, Atlanta, Georgia, USA

Anthony P. Weetman Clinical Sciences Centre, University of Sheffield, Northern General Hospital, Sheffield, UK

Senga Whittingham Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia

Georg Wick Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University, Innsbruck, Austria

Allan Wiik Statens Serum Institut, Copenhagen, Denmark

Margitta Worm Department of Dermatology and Allergy, Charité University of Berlin, Campus Mitte, Berlin, Germany

Yang Yang Department of Biochemistry & Molecular Biology, The University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada

Renato Zanchetta Endocrinologist, Endocrine Unit, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy

Rolf M. Zinkernagel Institute of Experimental Immunology, University Hospital, Zurich, Switzerland

Prospectus: The Road to Autoimmune Disease

NOEL R. ROSE*

**Departments of Pathology and of Molecular Microbiology and Immunology, The Johns Hopkins University, Baltimore, Maryland, USA*

IAN R. MACKAY†

†Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

With this, the 21st year since the first edition of The Autoimmune Diseases in 1985, we turn to some historical reflections. The term "immunity" as used in Roman times had a legal sense (*immunitas*, exemption from duty), but came into common parlance to describe the observation that people recovering from certain infectious diseases became resistant to those diseases. Immunity was given biologic sense by Pasteur in the late 19th century to describe the protection conferred by the use of vaccines, a term Pasteur coined in deference to Jenner. Immunity took on a more precise meaning in 1891 when Behring and Kitasato showed that this protection depended upon the serum. Serologic science developed hand-in-hand with bacteriology, and Metchnikoff and Ehrlich promulgated their alternative views, cellular versus humoral, on the provision of immunity against infection. Indeed, even then, around the year 1900, views were current on the possibility (or impossibility) of autoimmunity, followed by a long period of neglect and eventual reawakening 50 years later, as recounted by Silverstein in Chapter 1. During this eclipse the science of immunology itself, and its associated pathologies in the form of hypersensitivity, anaphylaxis, and allergy, flourished and spread rapidly and far afield from Europe. For example, The American Association of Immunologists was established in 1913 largely to promote the use of vaccines, as recounted in the first presidential address by Gerald B. Webb. The Journal of Immunology began publication in 1916, edited by Arthur F. Coca, a pioneer in allergy. Immunology had its "second coming" after the 1950s with the beginning of a clearer recognition of the cellular basis of immune responsiveness, and the complex inner workings of the immune system, in both health and disease, became even more evident.

This brief prospectus to introduce our fourth edition cannot claim to demystify contemporary immunology, but rather seeks to annotate a few of the landmarks in the transition from immunity as a mechanism of protection to autoimmune disease, the pathologic consequence of an autoimmune response.

I. AN IMMUNOLOGIC ROADMAP

The same immune system that guards the body against infection can also cause autoimmune disease. Whether directed against self-antigens or foreign antigens, the immune system follows the same rules of the road, once natural immune tolerance has failed. Our growing knowledge of the intricate highways and byways of the immune response helps us to understand the seamless transition from protection to pathogenic autoimmunity.

The present vertebrate immune system represents a melding of two evolutionarily distinct systems. The earliest immune response descends from the major defense mechanisms passed down from our invertebrate ancestors. It was adopted and modified in vertebrates to form the natural or innate immune response. This response depends upon broad patterns of recognition of noxious agents and can be set into motion with little delay. Since the patterns that prompt the innate immune system appear mainly on invading microorganisms we would not expect this innate immune response to react against self-antigens. Yet, naturally occurring autoantibodies and their precursor cells are a component of the innate immune system, and indeed in some circumstances have beneficial functions, including the "sanitary" disposal of waste and debris accruing from cell turnover. Although the origin of natural autoantibodies is still uncertain, they provide a measure of initial protection against infection because of their broad cross-reactivity. But might they represent the precursors of later, injurious autoimmune responses? The evidence is not in.

Most of the cells of the adaptive immune system are recycled by vertebrates from the earlier innate immune system. The distinctive components of the adaptive immune

system include thymus-derived T lymphocytes and bone marrow-derived B lymphocytes, the major histocompatibility complex (MHC), and the antigen-processing and antigen-presentation systems. Over many millennia, these elements have been further adapted by evolutionary modification to include cell–cell collaboration, secretion of cytokines for cell-to-cell signaling, chemokines to control cell trafficking, and cellular adaptations for provision of immunologic memory. The adaptive immune system recognizes specific antigens through precisely structured receptors on lymphocytes generated by a unique system of genetic recombination and reassortment. The more focused and potent immune response of adaptive immunity requires the generation of a galaxy of different antigen receptors, each tailored for optimal affinity of reactivity with a specific antigen. Inevitably many of these receptors on B cells and T cells will react with constituents of the host, albeit in most cases at lower affinity.

The mounting of an efficient immune response involves cooperation of the two immune systems; the ancient innate system and the more recent adaptive system. To achieve this goal, three major cell populations must participate; antigen-presenting cells (APCs) that take up, prepare and present antigen, and T cells and B cells that alone have the ability to recognize specific antigenic determinants (epitopes). Cells capable of serving as APCs come in many guises; the prototypic APC is the dendritic cell (DC), but macrophages are also capable as well as B cells, which, in their role as APCs, can strongly promote the persistence of autoimmunity. Lymphocytes of either T or B lineage can exist functionally as naïve, activated (antigen-experienced and antigen-reactive) or memory cells, a distinction of relevance to autoimmunity.

Repeated stimulation of the adaptive immune system raises the possibility of infinite expansion of constituent T and B lymphocytes. This outcome is obviously untenable and indicative of the requirement that the immune system be carefully regulated by processes that restore homeostasis among the constituent cell populations. Immune responses to invading pathogens typically continue for only a limited period of time because the antigen driving them is eliminated; thus, immunity wanes unless refreshed or restimulated. Processes intrinsic to the immune system itself also regulate the immune response. In fact, the failure of any of the intrinsic control mechanisms could lead to unchecked lymphoproliferation with dire consequences, including autoimmunity and malignancy. Metaphorically, the immune domicile requires efficient housekeeping to ensure that it is neither underpopulated with consequent immunodeficiency (and possible responses to self-proteins developed to "fill the gap"), nor overpopulated with insufficient flexibility for new responses. Finally, in the process of winding down after an antigenic encounter, there is a crucial need for a lasting imprint in the form of immunologic memory to provide for a prompt response on the next encounter. This anamnestic response can produce great benefits (witness the medical triumph of wide-scale vaccination) but has the potential for great harm if, somehow, a nascent autoimmune response cannot be snuffed out before memory cells develop.

Although immunity proceeds as a continuum we can, for discussion purposes, consider four distinct stages: initiation, maintenance, termination, and memory, as shown schematically in Figure 1.

The immune response begins when a particulate antigen is ingested by a macrophage and degraded so that peptide fragments can be presented at the cell surface in conjunction with molecules of the MHC to CD4-bearing lymphocytes expressing the appropriate antigen receptor. DCs, which are richly present at mucosal surfaces as well as in other tissue sites, stand ready to endocytose, prepare, and present soluble antigen, as described in Chapter 4. If the antigen engages a toll-like receptor (TLR) on the DC, it transduces an activation signal that stimulates production of facilitating cytokines by the DC. Upregulation of MHC further enhances the capacity for presentation of antigen.

The activated DC migrates to a regional lymph node or to the splenic white pulp. There the DC may encounter a naïve or memory T cell with the corresponding receptor. This event sets into motion the formation of germinal centers to serve as factories for production of additional antigen-specific lymphocytes and sites that maximize the chance of an encounter between antigen-reactive T and B cells with the APCs, as described in Chapter 2.

Germinal center formation is a critical step in the development of a robust immune response. The reaction depends upon a subset of $CD4^+$ T cells, follicular homing T cells (Tfh), which are chemotactically attracted to the germinal center. These T cells, in turn, direct naïve B cells that have received an antigenic signal (called signal 1) to respond to this second T-helper signal (signal 2) by proliferation and affinity maturation of their immunoglobulin-like antigen receptor. Second signals involve costimulatory molecules on the participating APC and T cells such as CD80/86 and CD28, and between participating T and B cells, such as CD40 and CD154 (CD40L). Affinity maturation of B cells occurs in germinal centers by the process of somatic hypermutation among genes that encode the B-cell receptor.

The immune response naturally terminates after the elimination of the provocative antigenic stimulus following removal of antigen, assisted by the regulatory processes intrinsic to the immune system mentioned above. These include expression of the molecule CD152 (CTLA-4) by activated T cells which, when engaged by CD80/86 (B7-1/2) on APCs, transduces a negative signal to the T cell. Other downregulatory events include activation-induced cell death (AICD), production of particular cytokines such as trans-

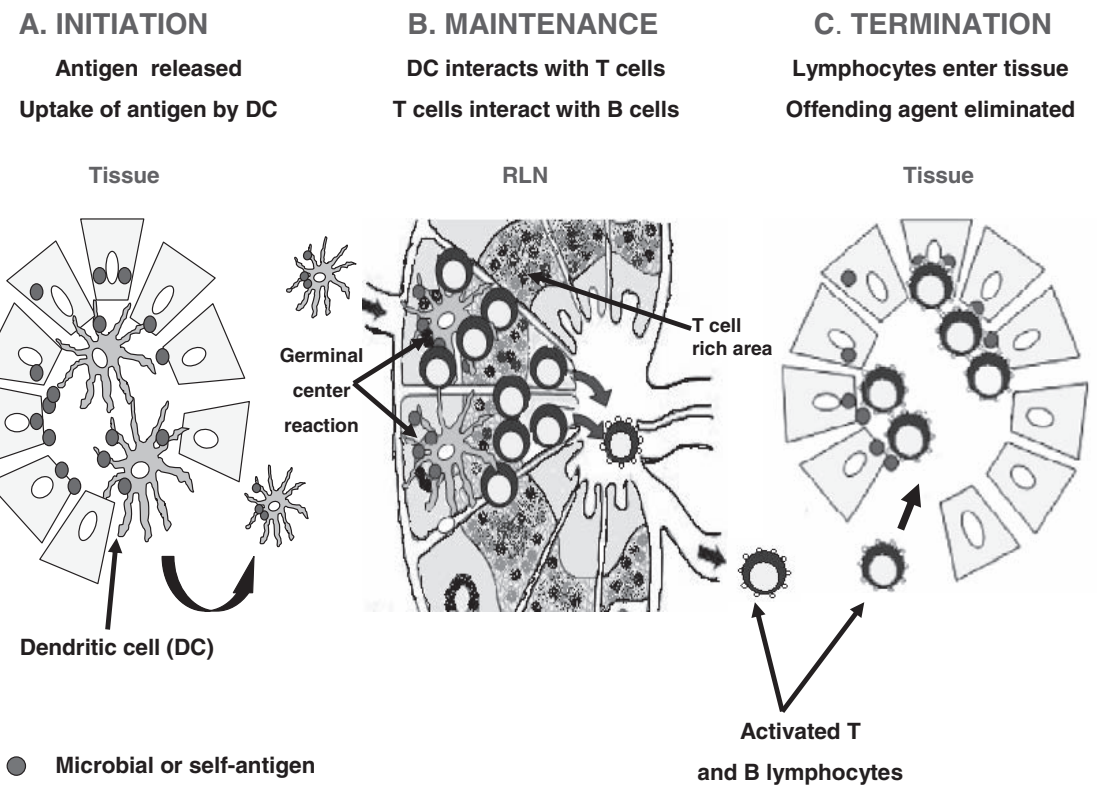


FIGURE 1 *A, Initiation*. The immune response begins with uptake of microbial antigens and self-antigens from degraded tissue by dendritic cells (DCs) that undergo maturation and migrate to regional lymph nodes (RLNs). *B, Maintenance*. The DC enters the primary follicle of the lymph node and may meet a cognate $CD4^+$ T cell, whereupon the germinal center (GC) reaction develops. T-cell proliferation and activation provide helper signals for $CD8^+$ T and B cells. Activated cells exit the RLN. *C, Termination*. Activated lymphocytes specific for microbial antigens enter the infected tissue, eliminate the offending agent, and the immune response winds down. But, if the incoming lymphocytes are specific for self-antigens, and tolerance is weak, self-sustaining autoimmunity ensues. Not shown are the accompanying development of immunologic memory, the costimulator cytokine- and chemokine-dependent events that are essential to the above processes, and the variety of processes that operate in the termination phase.

Reproduced from Seminars in Liver Disease (2005), with permission of Dr Paul Berk, Columbia University, New York, NY, and Thieme Medical Publishers, Norwalk, CT.

forming growth factor (TGF)-β, and the mobilization of suppressive types of regulatory T cells, as described in Chapter 9.

Memory confers major advantages on the adaptive immune system by providing a more efficient and effective response on second encounter with a particular pathogen. Yet, the basis of immunologic memory is still not fully understood, as discussed in Chapters 6 and 12. It was first studied in relation to B-cell function, being discernible after antigen challenge by conversion of a delayed low-titer primary response to an early higher-titer secondary response. Later the memory B cell was characterized by the conversion of variable regions of the immunoglobulin gene from a germline to a mutated sequence. Memory T cells express antigen-specific receptors with higher affinity for the peptide–MHC complex and have a particular phenotype that confers the capacity for ready egress from the lymphatic circulation, as described in Chapter 18.

The immune system is endowed with a wide range of effector elements designed to combat infections. These same agents come into action in unison in autoimmune reactions. They include soluble antibody, which in combination with complement, can effect target-cell destruction. $CD4^+$ T cells secrete a variety of cytokines, some of which can be cytotoxic, including lymphotoxin (LT) and tumor necrosis factor (TNF)-α. $CD8^+$ T cells may themselves be directly cytotoxic to target cells that express the requisite peptide–MHC complex on their surface. In addition, both $CD4^+$ and $CD8^+$ T cells may produce cytokines that activate phagocytic cells. Any of these mechanisms can be called upon under certain circumstances depending largely upon the accessibility and vulnerability of a particular antigenic target.

II. IMMUNE TOLERANCE

With an immune system poised to respond to a nearly infinite number of antigens, specialized regulatory devices are required to minimize the harmful effects that may occur as the result of the concomitant autoimmunity that would seem inevitable, given the similarities in constituents of mammalian and microbial cells. These processes involve tolerance to self-antigens in both the T- and B-cell compartments, as described in Chapters 8, 9, and 13. For convenience, as much as logic, these mechanisms are generally grouped as central or peripheral.

Central tolerance develops during the generation of the immune repertoire in primary lymphoid tissues. It requires specific deletions of nascent lymphocytes that undergo high-affinity interaction with their cognate antigen. In the thymus, developing lymphocytes encounter stromal cells that can express a self-antigen associated with an appropriate MHC molecule. If the affinity of the interaction is sufficiently high, the nascent lymphocyte experiences a lethal signal and undergoes apoptosis. This purging tilts the T-cell repertoire strongly away from self-reactivity. The process is, however, necessarily imperfect, because the repertoire of "self" is so large and complex that complete purging of the antiself repertoire might well leave almost no remaining specificities to react with nonself. Accordingly, it would appear inevitable that the developing lymphoid system must make compromise decisions concerning survival versus death based on affinity, where clones with sufficiently low affinity for self are allowed to survive. The threshold for these decisions has presumably been made over evolutionary timespans to give a reasonable compromise between threats from outside the body versus the threat to the body posed by autoreactivity.

In addition, it is conceivable that such an affinity-based selection system in primary lymphoid organs could occasionally fail and let a high-affinity antiself clone escape to the periphery, perhaps because of a failure to encounter the relevant self-antigen while developing. Efficient thymic deletion of self-reactivity requires that the greatest possible array of autoantigens be available in the thymus for presentation by specialized cells in the thymus to antigen receptors of developing T cells. Sources of self-antigens might include constituents of the blood that pass through the thymus, as well as antigens of the thymus tissue itself, along with cell debris that may happen upon the scene.

General self-tolerance also requires that self-antigens of tissue-specific character be presented in the thymus. A number of years ago, one of us (IRM) suggested that the thymus contains representatives of various other peripheral self-components that would initiate natural self-tolerance: in other words, the thymus might serve as an "immunologic homunculus." Recently, the concept of broad intrathymic representation of peripheral cells has been substantiated by an intriguing discovery in the clinic. It emanated from a disease of children, autoimmune polyendocrine syndrome type 1 (APS1) or, alternatively, autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia (APECED). This disease follows recessive inheritance of a mutated dysfunctional autoimmune regulator (*AIRE*) gene. The encoded AIRE protein, an E3 ubiquitin ligase, determines the intrathymic expression by thymic stromal cells of a wide variety of tissue-specific autoantigens, although exactly how this occurs has still to be worked out. In mice, homozygous disruption of *aire* by gene knock-out results in multiple and diverse autoimmune expressions depending upon the strain of mice. These features sometimes simulate APS1, as described in Chapter 38. Thus, intrathymic deletion of autoimmune T cells, based on ectopically expressed peripheral autoantigens, is an important mechanism for establishing tolerance to self. We now note that AIRE-based thymic deletion likely occurs in the periphery as well as in the thymus.

Central tolerance of B cells probably occurs by mechanisms of clonal deletion similar to those for T cells, although the details are less well known. Deletion, moreover, is accompanied by a process of receptor editing, such that engagement of an antigen receptor on the developing B cell by an autoantigen induces a signal to the immunoglobulin recombinase genes to express an alternative receptor molecule. Receptor editing operates in both the bone marrow and the periphery, and has been described in T cells as well as B cells.

The existence of self-reactive T and B cells in the periphery shows that central tolerance is indeed incomplete. Cells that escape central tolerance are controlled by multiple mechanisms. First, self-reactive "naïve" cells are kept in ignorance of their autoantigen by the absence of appropriate localization signals. In the case of T cells, they remain confined to the lymphatic circulation and lymphoid tissues. Second, self-reactive T or B cells do not receive the signals needed to ensure their survival, namely the necessary helper or costimulatory signals (signal 2) and so undergo anergy and eventually apoptosis. This could apply particularly to those B cells that are highly dependent on T-cell help. Third, there are mechanisms for tolerance that involve active suppression by regulatory cells. Quite a number of cells can provide downregulatory signals, including different types of T cells, and also APCs, as described in Chapter 9.

III. AUTOIMMUNITY: A FAILURE OF SELF TOLERANCE

As explained above, self-reactive T and B cells often avoid central deletion and take up residence in peripheral sites. The development of autoimmunity, therefore, presents a constant albeit generally low-grade hazard. DCs take up

self and foreign antigens alike, but foreign antigens such as microbial constituents are much more likely to engage a TLR and so transduce a vigorous activation signal. In previous publications, one of us (NRR) referred to the contribution to a self-antigen of a microbial product as the "adjuvant effect," recalling the significant role that complete Freund adjuvant plays in inducing experimental autoimmune disease. Spillage of autoantigens, during tissue damage or apoptopic cell death, should not normally activate a DC by a TLR and therefore should be less likely to engender a vigorous and therefore pathogenic autoimmune response. Yet, the diversity of molecules capable of binding a TLR or similar receptors is ever widening. There is no doubt that harmless autoantibodies frequently arise and may even help to mop up cellular debris. Perhaps endocytosis of autoantigens by DCs that are activated by an inflammatory stimulus does result in efficient presentation. In any event, activated DCs loaded with autoantigen can migrate in their usual pattern to the regional lymph nodes where they may encounter recirculating self-reactive T cells and B cells. Fortunately, such cells are greatly outnumbered in the recirculating pool by foreign-reactive cells.

Yet, when all is said and done, the occurrence of autoimmunity is a relatively common event. It is signified by the presence of broadly reactive T cells and of autoreactive antibodies of low affinity, often of the IgM class. Switching to high-affinity IgGs depends upon the active cooperation of B cells with corresponding T cells that have escaped mechanisms of self-tolerance. DCs, depending upon their state of activation, may provide stimulatory or suppressive signals. T cells themselves are constrained by the requirement for appropriate costimulatory signals. Furthermore, they must overcome the effects of suppressive regulatory T cells or other suppressive cells. A number of years ago, one of us (NRR) coined the term "clonal balance" to emphasize that an autoimmune response usually depends upon a subtle shift in the equilibrium between positive and negative factors, rather than the emergence of a somatically mutated "forbidden clone" as proposed in earlier days. However, we concede the possible emergence of harmful self-reactive clones as a result of somatic mutations in genes for antigen receptors or genes controlling thresholds for activation or apoptosis.

Unlike responses to foreign antigens, autoimmune diseases are concerned with antigens that have a continuing presence in the body. Therefore, termination of an autoimmune response due to the disappearance or masking of antigen is unlikely. Intrinsic downregulatory signals provide for AICD, which normally limits immune responses. Since AICD depends upon T-cell apoptosis, a defect in the apoptotic cascade can prevent AICD, prolong lymphocyte multiplication, induce lymphoproliferative disease, and enhance autoimmune responses, as described in Chapters 69 and 70. Cytokines generated during the immune response, like TGF-β (which inhibits T-cell activation) or interleukin (IL)-10 (which deviates the immune response toward Th2) also avert harmful, progressive self-responses. Since they are more easily stimulated than naïve cells, and are more resistant to ablative therapies, memory T cells are ranked highly among the components of unwanted autoimmunity that are targets of potential therapies.

IV. AUTOIMMUNE DISEASES

An autoimmune disease can be defined as a disease in which autoimmunity plays a directly causative or a significantly contributory role. It depends upon the induction and mobilization of immune effector mechanisms with the capability of injuring host cells. The effector mechanisms are the same as those associated with conventional immune responses to invading pathogens, including soluble antibody, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, and other phagocytic cells and mast cells. Antibody can induce injury by binding directly to a cellular antigen and activating complement, by blocking or stimulating a receptor or by producing damaging immune complexes. The receptors for the Fc piece of antibody can variously modulate the course of immune and autoimmune responses, as described in Chapter 16. In organ-localized pathologies, $CD4^+$ and $CD8^+$ T cells are most often implicated because antibody would seem to be unable to reach the intracellular antigen in sufficient quantities. Frequently, however, it is difficult to sort out the relative roles of antibody- and cell-mediated damage in human autoimmune conditions. It should be remembered that B cells not only produce antibody but serve very effectively as antigen-capturing cells and APCs, particularly for activated/memory T cells, and thereby help to perpetuate the injurious autoimmune process.

A recurring problem, and one that confronted the Editors from the outset, was the definition of an autoimmune disease and the criteria for inclusion as such in this text. Unfortunately, since no set of criteria has succeeded in covering all the contingencies, we have simply adopted a listing of characteristics preparatory to a Bayesian type of solution when all the necessary data are in, as shown in Table 1.

The occurrence of autoimmune disease is associated with a confluence of genetic predisposition and environmental exposure combined with a large portion of chance. Mechanisms that could result in chance events include environmental influences, somatic mutations, and random receptor mutations. The importance of heredity in autoimmune disease is abundantly clear from human studies wherein familial aggregations of autoimmune diseases have been long recognized, and comparisons of genetically identical with nonidentical twins show increased frequencies in the former. In animals, the evidence is more direct and telling. Not only can animals be inbred so that they spontaneously

TABLE 1 Defining criteria for autoimmune diseases*

Level of evidence	Comments, caveats†
Level 1: Direct evidence	
1. Transfer of disease with autoreactive serum	
a. Person to person	
Experimentally	Autoimmune thrombocytopenic purpura is the classic example (42)
Naturally, transplacental transfer	Thyrotoxicosis (35), myasthenia gravis (48), congenital heart block (71), and others
b. Transfer with human antisera to animals	Pemphigus vulgaris (57), myasthenia gravis (48)
2. Autoantibody (aab) binding in vitro	Autoimmune hemolytic anemia (41)
3. Transfer of disease with autoreactive lymphocytes	Inadvertent transfer with bone marrow transplant (type 1 diabetes)
Level 2: Indirect evidence	
1. Animal model replicating human counterpart	
a. Spontaneous (genetic basis)	NOD mouse, NZB mouse, BB-W rat, OS chicken, and others (26)
b. Experimentally induced	
Immunization with culprit antigen	Experimental autoimmune encephalomyelitis, thyroiditis, orchitis, etc; adjuvants usually required (26)
Neonatal thymectomy	Gastritis, BALB/c mouse, illustrative of peripheral tolerance (39)
Genetic manipulation, transgenics, knock-outs	Numerous examples (26)
c. T-cell transfer of disease in animals	Type 1 diabetes in NOD mice (36), collagen-induced arthritis in mice
Level 3: Circumstantial evidence	
1. Other causes excluded, particularly chronic infection	Infection and autoimmunity may coexist!
2. Marker autoantibody (aab) to high titer	The basic pointer, but sometimes aab specificity "incongruent" with site of disease, e.g. primary biliary cirrhosis (54)
3. Female bias (25)	Some exceptions, e.g. type 1 diabetes, myocarditis
4. Disease clustering, within an individual or among family members, clinical and/or serologic	A long recognized feature (21)
5. Histopathology: lymphoid aggregates, germinal centers	Seen in some chronic infections as well
6. Responsiveness to immunosuppression	Characteristic feature—a diagnostic therapeutic! (76)
7. T-cell reactivity in vitro to putative autoantigen	Assays remain difficult (73)
8. MHC (HLA) associations	Usually class II, HLA-D (5 and 20)

*Modified from Rose, N. R. and Bona, C. (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today 14,* 426–430.

†Chapters citing relevant data are given in parentheses.

develop autoimmune disease, but more and more susceptibility genes are being identified and their functions defined. The dominant contributors to autoimmune diseases are susceptibility alleles at the MHC class II locus, as described in Chapters 5 and 20. It is generally believed, although it has never been clearly established, that the role of MHC class II depends on the presentation to T cells of a critical self-peptide. But does this involve hyperefficient presentation, which in the thymus would enforce the deletion of "rogue" T cells (or in the periphery favor their reactivity), or hypoefficient presentation with just the reverse effects? There remains much to be understood. Numerous other genes have more recently been implicated in affecting the susceptibility to autoimmune disease, as described in Chapter 21, including the *AIRE* genes and apoptosis genes, such as Fas and FasL. Often these turn out to be genes engaged in the regulation of the immune response, such as alleles at the CD152 (CTLA-4) locus, or allelic forms of genes or their promoters encoding cytokines involved in immune responses. And not to be overlooked are nonimmune genes of which the product is prejudicial to the integrity of the target tissue.

Despite the established role of genetic predisposition in virtually all autoimmune diseases, its total effect seems at first sight to be relatively minor, since rarely is more than a third of the risk of a human autoimmune disease attributable to inheritance. But this may change as knowledge of the genome increases. Alternatively, a major portion of remaining risk should be contributed by the environment, as described in Chapters 22 and 23. The most likely suspects include infectious organisms, although a direct connection has been firmly established only in a very few instances, such as some cases of Guillain–Barré syndrome or rheumatic fever, as described in Chapters 47 and 62. A virus that keeps popping up as a suspect without ever a direct conviction is the Epstein–Barr herpes type virus, of interest because this is lymphomagenic as well. Perhaps the best examples of environmental chemical causation can be found among the medicinal drugs that can induce signs and symptoms similar to classical autoimmune disease. Procainamide

and hydralazine are two drugs that can induce a "lupus-like" response with all the serologic concomitants, and their causative role can be substantiated by withdrawal of the drug inducing full remission, and by the autoimmune state recurring in an accelerated manner with rechallenge.

Finally, we must return to the role of chance in the development of autoimmune disease. Identical twins, after all, do not have identical immune systems because of the stochastic mechanisms integral to generating the diversity of the immune response. This randomness must certainly account for some measure of the nonconcordance of identical twins. Experimentalists are constantly reminded that even genetically identical mice immunized in exactly the same way show great variability in their responses. There are also strong suggestions that autoimmune diseases evolve in stepwise fashion, and that preclinical autoimmunity may lie dormant for long periods. Perhaps further environmental triggers and/or chance variations determine progression to symptomatic disease. We are reminded here of precancerous lesions that are on the way to, but may never reach, overt disease.

V. INTO THE FUTURE

Autoimmune diseases can affect virtually any site in the body and, accordingly, their clinical presentation varies widely. Therefore, each disease is usually considered separately. Only recently have the actual dimensions of the autoimmune disease problem become evident. Estimates suggest that 5–8% of people in developed countries (based on data from the USA) suffer from an autoimmune disease. To illustrate the breadth and depth of the autoimmune disease problem, in our first edition in 1985, we included some 25–30 disease entities, whereas in this fourth edition, 20 years later, there are over 60 well-credentialled examples. Moreover, for some, the population prevalence is notably high, e.g. rheumatoid arthritis, autoimmune thyroid disorders, and Sjögren syndrome; and the population morbidity is, for some others, extreme, particularly systemic lupus erythematosus, type 1 diabetes, and multiple sclerosis. Thus, we assert that in developed, and increasingly in developing countries too, autoimmune disease ranks well up with the major global health concerns of cancer, cardiovascular disease, chronic pulmonary disease, diarrhea, and obesity-related disorders. It is evident that autoimmune diseases are a substantial and increasing public health issue. In the past it may have been reasonable to approach each disease individually, but the magnitude of the autoimmune disease problem prompts us to emphasize the urgent need to look at the autoimmune diseases collectively. After all, many fundamental pathogenetic mechanisms link all of the autoimmune diseases and research on all will be strengthened if the common features are explored. Sometimes a new treatment of one autoimmune disease may be beneficial for another (although we do recognize, given the diversity of the immune response, a treatment benefiting one disease may be inert or even detrimental for another). Shared genetic traits dictate that individuals with one autoimmune disease are at risk of developing a second or even a third one, and that multiple autoimmune diseases may cluster in certain families. A deeper understanding of these fundamental principles will benefit all of the autoimmune diseases.

In Greek mythology, Aesculepius, the god of medicine, had two daughters, Panacea the goddess of cures and Hygeia the goddess of prevention. As we approach patients with autoimmune diseases in this millennium, we invoke blessings of both goddesses. Successful treatments will come from our better understanding of the mechanisms regulating the immune response and will undoubtedly encompass both antigen-specific and immunomodulatory approaches. Given our ultimate goal of prevention, we will garner our growing knowledge about genetic predisposition and early biomarkers to identify individuals and populations at inordinate risk. The diabetes community is showing the way in this respect, as described in Chapters 74 and 75. Separating the vulnerable person from the external, environmental trigger has already proved to be a highly successful strategy in celiac disease, as described in Chapter 51. But even for an established disease, there is growing optimism over the prospects for new biologic remedies, as described in Chapter 76. We may soon reach a point at which therapists can effectively arrest or even cure established autoimmune disease before there is full understanding of its cause. Early intervention may restore immunologic homeostasis and correct immunologic imbalance before disease produces irreversible destruction: this is a goal truly worthy of the effort.

Reviewers

The Publisher and Editors are indebted to the following individuals for their very helpful reviews of chapters in this book.

Gordon Ada Division of Immunology and of Cell Biology, John Curtin School of Medical Research, The Australian National University, Canberra City, New South Wales, Australia

Elaine Alexander Experimental and Clinical Research, Arena Pharmaceuticals, Inc., San Diego, California, USA

Mark Anderson Diabetes Center, University of California/San Francisco, San Francisco, California, USA

Robert Anderson Immunology Division, Walter & Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia

Grant Anhalt Departments of Dermatology and Pathology, Johns Hopkins University, School of Medicine, Baltimore, Maryland, USA

Jacques Banchereau Baylor Institute of Immunologic Research, Dallas, Texas, USA

Alan Baxter Comparative Genomics Centre, James Cook University-Townsville, Queensland, Australia

Richard Boyd Monash Immunology and Stem Cell Laboratories, Monash University, Clayton, Victoria, Australia

Ware Branch University of Utah Health Sciences Center, LDS Hospital, Salt Lake City, Utah, USA

Robert Brodsky Oncology-Hematologic Malignancies Division, Johns Hopkins University, School of Medicine, Baltimore, Maryland, USA

James Bussel Department of Pediatrics, Division of Allergy and Immunology, Mount Sinai Medical Center, New York, New York, USA

Stanley Cohen Department of Pathology and Laboratory Medicine, UMDNJ-New Jersey Medical School, Newark, New Jersey, USA

Antonio Coutinho Instituto Gulbenkian de Ciencia, OEIRAS, Portugal

Terry Davies Department of Medicine, Mount Sinai Medical Center, New York, New York, USA

James De Jersey Department of Immunology, Monash University Medical School, Prahran, Victoria, Australia

Barbara Detrick Department of Pathology, Johns Hopkins University, School of Medicine, Baltimore, Maryland, USA

Daniel Drachman Department of Neurology, Johns Hopkins University, School of Medicine, Baltimore, Maryland, USA

Marc Feldmann Kennedy Institute of Rheumatology Division, Imperial College London, Charing Cross Hospital Campus, London, UK

Dori Germolec Laboratory of Molecular Toxicology, NIEHS–NIH, Research Triangle Park, North Carolina, USA

James Goding Departments of Immunology and Physiology, Monash University, Clayton, Victoria, Australia

Michael Good The Queensland Institute of Medical Research, The Bancroft Centre, Royal Brisbane Hospital, Queensland, Australia

Malcolm Greaves Institute of Dermatology, GKT School of Medicine, Guys Hospital, London, UK

Peter Gregersen Robert S. Boas Center for Genomics and Human Genetics, North Shore Long Island Jewish Research Institute, Manhasset, New York, USA

Andrew Grigg Departments of Clinical Haematology and Medical Oncology, Royal Melbourne Hospital, Parkville, Victoria, Australia

Mark Hedger Institute for Reproduction and Development, Monash University, Clayton, Victoria, Australia

Phil Hodgkin The Walter & Eliza Hall Institute of Medical Research, Bundoora, Victoria, Australia

Mark Hogarth Austin Research Institute, Heidelberg, Victoria, Australia

Peter Hollingsworth Department of Clinical Immunology and Biochemical Genetics, Royal Perth Hospital, Perth, Western Australia, Australia

Derek Jewell Gastroenterology Unit, University of Oxford, Radcliffe Infirmary, Oxford, UK

David Jones School of Clinical Medical Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

Thomas Kay St. Vincent's Institute, Fitzroy, Victoria, Australia

Vijay Kuchroo Department of Neurology, Harvard Medical School, Boston, Massachusetts, USA

Michael Lenardo Laboratory of Immunology, NIAID-NIH, Bethesda, Maryland, USA

Peter Lipsky Intramural Research Program, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, USA

Mark Mamula Yale University School of Medicine, New Haven, Connecticut, USA

Geoffrey McCaughan Faculty of Medicine and Surgery, Royal Prince Alfred Hospital, Camperdown, Sydney, New South Wales, Australia

Hugh McDevitt Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California, USA

Ruslan Medzhitov Section of Immunobiology, HHMI, Yale University School of Medicine, New Haven, Connecticut, USA

Stephen Miller Interdepartmental Immunology Center, Northwestern University Medical School, Chicago, Illinois, USA

Grant Morahan Diabetes Research Centre, Western Australian Institute for Medical Research, Perth, Western Australia, Australia

Kamal D. Moudgil Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, USA

Robert Nakamura Department of Pathology, Scripps Clinic, La Jolla, California, USA

Dolores Njoku Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University, School of Medicine, Baltimore, Maryland, USA

Abner Notkins Experimental Medicine Section, Oral Infection and Immunity Branch, NIDCR-NIH, Bethesda, Maryland, USA

Klaus Rajewski Department of Pathology, Center for Blood Research, Harvard Medical School, Boston, Massachusetts, USA

Westley Reeves Division of Rheumatology and Clinical Immunology, University of Florida, Gainesville, Florida, USA

Morris Reichlin Immunology Section, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

Peter Roberts-Thomson Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, Bedford Park, Adelaide, South Australia, Australia

Ethan Shevach Cell Immunology Section, Laboratory of Immunology, NIAID-NIH, Bethesda, Maryland, USA

Ken Shortman Immunology Division, Walter & Eliza Hall Institute of Medical Research, IG Royal Parade, Parkville, Victoria, Australia

Lawrence Steinman Departments of Neurology and Neurologic Sciences, Stanford University School of Medicine, Stanford, California, USA

Cory Teuscher Immunobiology Program, University of Vermont, Burlington, Vermont, USA

Argyrios Theophilopoulos Department of Immunology, Scripps Research Institute, La Jolla, California, USA

Tony Tiganis Department of Biochemistry and Molecular Biology, Monash University School of Biomedical Sciences, Clayton, Victoria, Australia

John Todd JDRF/WT Diabetes & Inflammation Laboratory, CIMR, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK

Ban-Hock Toh Department of Immunology, Monash Medical School, Alfred Hospital Center, Prahran, Victoria, Australia

George Tsokos USUHS/WRAIR, Silver Spring, Maryland, USA

John Vierling Department of Hepatology, Cedars-Sinai Medical Center, Los Angeles, California, USA

Gabriel Virella Department of Immunology and Microbiology, Medical University of South Carolina, Charleston, South Carolina, USA

Raivo Uibo Department of Immunology, University of Tartu, Biomedicum, Tartu, Estonia

Emil Unanue Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri, USA

Clive Wasserfall Departments of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida, USA

Senga Whittingham Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

Ian Wicks Reid Rheumatology Laboratory, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Acknowledgments

The editors are pleased to acknowledge the outstanding editorial assistance of Ms. Hermine Bongers. Ian R. Mackay would also like to thank Professor Christina Mitchell, Head of the Department of Biochemistry and Molecular Biology at Monash University Melbourne, for generous provision of facilities, Dr. Merrill Rowley and Professor Ban-Hock Toh for stimulating laboratory interactions and collegiality, and also Ms. Elaine Pearson for skilled secretarial assistance.

The editors are most appreciative of the sustained assistance and advice in the preparation of this book from Ms. Margaret MacDonald, Ms. Victoria Lebedeva, Mr. Stephen Packwood at the publishing office of Elsevier, London and Ms. Nora Naughton at Naughton Project Management.

The editors also thank their wives for their extraordinary patience.

Noel R. Rose
Ian R. Mackay

CHAPTER

1

Autoimmunity: A History of the Early Struggle for Recognition

ARTHUR M. SILVERSTEIN

Institute of the History of Medicine, Johns Hopkins Medical School, Baltimore, Maryland, USA

> ". . . 1955–1965 [was] the decade marked by the question, Does autoimmunity exist?"
>
> (Rose and Mackay, 1985)

It is one of the curious situations in science that certain well-demonstrated facts are refused entry into the body of accepted knowledge, and may become so effaced from the collective memory that they must be rediscovered many years later in order to gain acceptance. Such was the case in immunology with Donath and Landsteiner's (1904) discovery that paroxysmal cold hemoglobinuria is an autoimmune disease, or with Clemens von Pirquet's (1910) explanation of immune complex disease. Sometimes the cause of this selective amnesia is merely an earlier pronouncement by a respected leader in the field; sometimes it lies in an inability to fit the new finding into the working paradigm that guides thought in the field, as the historian of science Thomas Kuhn (1970) has suggested. In the end, it may be that Ludwik Fleck (1979) was right when he proposed that acceptance of a fact in science depends less upon its truth than upon its acknowledgment by the leaders in the discipline (whom Fleck called the *Denkkollektiv*). However, the truth in science ultimately emerges, although sometimes it takes a very long time.

The earliest discoveries in immunology were made in the context of the battle to ward off infectious diseases. These included Louis Pasteur's (1880) preventive vaccines, Ilya Metchnikoff's (1884) bacteria-eating phagocytes, and Behring and Kitasato's (1890) curative antidiphtheria and antitetanus sera. It seemed evident that these efficient mechanisms for the protection of the body were Darwinian adaptations designed to prevent or control infectious disease: a widespread view in the 1890s even after it was demonstrated that specific antibodies might be formed against such innocuous antigens as egg albumin, bovine serum proteins, and sheep red cells. It seemed unthinkable at the time that mechanisms designed to prevent disease might turn the tables and cause disease. So well established did this concept of a benign immune system become that demonstrations that antibodies might cause disease were either disregarded entirely, or else ascribed to "aberrant" antibodies acting under the influence of a "misdirected" immunity (Silverstein, 1989). This was how the early discoveries of serum sickness, hay fever, asthma, and a variety of immunopathologic phenomena were treated by mainstream immunology during the first half of the 20th century.

It is beyond the scope of this chapter to discuss the entire history of the unwillingness to accept that the immune response might lead to a variety of harmful outcomes (which we now describe under the rubric "immunopathology"). We shall limit the present discussion to the way that a subset of the whole, autoimmune diseases, was regarded (or rather disregarded) during the first half of the 20th century. This sample should provide an adequate representation of the way that early immunologists dealt with the paradox presented by the almost oxymoronic word *immunopathology*.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

THE SEARCH FOR AUTOANTIBODIES

Horror Autotoxicus

A new mechanism that functions to mediate immunity was discovered by Richard Pfeiffer (1894)—the destruction of bacteria by humoral antibodies. Jules Bordet (1899) showed that not only were bacteria lysed by thermostable antibody and a thermolabile substance that he called *alexine* (later termed *Komplement* by Paul Ehrlich), but that mammalian erythrocytes could be hemolysed specifically by the same two agents. Here was a technique that would see broad application in many areas of immunology (Silverstein, 1994), not least in connection with the question of whether the individual could form antibodies against his/her own self.

Two consequences of Bordet's report were immediately apparent. Karl Landsteiner (1900) became interested in red cells and discovered blood groups in humans (for which he received the Nobel Prize in 1930). Then Paul Ehrlich and Julius Morgenroth launched a series of studies of immune hemolysis in order to develop additional support for Ehrlich's side-chain theory of how antibodies are produced and how they function (Ehrlich, 1897; 1900). It is Ehrlich's interpretation of his hemolysis experiments that would play a major role in the early history of autoimmunity. These hemolysis experiments are described and analyzed in detail by Silverstein (2002).

During the course of these experiments, Ehrlich and Morgenroth immunized many different species with the red cells of other species. They also immunized animals with the red cells of other members of their own species, and even tried to immunize animals with their own red cells. In every case, they were able to demonstrate the production of xenohemolysins and isohemolysins, but autohemolysins were never observed. This led inexorably and logically to the conclusion that animals could not make autotoxic antibodies to any self-antigens, a postulate that Ehrlich named *horror autotoxicus*. Indeed, he would conclude that, "It would be dysteleological in the highest degree, if under these circumstances self-poisons of the parenchyma—autotoxins—were formed" (Ehrlich, 1902).

But Ehrlich was not the only one who responded to Bordet's publication on immune hemolysis. If red cells could stimulate an immune response, why not other tissues and organs? In no time, attempts were undertaken to immunize animals with all types of cells and tissue extracts, especially at the Pasteur Institute in Paris, where Bordet had worked. As expected, cytotoxic xenoantibodies against a variety of tissues were reported; indeed, volume 14 of the *Annales de l'Institut Pasteur* was largely devoted to these studies, including a review of antitissue antibodies by Metchnikoff (1900). Most surprising was the report by Metalnikoff (1900) that some animals were able to form antibodies against their own spermatozoa. But while these autoantibodies could destroy the sperm *in vitro*, they seemed to have no effect *in vivo* on the viable sperm in the immunized animal.

Ehrlich was not impressed. He commented that these are not "autocytotoxins within our meaning," since they do not cause disease (Ehrlich and Morgenroth, 1901). Here was the true meaning of *horror autotoxicus*: not that autoantibodies cannot be formed, but that they are prevented "by certain contrivances" from exerting any destructive action (Goltz, 1980). Due in part to Ehrlich's worldwide prestige and to the fact that an autoantibody seemed so obviously counterintuitive, *horror autotoxicus* found broad acceptance as a guiding principle. Indeed, so firm was the conviction that autoimmune disease was impossible that everyone soon forgot Ehrlich's suggestion that an autoantibody might exist without causing disease. It would be some 80 years before the important distinction would be made between autoimmunity and autoimmune disease (Rose and Mackay, 1985).

Nature of Ehrlich's "Contrivances"

Paul Ehrlich was nothing if not logical. He proposed one of his typical thought-experiments to examine the possible outcomes (Ehrlich and Morgenroth, 1900). Suppose the existence of a self-antigen α. Then, since antibody formation results from the interaction of antigen with preformed cell receptors according to the side-chain theory (the first selection theory), two possibilities are seen:

1. The host possesses no anti-α cell receptors. Therefore, no autoantibody response and thus no disease can occur. (Here is, in embryo, Burnet's later clonal deletion idea.)
2. The host does possess anti-α cell receptors on its cells. Therefore, autoanti-α is formed. But the host also possesses the self-antigen α on its cells, with which the anti-α may react to stimulate the formation of anti-anti-α. (Remember, Ehrlich knew nothing about lymphocytes, and conceived that all cells possess receptors and may form antibodies.) But the specific site on the anti-antibody should be identical with that on the original antigen, since they both should react specifically with the antibody combining site. Thus, Ehrlich proposed that a self-regulating equilibrium would be established between autoantibody and antigen (= anti-antibody) to suppress the development of autoimmune disease. [Here was a regulatory network theory 70 years before Niels Jerne's (1974) idiotype–anti-idiotype theory.]

CHALLENGES TO THE THESIS

Lens Autoantibodies

The initial flurry of interest in antitissue antibodies quickly subsided as the implications of *horror autotoxicus*

gained broad acceptance. But in 1903, Paul Uhlenhuth (1903) demonstrated the existence of organ-specific antigens by showing that the proteins of the lens are unique to that tissue; they are found nowhere else in the body. Moreover, these antigens are shared by the lenses of different species. Ophthalmologists seized upon this finding to suggest that an immune response to an individual's own lens might be responsible for the development of senile cataract (Römer, 1905). They showed further that an intraocular inflammation may be induced by the experimental rupture of the lens in the eye of a lens-immunized animal (Krusius, 1910).

Here were observations that would fascinate both ophthalmic clinicians and a later generation of immunopathologists interested in the possible workings of autoimmune disease. First, there was this early preview of what would later be called the "sequestered antigen" concept. Since "self"-antigens by definition cannot elicit an immune response, then such antigens as do must be "foreign," that is, isolated from the immunologic apparatus of the host, like sperm and lens. Secondly, Römer and Gebb (1912) concluded that if indeed disease does result from the formation of autoantibodies, this would represent a most unusual occurrence and must be considered as an aberration due to a malfunction of Ehrlich's "contrivances." Here they showed that, unlike a future generation, they understood Ehrlich's "law of immunity research" completely.

Interest in the possibility that autoimmunity to lens might lead to disease did not disappear in the years that followed. But whereas the initial studies had been done in the context of the new immunology and were known to all workers in the field, further work was restricted to ophthalmologists and eye departments. Thus a broad clinical study led Verhoeff and Lemoine (1922) to identify numerous cases of lens-induced inflammatory disease, to which they gave the name *phacoanaphylaxis*. Thenceforth, the description would appear routinely in textbooks of ophthalmic pathology, and clinical diagnoses would be made.

Paroxysmal Cold Hemoglobinuria

Fast on the heels of the lens antigen demonstration came an even more convincing case involving erythrocyte antigens. Paroxysmal cold hemoglobinuria (PKH) was a rare disease presenting with signs of intravascular red cell lysis and a resulting hemoglobinuria, following exposure of the patient to the cold. Donath and Landsteiner (1904; 1906) published reports that reproduced *in vitro* all features of the disease. They demonstrated beyond question that it was due to a peculiar autoantibody in the patient's serum that affixes to his/her own red cells only in the cold, and mediates hemolysis with complement when the sensitized cells are rewarmed.

It was clear from the outset that Landsteiner understood fully the implications of this discovery and its meaning for Ehrlich's *horror autotoxicus*. Indeed, even Ehrlich's student Hans Sachs (1909) gave a somewhat grudging acceptance of the phenomenon and its interpretation. But again, the implication seemed to be that this was an unusual exception to the regular scheme, and the implications of PKH as the prototypical autoimmune disease soon almost vanished.

Sympathetic Ophthalmia

It had always seemed odd to clinicians that after traumatic injury to one eye, the second eye might spontaneously develop a blinding inflammatory disease, even years later. Soon after the discovery of cytotoxic antibodies, the proposal was advanced that sympathetic ophthalmia might be caused by the formation of "autocytotoxins" (Santucci, 1906). This concept was picked up and given broad currency by one of the foremost ophthalmologists of the day, Elschnig (1910a; 1910b) of Prague. As with autoimmunity to lens, work on the immunology of sympathetic ophthalmia continued, but in ophthalmology departments. Woods (1921; 1933) reported the presence of antiuveal antibodies in patients with perforating injuries of the globe, and uveal pigment was implicated as the causative antigen (Woods, 1925; Friedenwald, 1934).

The Wassermann Antibody

The discovery of the role of complement in immune hemolysis was soon followed by the finding that *any* antigen–antibody interaction would fix complement nonspecifically (Bordet and Gengou, 1901). The ability to measure this uptake using a hemolytic assay meant that antibody could be titered if specific antigen were available. With the recent identification of *Treponema pallidum* as the cause of syphilis, a serologic test for this disease was sought. But since the organism could not be grown in culture, von Wassermann et al. (1906) and, independently, Detré (1906) used extracts of tissues from syphilitic patients as the antigen, and a valuable diagnostic test was born.

Most perplexing, however, was the report from many laboratories that positive tests for syphilis might be obtained also using extracts of normal tissues as antigen. This ran counter to the prevailing view that only *specific* antigen can interact with antibody to fix complement. It appeared necessary, therefore, to conclude that the "Wassermann antigen," being native, must be measuring an autoantibody rather than an antitreponemal antibody. This suggestion was made by Weil and Braun (1909) who speculated that the Wassermann antibody is an autoantibody specific for the tissue breakdown products generated in the syphilitic lesions. They suggested further that these autoantibodies exacerbate the disease, and that the brain lesions in tertiary

syphilis (paresis) may represent an autoimmune disease directed against neural antigens. (A century later, the antigen involved in the Wassermann reaction has been identified as a lipid, named cardiolipin, but why these antibodies are formed is still a mystery as is their role in the disease process.)

THE SHIFT TO IMMUNOCHEMISTRY

Despite all these hints that autoimmune diseases might exist, interest in the question waned in mainstream immunology—indeed almost disappeared—for some 40 years, from just before the First World War to the mid to late 1950s. This was due in part to the continuing sway of Ehrlich's *horror autotoxicus*. But there was another factor at play: the change in the overall direction of the field of immunology.

During the quarter century prior to the First World War, immunology had been concerned chiefly with medical problems, and was pursued almost exclusively by physicians. It had achieved notable successes in the prevention of infectious diseases (vaccine development), their cure (serotherapy), and their diagnosis (serology). It had even begun to define several immunogenic diseases (anaphylaxis, serum sickness, hay fever, and asthma). But most of the easy problems had been solved, and further successes in these areas became disappointingly rare. Vaccines were sought, generally unsuccessfully, for the remaining great scourges of mankind: syphilis, tuberculosis, typhus, and the many serious tropical diseases. Few diseases were caused by exotoxins like diphtheria and tetanus, and thus new serotherapeutic approaches were rare. Yet other forces were at work. The Wassermann test and its offshoots became so widespread for the diagnosis of disease that it moved from the immunologic research laboratory to the clinic. A new discipline, serology, arose and soon became independent of the mother discipline, immunology. In the same way, experimental anaphylaxis and its human disease relations, hay fever and asthma, stimulated the interest of clinicians, who soon took over work in this field and called their new discipline "allergy."

When in a science one research direction reaches the point of severely diminishing returns, its practitioners will usually move to more productive pursuits. So it was with immunology, beginning shortly after the end of the First World War. Karl Landsteiner (1962) started working with haptens, and soon devoted himself almost entirely to a chemically-oriented study of the structural basis of immunologic specificity and cross-reactions. Then, Michael Heidelberger (Heidelberger and Avery, 1923) studied the immunochemistry of pneumococcal polysaccharides and introduced a variety of quantitative methods for the estimation of antigens and antibodies, best typified by the popular text written by his students, *Quantitative Immunochemistry* (Kabat and Mayer, 1949). For more than three decades, the field was devoted largely to studies of structure, specificity, and the thermodynamics of antigen–antibody interactions. The texts and monographs were primarily chemically oriented, and the practitioners were either chemically trained or at least chemically oriented. Even the theories of antibody formation that guided the field, Breinl and Haurowitz's (1930) and Pauling's (1940) antigen-instruction concept, were chemical (i.e., nonbiologic and non-Darwinian) in spirit. It was easy to assume that a protein might be synthesized according to external instruction; for the chemist, molecules have no evolutionary history.

Given the continuing influence of Ehrlich's dictum, and the generally nonmedical orientation of the most prominent immunologic investigators, it is not surprising that autoantibodies and autoimmune diseases were not among the most popular topics in the research laboratory. This is not to say, however, that there was no work along these lines. As we have seen, ophthalmologists reported findings in lens-induced disease and in sympathetic ophthalmia, but these were published in specialty ophthalmic journals. In the early 1930s, Rivers et al. published a series of papers on the production of an experimental autoimmune encephalomyelitis (EAE) (Rivers et al., 1933; Rivers and Schwentker, 1935). While these studies are viewed today as important milestones in autoimmunity research, they attracted little attention at the time among immunologists.

The contemporary view of autoimmunity during the 1940s and early 1950s is perhaps best exemplified by the position of Ernest Witebsky, who was trained in immunology by Ehrlich's student Hans Sachs and was himself a disease-oriented physician. He could say as late as 1954 at the celebration of the centenary of the birth of Ehrlich that, "The validity of the law [sic] of *horror autotoxicus* certainly should be evident to anyone interested in blood transfusion and blood disease. Autoantibodies—namely, antibodies directed against receptors of the same individual—are not formed" (Witebsky 1954). This was said by the individual who, only 2 years later with his student Noel Rose, would help refocus interest on autoimmunity with the demonstration of the production of experimental autoimmune thyroiditis (Rose and Witebsky, 1956; Witebsky et al., 1957).

THE RETURN OF IMMUNOBIOLOGY

During the late 1930s and 1940s, a series of observations began to challenge the assumptions that had guided recent thought and experiment in immunology. How could the enhanced booster antibody response, or the change with time of the specificity and affinity of the antibodies formed,

be explained in chemical terms? How to explain the persistence of antibody formation in the apparent absence of antigen? Even more troubling was the lack of relationship between immunity to certain viral diseases and the titer of antiviral antibodies. Here were basic biologic questions that demanded answers—questions with which current theory was unable to cope, and for which it could not even provide experimental approaches. But even more difficult questions arising from biology and medicine would pose further challenges.

Peter Medawar's (1945) experiments showed that the rejection of tissue grafts was somehow mediated by immunologic mechanisms. Then, Ray Owen (1945) described the paradoxical situation in which dizygotic twin cattle might share one another's red cells without being able to mount an immune response to these foreign antigens. Macfarlane Burnet, biologist *par excellence*, called attention to all of these inexplicable phenomena and hypothesized the existence of a fundamental biologic mechanism to explain Owen's finding—an embryonic interaction that would suppress the ability of an individual to respond to his/her own native antigens (Burnet and Fenner, 1949). This was soon confirmed by Medawar's group (Billingham et al., 1953), and would be termed *immunologic tolerance*. Yet another observation to emphasize the awakening biomedical movement in immunology was the description of a group of immune deficiency diseases.

Taken together, these new questions and phenomena foretokened a radical change of direction—termed elsewhere the "immunobiologic revolution" (Silverstein, 1991). Not only did these questions challenge the accepted dogma but they also served to stimulate the entry of a new group of investigators into the field. These were basic scientists from such fields as genetics and physiology, and clinicians from a variety of medical disciplines. They were unfettered by any allegiance to earlier ideas and techniques, and thus could entertain iconoclastic ideas and design novel experiments.

Perhaps the best illustration of the long period during which immunologists showed little interest in disease is provided in Table 1.1. Here, for each organ or disease entity, the interval is given between the last significant study during the "classical" period and the first significant contribution of the "modern" era. The average hiatus, where both end and restart dates can be identified, is about 44 years. This is an extremely long interlude for a field that was only some 70 years old in 1950.

Thus, in the context of a growing interest in the more biomedical aspects of the immune response, work on autoimmunity became respectable. This was due also to the increasing use of Freund adjuvant, which made animal models of the various autoimmune diseases more readily available and more reproducible. Advances came rapidly.

TABLE 1.1 Dark ages of autoimmunity*

Disease/system	Last "classical" contribution	First "modern" contribution
Hemolytic disease	1909	1945 (Coombs et al.)
Sperm and testicular	1900	1951 (Voisin et al.)
Encephalomyelitis	1905	1947 (Kabat et al.)
Sympathetic ophthalmia	1912	1949 (Collins)
Phacoanaphylaxis	1911	1957 (Halbert et al.)
Thyroid	1910	1956 (Rose and Witebsky; Roitt et al.)
Wassermann antibody	1909	—
Platelet disease	—	1949 (Acroyd)

*Modified from Silverstein (1989).

Coombs et al. (1945), using the antiglobulin test, showed that many cases of acquired hemolytic anemia were due to the "incomplete" (nonagglutinating) antibodies, Kabat et al. (1947) refocused attention on the immunopathogenesis of "allergic" encephalomyelitis. Collins (1949) introduced a reproducible animal model of sympathetic ophthalmia. Voisin et al. (1951) showed how to produce an experimental allergic orchitis. Finally, Rose and Witebsky (1956) demonstrated in experimental animals and Roitt et al. (1956) in human Hashimoto's disease that some forms of thyroid disease might be based on autoimmune processes. In addition, an understanding of the pathogenesis of some of these diseases was made easier by the increasing appreciation of the fact that not all these diseases were mediated by circulating antibodies; some involved the action of subclasses of lymphocytes that originate in the thymus.

These new findings not only opened wide the floodgates of autoimmunity studies, but stimulated further interest in the more general field of immunopathology as well. This new movement was provided with a theoretical base with Talmage's (1957) suggestion and Burnet's (1959) clonal selection theory, emphasizing for the first time the biologically important role of cell dynamics in the antibody response. It is no accident that the late 1950s saw the first international conferences on immunopathology (Miescher and Vorlaender, 1958; Grabar and Miescher, 1959) and on the fundamentals of hypersensitivity (Lawrence, 1959; Shaffer et al., 1959). For the first time, in 1963, there appeared a textbook aimed at medical students (Humphrey and White, 1963), and then two comprehensive descriptions of immunologic diseases aimed at clinicians (Gell and Coombs, 1963; Samter et al., 1965). It was in the spirit of the new immunology that Mackay and Burnet (1963) could summarize contemporary knowledge in the increasingly active field of the autoimmune diseases.

CONCLUDING REMARKS

This, then, is the story of the early stirrings of interest in the possibility that disease might result from an immune response to an individual's own autochthonous antigens. Perhaps the initial reports were too premature to be incorporated into the received wisdom of the young field of immunology, just as the discovery of several allergic diseases could not at first be integrated. Certainly Paul Ehrlich's dictum of *horror autotoxicus* contributed to an unwillingness to recognize the full significance of the initial findings of a response to spermatozoa, erythrocytes, and retina. But the mounting challenges to the dogma would eventually prove irresistible and the field of autoimmunity would finally flourish, as the following chapters in this volume attest.

References

Ackroyd, J. F. (1949). The pathogenesis of thrombocytopenic purpura due to hypersensitivity to sedormid. *Clin. Sci. 8*, 269–287.

Behring, E. and Kitasato, S. (1890). Über das Zustandekommen der Diphtherie-immunität und der Tetanus-immunität bei Tieren. *Dtsch. Med. Wochenschr. 16*, 1113–1114.

Billingham, R. E., Brent, L., and Medawar, P. B. (1953). Actively acquired tolerance of foreign cells. *Nature (London) 172*, 603–606.

Bordet, J. (1899). Sur l'agglutination et la dissolution des globules rouges par le sérum d'animaux imjectés de sang défibriné. *Ann. Inst. Pasteur 12*, 688–695.

Bordet, J. and Gengou, O. (1901). Sur l'existence des substances sensibilisatrices dans la plupart des sérums anti-microbiens. *Ann. Inst. Pasteur 15*, 289–302.

Breinl, F. and Haurowitz, F. (1930). Chemische Untersuchung des Präzipitates aus Hämoglobin und Anti-Hämoglobin und Bemerkungen über die Natur des Antikörpers. *Z. Physiol. Chem. 192*, 45–57.

Burnet, F. M. (1959). *The Clonal Selection Theory of Acquired Immunity*. Cambridge: Cambridge University Press.

Burnet, F. M. and Fenner, F. (1949). *The Production of Antibodies*, 2nd ed. New York: Macmillan.

Collins, R. C. (1949). Experimental studies on sympathetic ophthalmia. *Am. J. Ophthalmol. 32*, 1687–1699.

Coombs, R. R. A., Mourant, A. E., and Race, R. R. (1945). A new test for the detection of weak and "incomplete" Rh agglutinins. *Br. J. Exp. Pathol. 26*, 255–266.

Detré, L. (1906). Über den Nachweis von spezifischen syphilis und der Antigenen bei Luetikern. *Wien. Klin. Wochenschr. 19*, 619–689.

Donath, J. and Landsteiner, K. (1904). Über paroxysmale Hämoglobinurie. *Münch. Med. Wochenschr. 51*, 1590–1593.

Donath, J. and Landsteiner, K. (1906). Über paroxysmale Hämoglobinurie. *Z. Klin. Med. 58*, 173–189.

Ehrlich, P. (1897). Die Wertbemessung des Diphtherieheilserums und deren Theoretischen Grundlagen. *Klinische Jahrb. 6*, 299–326.

Ehrlich, P. (1900). On immunity with special reference to cell life: Croonian Lecture. *Proc. R. Soc. London 66*, 424–448.

Ehrlich, P. (1902). Die Schutzstoffe des Blutes. *Verh. Ges. Dtsch. Naturforsch. Aerzte 1*, 250–275.

Ehrlich, P. and Morgenroth, J. (1900). Über Hämolysine. Dritte Mittheilung. *Berlin Klin. Wochenschr. 37*, 453–458.

Ehrlich, P. and Morgenroth, J. (1901). Über Hämolysine. Fünfte Mittheilung. *Berlin Klin. Wochenschr. 38*, 251–255.

Elschnig, A. (1910a). Studien zur sympatischen Ophthalmie. *von Graefes Arch. Ophthalmol. 75*, 459–474.

Elschnig, A. (1910b). Studien zur sympatischen Ophthalmie: Die Antigene Wirkung des Augenpigmentes. *von Graefes Arch. Ophthalmol. 76*, 509–546.

Fleck, L. (1979). *Genesis and Development of a Scientific Fact*. Chicago: University of Chicago Press.

Friedenwald, J. S. (1934). Notes on the allergy theory of sympathetic ophthalmia. *JAMA 17*, 1008–1018.

Gell, P. G. H. and Coombs, R. R. A. (1963). *Clinical Aspects of Immunology*. Oxford: Blackwell Scientific.

Goltz, D. (1980). *Horror Autotoxicus: Ein Beitrag zur Geschichte und Theorie der Autoimmunpathologie im Spiegel eines vielzitierten Begriffes*. Thesis, University of Münster.

Grabar, P. and Miescher, P. (1959). *Immunopathology-Immunopathologie*. Basel: Benno Schwabe.

Halbert, S. P., Locatcher-Khorazdor, D., Sonn-Kazar, C., and Swick, L. (1957). Homologous immunological studies of ocular lens II. Biological aspects. *J. Exp. Med. 105*, 453–462.

Heidelberger, M. and Avery, O. T. (1923). The soluble specific substance of pneumococcus. *J. Exp. Med. 38*, 73–79.

Humphrey, J. H. and White, R. G. (1963). *Immunology for Students of Medicine*. Oxford: Blackwell Scientific.

Jerne, N. K. (1974). Towards a network theory of the immune system. *Ann. Immunol. (Paris) 125C*, 373–389.

Kabat, E. A. and Mayer, M. M. (1949). *Quantitative Immunochemistry*. Springfield, IL: Charles C. Thomas.

Kabat, E. A., Wolfe, A., and Bezer, A. E. (1949). The rapid production of acute encephalomyelitis in Rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants. *J. Exp. Med. 85*, 117–130.

Krusius, F. F. (1910). Über Empfindlichkeitsversuche vom Auge aus. *Arch. Augenheilk. 67*, 6–35.

Kuhn, T. (1970). *The Structure of Scientific Revolutions*, 2nd ed. Chicago: University of Chicago Press.

Landsteiner, K. (1900). Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkung des Blutserums und der Lymphe. *Centralbl. Bakteriol. 27*, 357–362.

Landsteiner, K. (1962). *The Specificity of Serological Reactions*, Reprint of 2nd ed., 1945. New York: Dover.

Lawrence, H. S. (1959). *Cellular and Humoral Aspects of Hypersensitivity States*. New York: Hoeber-Harper.

Mackay, I. R. and Burnet, F. M. (1963). *Autoimmune Diseases*. Springfield, IL: Charles C. Thomas.

Medawar, P. B. (1945). The behavior and fate of skin autografts and skin homografts in rabbits. *J. Anat. 78*, 176–199.

Metalnikoff, S. (1900). Études sur la spermotoxine. *Ann. Inst. Pasteur 14*, 577–589.

Metchnikoff, I. I. (1884). Über eine Sprosspilzkrankheit der Daphnien: Beitrag zur Lehre über den Kampf des Phagozyten gegen Krankheitserreger. *Arch. Pathol. Anat. 86*, 177–195.

Metchnikoff, E. (1900). Sur les cytotoxines. *Ann. Inst. Pasteur 14*, 369–377.

Miescher, P. and Vorlaender, K. O. (1958). *Immunpathologie in Klinik und Forschung*. Stuttgart: Georg Thieme.

Owen, R. D. (1945). Immunogenetic consequences of vascular anastomoses between bovine twins. *Science 102*, 400–401.

Pasteur, L. (1880). Sur les maladies virulentes et en particulier sur la maladie appelée vulgairement choléra des poules. *Compt. Rend. Acad. Sci. 90*, 239–248.

Pauling, L. (1940). A theory of the structure and process of formation of antibodies. *J. Am. Chem. Soc. 62*, 2643–2657.

Pfeiffer, R. (1894). Weitere Untersuchungen über das Wesen der Choleraimmunität und über spezifische baktericide Prozesse. *Z. Hygiene 18*, 1–16.

Rivers, T. M. and Schwentker, F. F. (1935). Encephalomyelitis accompanied by myelin destruction produced in monkeys. *J. Exp. Med. 61*, 689–702.

Rivers, T. M., Sprunt, D. H., and Berry, G. P. (1933). Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. Exp. Med. 58*, 39–54.

Roitt, I. M., Doniach, D., Campbell, P. N., and Vaughan-Hudson, R. (1956). Auto-antibodies in Hashimoto's disease (lymphadenoid goiter). *Lancet 2*, 820–821.

Römer, P. (1905). Die Pathogenese der *Cataracta senilis* vom Standpunkt der Serumforschung. *von Graefes Arch. Ophthalmol. 60*, 175–186.

Römer, P. and Gebb, H. (1912). Beitrag zur Frage der Anaphylaxie durch Linseneiweiss und Eiweiss aus andern Geweben des Auges. *von Graefes Arch. Ophthalmol. 81*, 367–402.

Rose, N. R. and Mackay, I. R. (1985). Introduction. In N. R. Rose and I. R. Mackay (eds), *The Autoimmune Diseases* (p. xxxiv). New York: Academic Press.

Rose, N. R. and Witebsky, E. (1956). Studies on organ specificity V. Changes in the thyroid gland of rabbits following active immunization with rabbit thyroid extracts. *J. Immunol. 76*, 417–427.

Sachs, H. (1909). Hämolysine und Cytotoxine des Blutserums. In Handbuch der Technik und Methodik der Immunitätsforschung, vol. 2. (pp. 896–897). Jena: Fisher.

Samter, M. et al. (1965). *Immunological Diseases*. Boston: Little Brown.

Santucci, S. (1906). Citotossine. *Riv. Ital. Ottal. Roma 2*, 213.

Shaffer, J. H., LoGrippo, G. A., and Chase, M. W. (1959). *Mechanisms of Hypersensitivity*. Boston: Little, Brown.

Silverstein, A. M. (1989). Allergy and immunity: The price of immunity. In *A History of Immunology* (pp. 214–251). New York: Academic Press.

Silverstein, A. M. (1991). The dynamics of conceptual change in twentieth century immunology. *Cell. Immunol. 132*, 515–531.

Silverstein, A. M. (1994). The heuristic value of experimental systems: The case of immune hemolysis. *J. Hist. Biol. 27*, 437–447.

Silverstein, A. M. (2002). *Paul Ehrlich's Receptor Immunology* (pp. 95–122). New York: Academic Press.

Talmage, D. W. (1957). Allergy and immunology. *Annu. Rev. Med. 8*, 239–256

Uhlenhuth, P. (1903). Zur Lehre von der Unterscheidung verschiedener Eiweissarten mit Hilfe spezifischer Sera. In *Festschrift zum 60 Geburtstag von Robert Koch* (p. 49). Jena: Fisher.

Verhoeff, F. H. and Lemoine, A. N. (1922). Endophthalmitis phacoanaphylactica. *Am. J. Ophthalmol. 5*, 737–745.

Voisin, G., Delaunay, A., and Barber, M. (1951). Sur des lésions testiculaires provoquées chez le cobaye par iso- et auto-sensibisation. *Ann. Inst. Pasteur 81*, 48–63.

von Pirquet, C. (1910). *Allergie*. Berlin: Springer. [English transl., *Allergy*. American Medical Association, Chicago, 1911.]

von Wassermann, A., Neisser, A., and Bruck, C. (1906). Eine serodiagnostische Reaktion bei Syphilis. *Dtsch. Med. Wochenschr. 32*, 745–746.

Weil, E. and Braun, H. (1909). Über das Wesen der luetischen Erkrankung auf Grund der neueren Forschungen. *Wien. Klin. Wochenschr. 22*, 372–374.

Witebsky, E. (1954). Ehrlich's side-chain theory in light of present immunology. *Ann. N.Y. Acad. Sci. 59*, 168–181.

Witebsky, E., Rose, N. R., Terplan, K., Paine, J. R., and Egan, R. W. (1957). Chronic thyroiditis and autoimmunization. *JAMA 164*, 1439–1447.

Woods, A. C. (1921). Immune reactions following injuries to the uveal tract. *JAMA 77*, 1217–1222.

Woods, A. C. (1925). Sympathetic ophthalmia: the use of uveal pigment in diagnosis and treatment. *Trans. Ophthamol. Soc. UK 45*, 208–249.

Woods, A. C. (1933). *Allergy and Immunity in Ophthalmology*. Baltimore: Johns Hopkins University Press.

CHAPTER

2

Structural and Functional Aspects of the Innate and Adaptive Systems of Immunity

PETER J. DELVES

Department of Immunology and Molecular Pathology, Division of Infection and Immunity, University College London, London, UK

Most cells of the immune system are derived from self-renewing hematopoietic stem cells initially generated in the embryonic yolk sac, later in the fetal liver and spleen, and then by the time of birth onwards in the bone marrow. The stem cells give rise to both the lymphoid lineage, which produces lymphocytes, natural killer (NK) cells, and some types of interdigitating dendritic cells (IDCs), and to the myeloid lineage which generates polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils), mast cells, monocytes and macrophages, megakaryocytes, erythrocytes, and IDCs. Development of the lymphoid and myeloid precursors is regulated by stromal cells (such as fibroblasts, endothelial cells, and macrophages) and by growth factors including stem cell factor and colony-stimulating factors.

The immune system can mount innate responses that occur to the same extent however many times the pathogen is encountered, and adaptive (acquired) responses that generate immunologic memory leading to quantitatively and qualitatively enhanced responses on subsequent encounters with the antigen. Innate responses exhibit broad specificity based on detection of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) (Janeway, 1989), and the binding of complement- and/or antibody-coated antigens. Among the PRRs that have been described are a number of Toll-like receptors (TLRs), which recognize microbial components, such as lipopolysaccharide from gram-negative bacteria (TLR4), peptidoglycan (TLR2), and the CpG motifs (unmethylated cytosine–guanosine dinucleotide sequence flanked by two 5′ purines and two 3′ pyrimidines) (TLR9). Other PRRs include the mannose receptor, which binds to terminal mannose and fucose, and scavenger receptors that bind a variety of microbial components. Only the lymphocytes, the dedicated cells of the adaptive response, bear receptors permitting exquisitely refined recognition of individual antigens. Each lymphocyte possesses many thousand antigen receptors which all have identical specificity. While the B-cell receptor (BCR) recognizes structures (epitopes) on the surface of native antigen, the epitopes recognized by the T-cell receptor (TCR) are short peptides. The peptides are produced by proteolytic processing of antigen within cells and are then presented to T cells by highly polymorphic major histocompatibility complex (MHC) class I and class II cell-surface molecules. The human class I comprises HLA-A, -B, and -C, and the class II comprises HLA-DP, -DQ, and -DR, whereas mice bear the H2-D, -K, and -L class I molecules, and I-A and I-E class II molecules. MHC class II expression is restricted to IDCs, macrophages, and B lymphocytes, which present 8–30 amino acid long peptides to the TCR on helper T (Th) cells. In contrast, MHC class I molecules, which present peptides of only 8–9 amino acids in length, are expressed by nearly all nucleated cells in the body and are concerned with alerting cytotoxic T lymphocytes (CTL) to the presence of intracellular infections.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

INNATE RESPONSES

Cellular Components

Cells of the innate immune response are generally either phagocytic (neutrophils, monocytes, and macrophages) or act by releasing inflammatory mediators (basophils, mast cells, eosinophils). NK cells, also a component of innate responses, induce apoptosis in infected cells.

The immediate consequence of encounter with an antigen that is deemed to pose a threat is often the generation of an acute inflammatory response in which cells and molecules of the immune system are rapidly recruited to the site of the stimulus. Inflammatory mediators, such as gram-negative bacterial endotoxin [lipopolysaccharide (LPS)], and the cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α lead to increased expression of vascular endothelial adhesion molecules, including P-selectin, E-selectin, and intercellular adhesion molecule (ICAM)-1. These alert inflammatory cells to the presence of a local infection. P- and E-selectin bind to P-selectin glycoprotein ligand (PSGL)-1 and ICAM-1 binds to the β_2 integrin CR3 on neutrophils (Ley, 2002). Initially the neutrophils are slowed down and roll along the blood vessel wall, eventually being brought to a halt by adhesion molecule interactions. The neutrophils undergo extravasation—squeezing between the endothelial cells—a process greatly facilitated by the deformable nature of the multilobed nucleus in these polymorphonuclear leukocytes. Histamine released from mast cells causes smooth muscle contraction and an increase in local vascular permeability, thus facilitating the passage of the neutrophil from the blood to the tissues. Activation of the complement system plays a central role in this process, triggering the mast cell degranulation and chemotactically attracting neutrophils. Chemokines (chemotactic cytokines) also induce the neutrophils to migrate to the site of the infection (Rot and von Adrian, 2004). The presence on the neutrophil cell surface of both Fc receptors for antibodies and complement receptors greatly facilitates phagocytosis if the antigen is opsonized (coated) with these agents (Underhill and Ozinsky, 2002). Engulfed microorganisms are killed by a plethora of toxic molecules, including superoxide anions, hydroxyl radicals, hypochlorous acid, nitric oxide, proteases, antimicrobial cationic proteins and peptides, and lysozyme.

Eosinophils are less phagocytic than neutrophils, but can kill large parasites by releasing cationic proteins and reactive oxygen metabolites. They also secrete leukotrienes, prostaglandins, and several cytokines (Wardlaw et al., 1995). Blood basophils and tissue mast cells are not phagocytic and share many features, although mast cells are probably not derived from basophils. Both cell types become sensitized with IgE antibodies bound to their high-affinity Fcε receptors (FcεRI) and, when antigen cross-links the IgE, release several preformed inflammatory mediators, including histamine, platelet-activating factor, neutrophil and eosinophil chemotactic factors, and TNF-α (Frossi et al., 2004). Newly synthesized leukotrienes, prostaglandins, and thromboxanes are also released. Two populations of mast cells have been described, those in the skin which contain both tryptase and chymase (MC_{TC}) and those in the lung and intestinal mucosa which do not contain chymase (MC_T) (Frossi et al., 2004). A number of immunoregulatory roles have been proposed for mast cells (Frossi et al., 2004), including the possibility that histamine release may regulate T- and B-cell responses via signaling through H1 and H2 receptors (Jutel et al., 2001).

The tissue macrophages and their circulating precursors, the blood monocytes, possess both Fc receptors and complement receptors, and they contain similar microbicidal substances to those present in neutrophils. However, they are not only much longer lived than neutrophils but are also able to process antigens for presentation to Th cells. An additional role of the macrophage is the removal of the body's own dead or dying cells. While tissue damage associated with necrotic cell death triggers inflammation, cells dying due to apoptosis are removed much more quietly. Loss of membrane symmetry is a feature of apoptotic cell death and exposes the molecule phosphatidylserine on the cell surface, marking the cell for phagocytosis by macrophages expressing phosphatidylserine receptors (Henson et al., 2001).

NK cells express sets of killer-activating receptors and -inhibitory receptors (Figure 2.1). The activating receptors, which can either possess immunoglobulin-like domains or be lectin-like, bind molecules ubiquitously expressed on the surface of nucleated cells, while the inhibitory receptors, which can also be either immunoglobulin- or lectin-like, recognize MHC class I molecules, which are also usually present on nucleated cells (Yokoyama et al., 2004). Loss of surface MHC class I expression can, however, occur in certain viral infections (e.g., herpesvirus) or sometimes as a result of malignant transformation. Any cell lacking MHC class I is therefore viewed by the immune system as being abnormal and signals from the NK-activating receptors are not blocked by the inhibitory receptors. Activation of NK cells leads to insertion of the pore-forming molecule perforin into the target cell membrane and subsequent injection of granzymes (serine proteases), which cause apoptosis in the target cell.

The IDCs, a heterogeneous population including the Langerhans cell in the skin, are bone marrow-derived cells that act at the interface of the innate and adaptive responses in that their recognition of antigen involves broadly-specific PRR, but their role is to present the antigen to the highly specific TCR on Th cells (Guermonprez et al., 2002) (Figure 2.2). IDCs constantly but quietly sample extracellular antigens by endocytosis and become activated to an

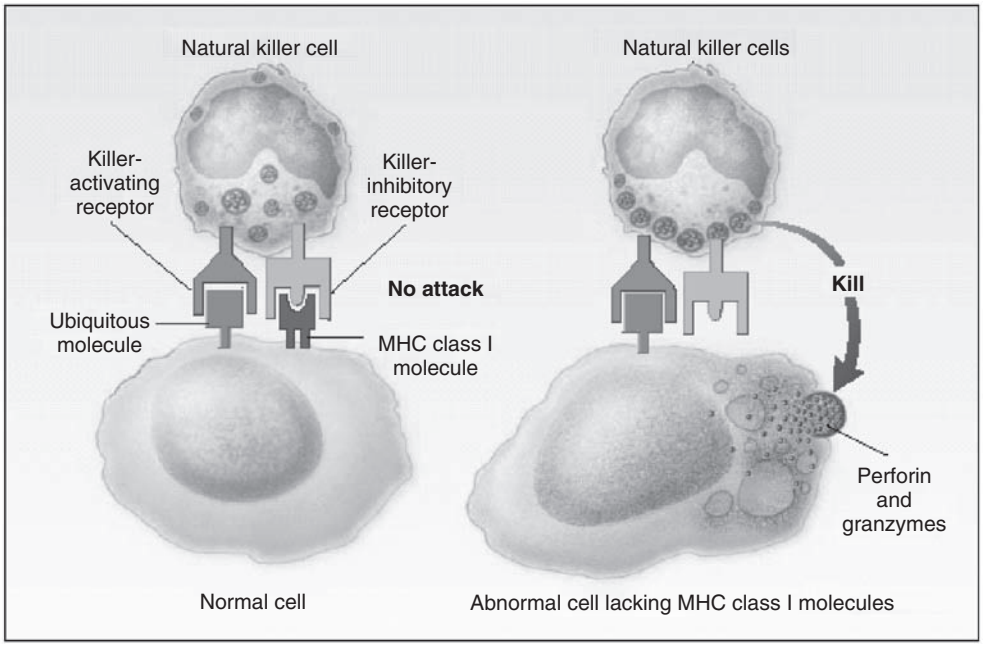


FIGURE 2.1 Natural killer (NK) cells can attack cells lacking MHC class I expression following recognition by killer-activating receptors in the absence of a downregulatory signal from killer-inhibitory receptors. The cytotoxic granules of the NK cells, which contain perforin and granzymes, become polarized to the interface between the cells and are then released into the target cell. See color plate section.

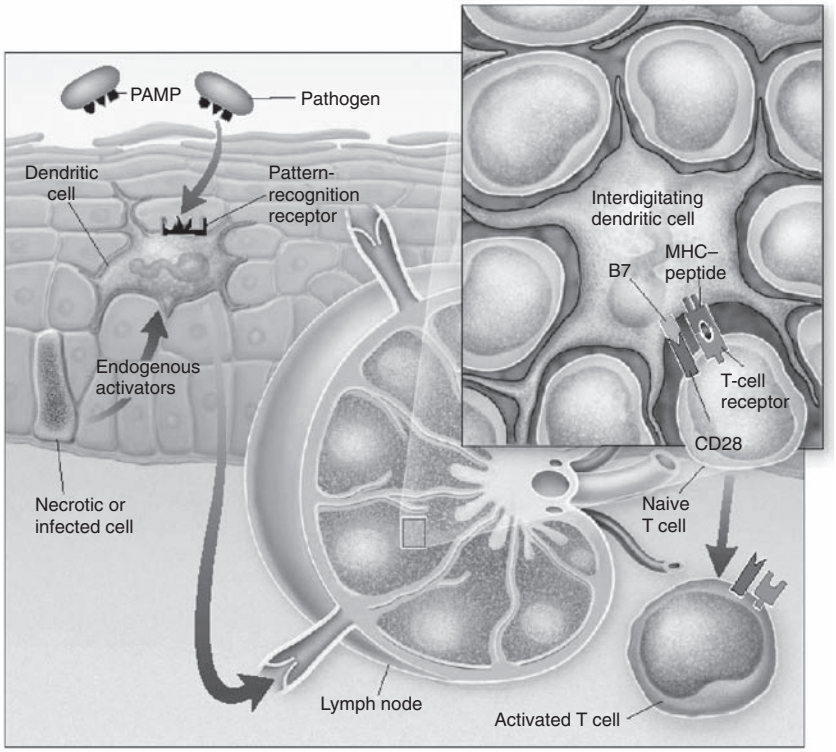


FIGURE 2.2 Pathogen-associated molecular patterns (PAMPs) allow the pattern-recognition receptors (PRR) on the interdigitating dendritic cells (IDCs) to differentiate between potentially harmful foreign microorganisms and self constituents. IDCs are also stimulated by endogenous activators such as interferon-α and heat-shock proteins released from infected or necrotic cells. The activated IDCs process the antigen to generate peptides that are presented by MHC molecules to the T-cell receptor (TCR) on T cells in the local draining lymph nodes. See color plate section.

antigen-presenting cell (APC) mode when their PRRs recognize PAMPs, such as LPS, terminal mannose, and microbial CpG motifs (Janeway and Medzhitov, 2002; Krieg, 2002). The activated IDCs then travel to the local draining lymph node where they present antigen to T cells. During their migration through the afferent lymphatics, they upregulate their cell-surface MHC class II molecules and CD80 (B7.1) and CD86 (B7.2) costimulatory molecules, which are ligands for CD28 on the T cell (Kroczek et al., 2004). Such costimulation is required, together with antigen, for the activation of naïve T cells. Within the dendritic cell (DC) the antigen is processed into short peptides and then expressed on the cell surface together with the MHC class II molecules for presentation to Th cells. IDCs are also able to "cross-present" exogenous antigens by transferring them into the MHC class I processing and presentation pathway for recognition by cytotoxic T cells (Ramirez and Sigal, 2004). T-cell interactions with immature DCs lacking CD80/CD86 expression lead to tolerance, often by inducing anergy (functional inactivation) in the T cell (Schwartz, 2003). Mature IDCs can also be tolerogenic in some cases, e.g., by suppressing T-cell proliferation via the production of indoleamine 2,3-dioxygenase (Mellor and Munn, 2003).

Although B cells are able to recognize antigen without the intervention of any other cell type, recognition is more efficient if multiple copies of the antigen are "presented" to the B cell, e.g., in the form of immune complexes held on the surface of follicular dendritic cells (FDCs) (van Nierop and de Groot, 2002). These are an entirely different cell type from the IDC, are not phagocytic, and lack MHC class II molecules. Furthermore, they are not bone marrow derived, probably arising from fibroblastic reticular cells in the B cell areas of lymphoid tissues. They can present immune complexes to B cells very efficiently by virtue of their FcγRIIB receptors for IgG, and CR1 and CR2 receptors for complement.

The role that erythrocytes perform in immune responses should not be overlooked. Their possession of CR1 complement receptors for C3b, C4b, and iC3b confers on these cells an important role in clearing immune complexes from the circulation to the liver and spleen, where they are destroyed by Küpffer cells and splenic macrophages (Birmingham, 1995).

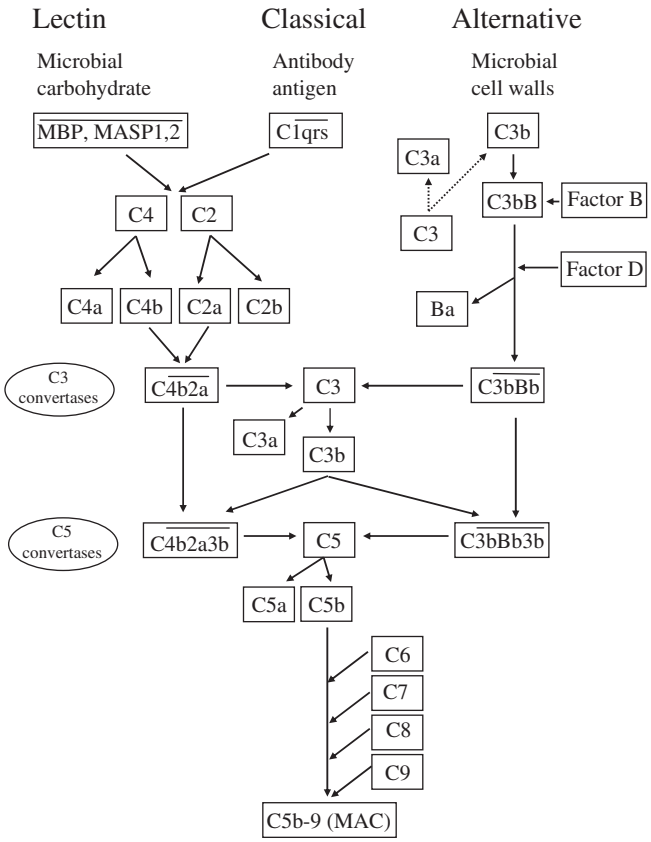


FIGURE 2.3 Complement system. For simplicity complement regulatory proteins have been omitted. For details see text. MAC, membrane attack complex; MASP, MBP-associated serine proteases; MBP, mannose-binding protein.

Soluble Mediators

The complement system is based on an enzymic amplification cascade that can be triggered using one of three pathways; classical, lectin, and alternative. These all lead to the cleavage of complement component C3 by a C3 convertase which "converts" C3 into C3a and C3b (Figure 2.3). The classical pathway is activated by IgG and IgM antibodies when they bind antigen, thereby creating an array of closely-associated immunoglobulin Fc regions to which complement component C1q binds, followed by C1r and C1s. This event initiates a series of enzymic reactions leading to the generation of the classical pathway C3 convertase, $C\overline{4b2a}$. The lectin pathway, which is essentially a variant of the classical pathway, leads to the generation of the same C3 convertase when microbial carbohydrates interact with mannose-binding protein (MBP) which then binds to the two MBP-associated serine proteases, MASP-1 and MASP-2. The initially quite separate alternative pathway is activated when complement component C3b becomes stabilized by binding to microbial cell walls. The C3b then combines with factor B which is cleaved by factor D, generating a different C3 convertase, $C\overline{3bBb}$. Both proteolysis and thioester hydrolysis of C3 constitutively generate very low levels of C3b. However, in the alternative pathway, it is only when $C\overline{3bBb}$ is generated that there is substantial splitting of C3 into C3a and C3b. Subsequently, a C5 convertase, either $C\overline{4b2a3b}$ (from the classical and lectin pathways) or $C\overline{3bBb3b}$ (from the alternative pathway), is produced by addition of C3b to the C3 convertase. This splits C5 into C5a

and C5b, ultimately leading to the generation of the membrane attack complex (MAC) composed of complement components C5b, C6, C7, C8, and C9. Because complement activation consists of a series of sequential enzyme reactions, there is a tremendous amplification of the initial response, and along the way a number of complement components with potent immunologic activities are generated.

The main function of C3b is to enhance the engulfment of antigens by phagocytic cells bearing the complement receptors CR1 and CR3. The C3a, C4a, and C5a components act as anaphylatoxins triggering the release of inflammatory mediators from mast cells. C5a is also a potent neutrophil chemoattractant. The MAC generates pores in cell membranes, ultimately leading to the demise of the target cell by apoptosis. Because of these many potent activities, the complement cascades are tightly controlled by a number of complement regulatory proteins, including C1 inhibitor, factor H, and factor I (both of which break down C3b in the alternative pathway); CD46 (membrane cofactor protein) and CD55 (decay accelerating factor), both of which limit the formation and function of the C3 convertases; and CD59 (homologous restriction factor, an inhibitor of MAC formation) (Morgan and Harris, 1999).

Complement components C3 and C9, and factor B are classed as acute-phase proteins. This diverse group of mediators, which includes C-reactive protein, serum amyloid A protein, proteinase inhibitors, and coagulation proteins, shares an ability to undergo a rapid change in plasma concentration in response to infection, inflammation, and tissue injury. Collectively, the acute-phase proteins facilitate host resistance to infection and promote the resolution of tissue damage (Gabay and Kushner, 1999).

Another group of proteins, which function in both the innate and adaptive response, are the cytokines (Fitzgerald et al., 2001). These soluble mediators facilitate communication both within the immune system and between the immune system and other cells of the body. To respond to a given cytokine a cell must express the relevant cytokine receptor. In addition to acting as communication molecules, some cytokines play a more direct role in immune defense. For example, the interferons produced by virally-infected cells establish a state of viral resistance in surrounding noninfected cells, thereby acting as a "firebreak" against the spread of the infection (Basler and Garcia-Sastre, 2002).

ADAPTIVE IMMUNE RESPONSES

The adaptive responses involve the clonal expansion of antigen-specific B and T lymphocytes. B cells produce the antigen-specific antibodies responsible for elimination of extracellular antigens. T cells kill virally-infected cells, secrete cytokines, and help B cells to make antibody.

T Cell Development

T-cell precursors migrate from the bone marrow to the thymus, an organ essential for the production of most T cells. T-cell development occurs in the thymus throughout life, despite the fact that it undergoes significant atrophy at puberty (Jamieson et al., 1999). The TCR on T cells comes in two versions, either a $\alpha\beta$ or a $\gamma\delta$ heterodimer; each chain of the dimer has one variable domain and one constant domain. T lymphocytes are capable of producing vast numbers of different TCR variable regions by recombining variable (V), diversity (D), and joining (J) gene segments from the pools of α, β, γ, and δ TCR genes. There are a number of different sequences for each segment and one of each set is utilized in the rearrangement event. The recombination-activating gene (RAG) products RAG-1 and RAG-2 mediate these processes following the recognition of recombination signal sequences (RSS) that flank the V, D, and J gene segments (Livak and Petrie, 2002). Splicing inaccuracies, and the insertion of additional nucleotides around the V–D–J junctions by the enzyme terminal deoxynucleotidyl transferase (TdT), further increase diversity (Benedict et al., 2000).

Rearrangement, and subsequent expression, of the TCR genes does not occur until the precursor T cells reach the thymus. For T cells that express an $\alpha\beta$ TCR, initially the TCR β chain gene segments are randomly rearranged and coexpressed with a nonrearranging pre-T α chain (which lacks a variable domain) to produce a preliminary version of the TCR. Subsequently the TCR α chain gene segments are randomly rearranged in order to generate the mature $\alpha\beta$ TCR. Both positive and negative selection of the T cells then occurs (Starr et al., 2003) (Figure 2.4). Positive selection ensures that the randomly generated TCR is able to interact with self MHC molecules. The T cells coexpress the cell-surface molecules CD4 and CD8 during their early development, allowing them to potentially interact with both MHC class I and class II molecules. CD4 binds to conserved (nonpolymorphic) residues on the MHC class II molecule, while CD8 binds to conserved residues on MHC class I. At this stage in their differentiation the T cells are programmed to undergo apoptosis and are only rescued from this default cell death if their TCR is capable of binding to MHC on the thymic cortical epithelium. Over 95% of the T cells fail at this first hurdle and are therefore eliminated. For the remaining cells the default pathway is switched to survival and apoptosis is induced in any lymphocytes capable of high-affinity binding to peptides presented by self-MHC on DCs, macrophages, and thymic epithelial cells. This clonal deletion process constitutes central tolerance of self-reactive T cells. Peptides are generated from the many organ- and tissue-specific self-antigens expressed in the thymus under the transcriptional control of the autoimmune regulator (AIRE) protein (Venanzi et al., 2004). Negative selection,

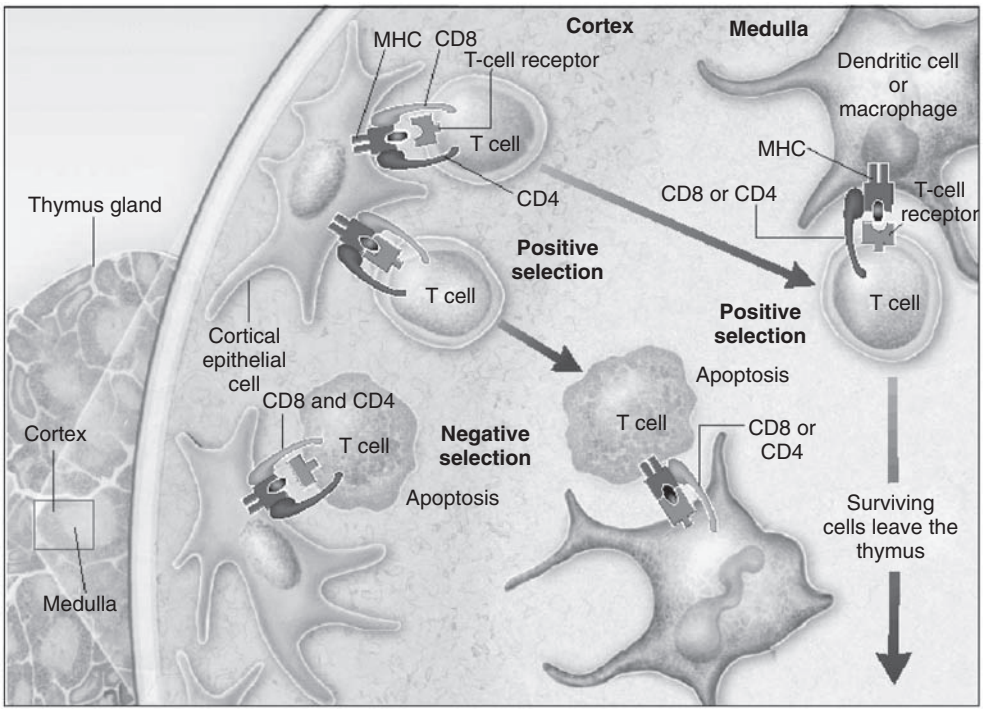


FIGURE 2.4 Positive and negative selection in the thymus. Cells with T-cell receptors (TCRs) of various affinities for self-MHC are positively selected on cortical epithelial cells. Any of these cells that bear a TCR with high affinity for self-peptide plus self-MHC (or even just MHC irrespective of the peptide contained) are subsequently eliminated by induction of apoptosis when they interact with dendritic cells, macrophages or epithelial cells in the thymic medulla (negative selection). This leaves T cells with only a weak affinity for self-MHC. These cells form the pool of T cells that are exported from the thymus as single $CD4^+$ or $CD8^+$ cells. In the periphery they have the potential to recognize foreign peptide plus self-MHC, and become activated if the affinity of the interaction is above a certain threshold and the recognition occurs in the presence of costimulatory signals. See color plate section.

like failure to be positively selected, results in extensive T-cell death within the thymus. The T cells lose expression of either CD4 or CD8 to become "single positive" CD4 or CD8 cells, and they exit the thymus and enter the periphery, a term used to denote any location outside of the primary lymphoid organs (bone marrow and thymus). These mature naïve T cells will be capable of recognizing foreign peptides presented by self-MHC.

Although γδ T cells also rearrange their TCR genes in the thymus, they do not undergo thymic selection and therefore leave the thymus early in their development. Indeed, the origin of some intestinal γδ T cells may be thymic independent (Carding and Egan, 2002).

Functional Activities of T Cells

The αβ and γδ TCRs are not themselves able to transmit activation signals to the cell nucleus, this function being assigned to the CD3 molecules (CD3γ, CD3δ, CD3ε) and the CD3-associated ζ chains (Figure 2.5). Receptor aggregation occurs within lipid rafts which also incorporate a number of adhesion and costimulatory molecules, including CD28, CD2, and CD43 (Dykstra et al., 2003), to form the immunologic synapse (Davis and Dustin, 2004). Stimulation through the synapse results in the phosphorylation of tyrosines within immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytoplasmic tails of the CD3 complex and the ζ chains. A number of protein kinases, including Lck, Fyn, and ZAP-70, are involved, with Lck also binding to the cytoplasmic tail of the CD4 or CD8 molecule (Hermiston et al., 2003). Downstream signal transduction results in the transcriptional activation of several genes, including that for IL-2. The CD45 phosphatase also plays a critical role in both T- and B-cell activation by its ability to act as both a positive and negative regulator of Lck and Fyn (Hermiston et al., 2003).

Broadly speaking, $CD4^+$ T cells act as helper T lymphocytes, while $CD8^+$ T cells are usually cytotoxic. However, some $CD4^+$ cells can exhibit cytotoxic activity (Appay et al., 2002), while $CD8^+$ cells secrete cytokines that

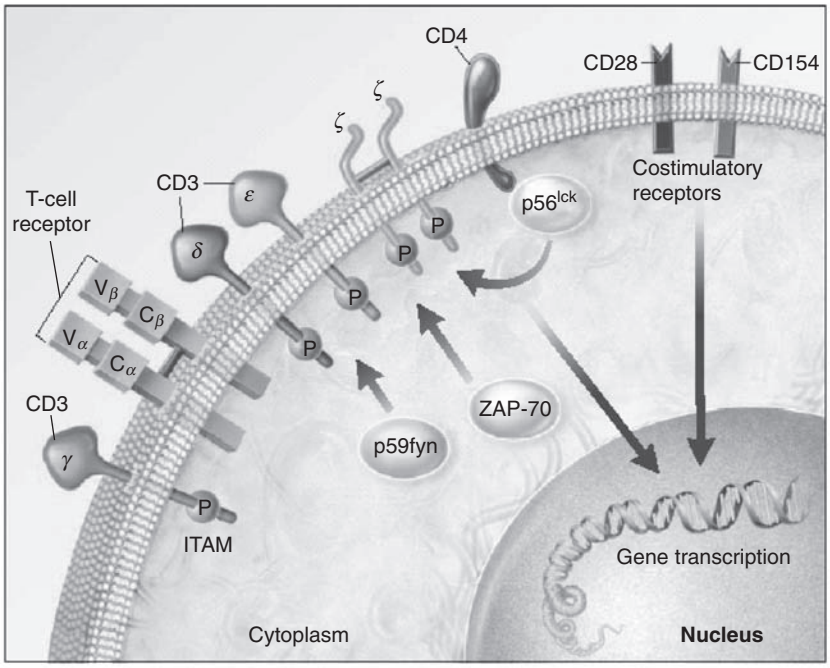


FIGURE 2.5 Lymphocyte activation involves a highly complex series of integrated events resulting from cross-linking of the antigen receptor on the cell surface. Because the antigen receptors have extremely short cytoplasmic tails they are associated with cell-surface molecules bearing cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs), which are subject to phosphorylation by protein kinases. These events lead to downstream signaling involving a number of different biochemical pathways that result in the transcriptional activation of genes involved in cellular proliferation and differentiation. The presence of signals from costimulatory molecules, such as CD28 and CD154 (CD40 ligand), is obligatory if the lymphocyte is to be activated; signals from the antigen receptor signal-transducing molecules alone lead to anergy or apoptosis. TCR, T-cell receptor. See color plate section.

can either help in the generation of immune responses or be cytotoxic (Mosmann and Sad, 1996; Woodland and Dutton, 2003).

The TCR on $CD8^+$ CTL binds to peptide–MHC class I on target cells. Endogenous antigens, including self-antigens and viral proteins, are broken down into peptides by a proteolytic structure known as the immunoproteasome (Shastri et al., 2002). If the TCR recognizes the peptide–MHC combination the CTL can kill the target either by engaging the Fas molecule on the target cell with Fas ligand on the CTL or by using the perforin/granzyme pathway (Russell and Ley, 2002). The outcome of either of these events is apoptosis of the target.

In stark contrast to the ubiquitously expressed MHC class I, MHC class II is only present on a few specialized cells, which are referred to as professional APCs; namely IDCs, macrophages, and B cells. These cells generate peptides by proteolytic cleavage of engulfed antigens within endosomal vesicles and then present the peptide–MHC class II combination to $CD4^+$ T cells. Th cells can be divided into different populations based on the cytokines they produce. Cells secreting IL-2 and interferon (IFN)-γ, but not IL-4, IL-5, and IL-6, are designated Th1 cells, while those secreting IL-4, IL-5, IL-6, IL-10, and IL-13, but not IL-2 and IFN-γ, are classified as Th2 cells (Mosmann and Sad, 1996). In general, cytokine production by Th1 cells facilitates "cell-mediated immunity," including macrophage activation and T-cell mediated cytotoxicity; but can also assist in the production of some humoral responses (e.g., IgG2a in the mouse), while the cytokines produced by Th2 cells are mostly involved in humoral immunity by helping B cells to produce antibodies, particularly those of the IgE and IgA isotypes (Fitzgerald et al., 2001). IL-12 from IDCs drives T cells towards a Th1 phenotype (Moser and Murphy, 2000) and Th cell responses tend to become polarized because the IFN-γ from Th1 cells downregulates Th2 activity, whereas IL-4 and IL-10 from Th2 cells downregulate Th1 cells.

An additional population of $CD4^+$ lymphocytes exist that have some properties of NK cells and some of T cells. These NK cells express lower levels of the αβ TCR than do conventional T-cells, and these TCRs exhibit a very limited diversity (Pear et al., 2004). They can recognize antigen presented by the non-classical MHC molecule CD1 and secrete both IL-4 and IFN-γ (Kronenberg and Gapin, 2002).

The antigen-specificity and functions of γδ T cells is much less well characterized than those of αβ T cells, but some γδ TCRs recognize native antigen directly or

recognize lipids, glycolipids, and lipopeptides presented by CD1 (Brigl and Brenner, 2004). γδ T cells take up residence throughout the body, being particularly prevalent in the intestinal epithelium where they contribute to mucosal defenses (Chen et al., 2002). They may also play an immunoregulatory role, secreting proinflammatory IFN-γ early in an immune response and then later producing immunosuppressive cytokines, such as IL-10, and acquiring cytotoxic activity against activated macrophages (Carding and Egan, 2002).

B Cell Development and Functions

A minor population of B cells, B1 cells, develops early during ontogeny and often expresses the CD5 cell-surface molecule (Berland and Wortis, 2002). These cells secrete low- to moderate-affinity IgM antibodies that can exhibit polyreactivity, i.e., recognize several different antigens, often including common pathogens and autoantigens. Such antibodies are sometimes called "natural antibodies" because of their presence in the absence of an obvious antigenic challenge. Most B cells, B2 cells, lack CD5 and develop slightly later in ontogeny. Like T cells, these B cells are capable of producing a huge number of different variable regions on their antigen receptors using rearrangement of immunoglobulin heavy and light chain gene loci (Figure 2.6). Initially, they express a pre-BCR composed of an immunoglobulin μ heavy chain generated by gene rearrangement and a surrogate light chain encoded by two nonrearranging genes termed VpreB and λ5. Expression of this pre-BCR on the immature B cell is obligatory for interaction with the bone marrow stromal cells that drive B-cell differentiation towards the mature naïve B cell coexpressing conventional IgM and IgD antibodies on the cell surface (Bradl et al., 2003). Once the B cells express a mature antigen receptor their survival and further differentiation becomes antigen dependent.

The antigen receptor is associated with several molecules, including Igα (CD79a), Igβ (CD79b), CD19, CD21 (the CR2 complement receptor), CD81 (TAPA-1), and CD225, which collectively transmit activation signals into the cell when receptor aggregation occurs following cross-linking of the surface immunoglobulin by antigen. This signaling includes phosphorylation of ITAM sequences on Igα and Igβ by the Src family kinases Lyn, Fyn or Blk, and the subsequent recruitment of Syk kinase which initiates downstream signaling (Wang and Clark, 2003). On binding to the BCR, antigen is endocytosed and then processed for presentation by MHC class II to Th cells (Clark et al., 2004). In addition to an antigen-presenting role, B cells secrete a number of cytokines, including IL-10, IL-12, IL-13, TNF-α, TNF-β (lymphotoxin), transforming growth factor (TGF)-β and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Fitzgerald et al., 2001). Following their activation, B cells undergo rounds of proliferation and differentiate into memory cells or plasma cells which produce high levels of soluble antibody. Most plasma cells have a half life of only a few days, but some survive for several weeks, particularly in the bone marrow (Slifka and Ahmed, 1998).

Antibodies

Antibodies are immunoglobulin molecules composed of two identical heavy polypeptide chains and two identical light polypeptide chains, held together by interchain disulfide bonds. All immunoglobulins are glycoproteins, containing between 3% and 13% carbohydrate, depending on the antibody class (Jefferis and Lund, 1997). The N-termini of the light and heavy chains are folded into a variable domain which contains three hypervariable loops, constituting the complementarity determining regions (CDRs) responsible for noncovalent binding of the antigen. Most epitopes recognized by antibodies are discontinuous, comprising amino acids that are only brought together on protein folding (Muller, 2000). The heavy chain C-terminal domains form the constant region, which specifies the class/subclass of antibody. The light chain constant domain determines the κ or λ isotype. The human antibody classes are IgG, IgA, IgM, IgD, and IgE, with four IgG (IgG1–4) and two IgA (IgA1, IgA2) subclasses. Each antibody can be produced either with a hydrophobic transmembrane sequence to anchor the molecule in the B-cell membrane, where it functions as the BCR, or as a secreted molecule lacking the transmembrane sequence.

The basic antibody monomer (biochemically a tetramer) is bivalent with two antigen-binding arms of identical specificity. Secretory IgA at mucosal surfaces is a tetravalent dimer, whereas circulating IgM is most frequently a decavalent pentamer with a minor proportion of hexamers and tetramers. IgA and IgM polymerization is stabilized by a polypeptide J (joining) chain (Johansen et al., 2000). Secretory IgA also includes a secretory component, a cleavage fragment of the poly-immunoglobulin receptor, which was used to transfer the IgA across epithelial cells to the mucosal surfaces (Johansen and Brandtzaeg, 2004).

Neutralizing antibody can inhibit the binding of microorganisms or biologic molecules (toxins, hormones, cytokines, and so forth) to their cellular receptors and thereby exert an effect independently of other immune system components. Usually, however, antibodies do not function in isolation but are employed to activate the classical complement pathway and/or link antigen to Fc receptor-bearing cells. Antigens opsonized with IgG, IgA or IgE bind to the appropriate Fc receptors (FcγR, FcαR or FcεR) on phagocytic cells (Underhill and Ozinsky, 2002). Alternatively, both IgG and IgE can mediate antibody-dependent cellular cytotoxicity (ADCC) in which NK cells,

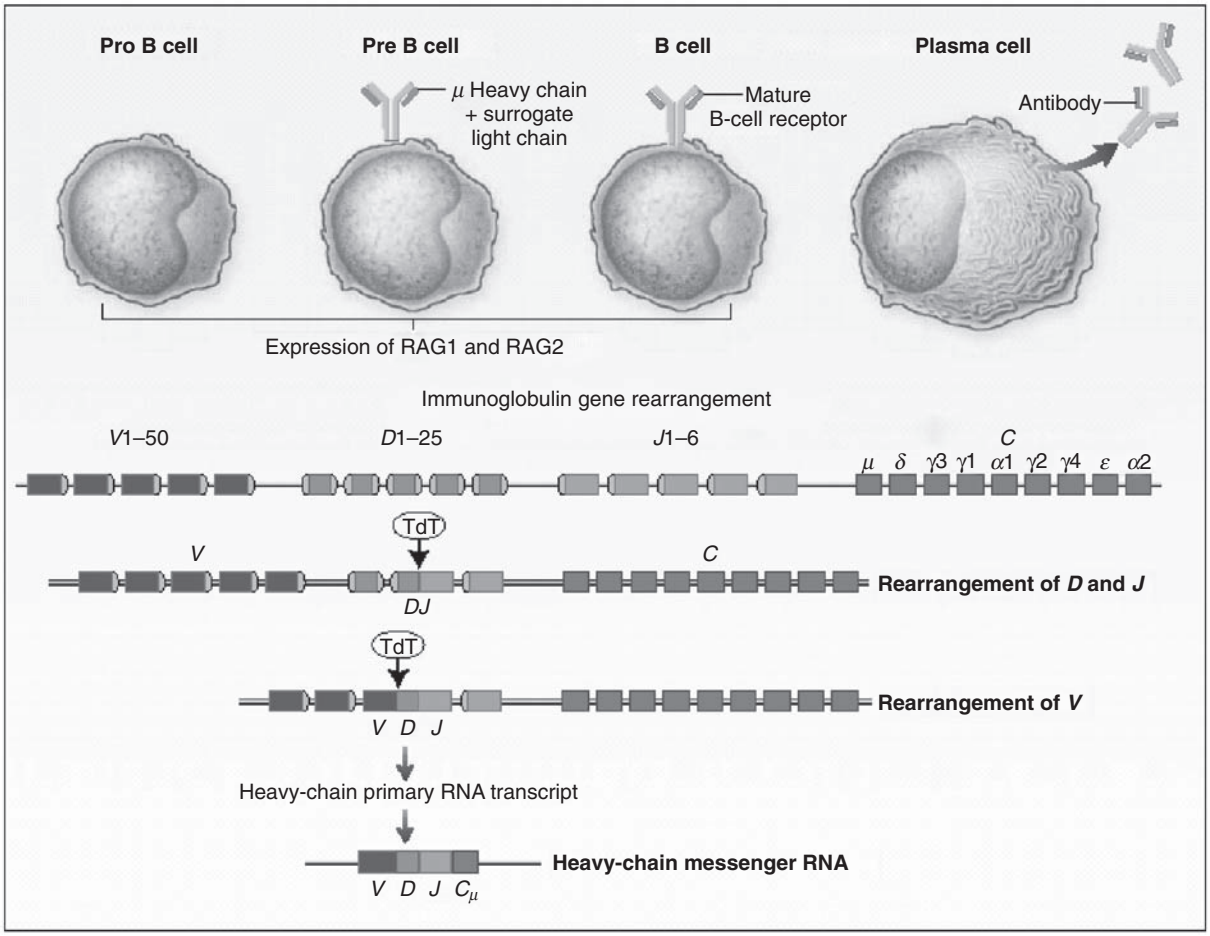


FIGURE 2.6 Diversity of antigen receptors. Early in B cell development, pro-B cells mature into pre-B cells, at which stage they express the recombination activating genes RAG-1 and RAG-2. Random rearrangement of any one of 25 diversity (D) gene segments next to any one of six joining (J) gene segments is followed by rearrangement of any one of approximately 50 variable (V) gene segments next to the already rearranged D–J segment. Different B cells will rearrange a different segment out of each pool, thereby creating one level of diversity. Additional diversity is brought about by junctional diversity due to splicing inaccuracies and by the incorporation of nucleotides mediated by the enzyme terminal deoxynucleotidyl transferase (TdT). The heavy chain primary RNA transcript is processed into messenger RNA (mRNA), with splicing of the rearranged V–D–J next to the Cμ constant region gene. This mRNA will encode a μ heavy chain which is placed on the surface of the pre-B cell together with the surrogate light chain V pre-B λ5. As the pre-B cell undergoes further maturation the immunoglobulin light chain V and J gene segments (for simplicity not shown) rearrange to produce a κ or λ light chain. This light chain replaces the surrogate light chain in order to produce a mature IgM B-cell receptor (BCR) on the cell surface. The BCR at this stage also usually comprises IgD antibodies of the same specificity, produced by alternative splicing of the rearranged V–D–J to either the Cμ or Cδ constant region genes. Expression of RAG-1 and RAG-2 is now switched off. Following encounter with antigen, and in the presence of costimulatory signals, the B cell further differentiates into either a plasma cell, which secretes high levels of the specific antibody, or into a memory B cell. The same general principles regarding the rearrangement process applies to the generation of αβ and γδ T-cell receptor. See color plate section.

monocytes, macrophages or neutrophils bearing Fcγ receptors or macrophages, eosinophils or platelets bearing Fcε receptors are focused onto antibody-coated target cells or parasites (Lucas, 1999). The target is destroyed by apoptosis using perforin and granzymes. IgE antibodies are also able to sensitize mast cells and basophils via the high-affinity IgE receptor FcεRI and, if cross-linked by antigen, will trigger the release of inflammatory mediators.

The epithelial cell poly-immunoglobulin receptor transports secretory IgA produced by plasma cells underlying mucosal surfaces (Johansen and Brandtzaeg, 2004). On the luminal side of the epithelium the IgA is released by proteolytic cleavage of the receptor and acts to prevent microbial adhesion to the epithelial cell wall. A second type of epithelial Fc receptor, FcRn, is present in both the placenta, where it transports IgG from the maternal to the fetal circulation

(Simister, 2003), and on the intestinal epithelium of the neonate where it is involved in the uptake of IgG from maternal milk (Ghetie and Ward, 2000).

Secondary Lymphoid Tissues

The primary lymphoid organs, bone marrow and thymus, are where fully differentiated mature naïve B and T cells are produced. However, activation of lymphocytes occurs in structurally-organized B- and T-cell compartments in the secondary lymphoid tissues; the lymph nodes, spleen, and mucosa-associated lymphoid tissues (MALT). Diffuse collections of lymphoid cells are also present throughout the lung and in the lamina propria of the intestinal wall. Only a handful of lymphocytes will be specific for a given antigen, requiring T and B cells to recirculate through the different lymphoid tissues. While responses to blood-borne antigens are usually initiated in the spleen, those to antigens in the tissues are stimulated in the local draining lymph nodes.

Lymphoid follicles within the secondary lymphoid tissues contain germinal centers where B-cell activation occurs within a meshwork of FDCs displaying immune complexes on their surface. Germinal centers are at the heart of the generation of adaptive responses for it is here that B cells proliferate, switch class, undergo affinity maturation, and differentiate into memory cells and plasma cell precursors (McHeyzer-Williams et al., 2001) (Figure 2.7). B cells can increase the binding affinity of their BCR by somatic hypermutation of the rearranged antibody variable genes. Higher affinity clones will then be preferentially selected by antigen. Class switching from IgM to IgG, IgA, and IgE involves switch sequences containing highly repetitive nucleotide motifs immediately upstream of each constant region gene (except Cδ). Both somatic hypermutation and class switching require the expression of activation-induced cytidine deaminase (AID) and of the heterodimeric protein Ku70/Ku80 (Sawchuk et al., 2004). Receptor editing, involving re-expression of RAG enzymes in the germinal centers, enables self-reactive B cells to replace the variable region gene in the rearranged VDJ sequence with a different variable region gene in order to eliminate autoreactivity.

Most pathogens enter the body through mucosal surfaces. The palatine tonsils and adenoids are the sites for induction of responses to intranasal and inhaled antigens (Brandtzaeg, 1999). Antigens from the gut are taken up by specialized epithelial microfold cells which transport the antigens across the epithelium for access to the Peyer patches where mucosal responses are induced (Kraehenbuhl and Neutra,

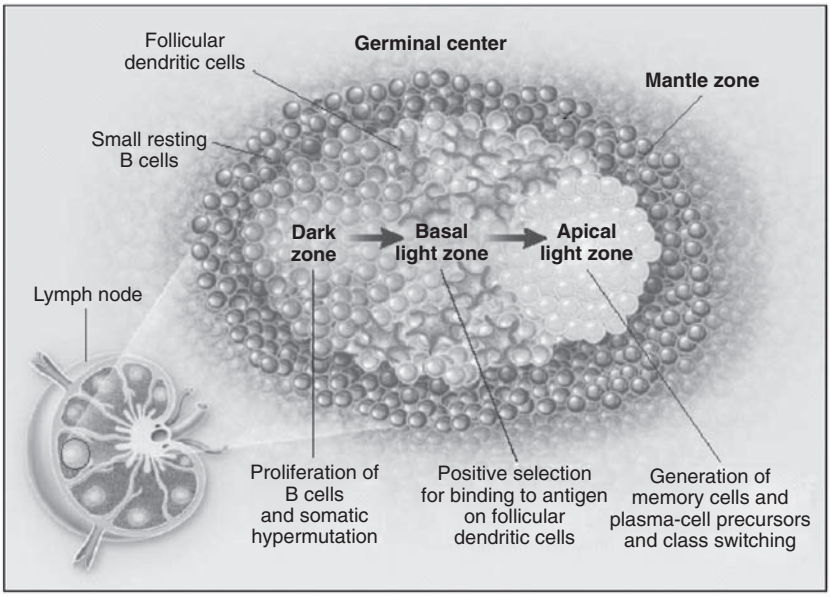


FIGURE 2.7 Germinal center. During the initiation of the acquired immune response, these structures form in the secondary lymphoid tissues in order to generate a microenvironment where all the necessary antigen-specific and innate antigen-presenting cells can interact. Antigen-stimulated B cell proliferation occurs in the dark zone and is accompanied by affinity maturation due to somatic hypermutation of the immunoglobulin variable region genes. Upon passage into the basal light zone, high-affinity antigen-specific B-cells are positively selected by interaction with antigen, which is present in the form of immune complexes on the surface of follicular dendritic cells. B cells which fail to be positively selected undergo apoptosis and are phagocytosed by tangible-body macrophages. The positively selected cells migrate to the apical light zone where proliferation continues, class switching occurs, and memory cells and plasma cell precursors are generated. See color plate section.

2000). Activated lymphocytes exit the Peyer patches via the efferent lymphatics, traffic through the blood, and then home to the lamina propria and other mucosal effector sites. Intraepithelial lymphocytes (IELs) interspersed between the gut epithelial cells also encounter antigens transported by microfold cells. Most IELs express CD8, can be either cytotoxic or immunoregulatory, and between 35% and 65% of murine IELs bear a γδ TCR (Hayday et al., 2001).

Lymphocytes enter lymph nodes, tonsils, and Peyer patches either via the afferent lymphatics or from the blood via high endothelial venules (HEV). L-Selectin is constitutively expressed on lymphocytes and constitutes a ligand for peripheral lymph node addressins (Ley, 2003). If expression of lymphocyte function-associated antigen (LFA)-1 is upregulated on the lymphocytes, their adhesion to HEV is enhanced and they migrate across the HEV into these lymphoid tissues. Although the spleen lacks HEV, circulating lymphocytes can directly access the marginal zone of this organ from the blood vessels. T cells locate mostly to the periarteriolar lymphoid sheaths, while B cells enter the lymphoid follicles. Lymphocytes leave the lymph nodes via the efferent lymphatics and the spleen via the splenic vein.

When naïve lymphocytes first encounter antigen in the secondary lymphoid tissues they mount a primary immune response, generating both effector and memory cells. The memory cells are responsible for the quantitatively and qualitatively superior secondary immune response that occurs on any subsequent encounters with the same antigen. Memory cells have a lower activation threshold than naïve cells and the secondary response is more rapid, involves larger numbers of lymphocytes, and, for B cells, produces higher levels of antibody with an improved affinity for antigen.

The term "T-independent antigen" refers to antigens that are capable of generating an antibody response without a requirement for Th cells. Polysaccharides, polymerized flagellin, and a number of other antigens have repetitive determinants which can extensively cross-link the BCR and thereby directly activate the B cell (Fagarasan and Honjo, 2000). Because they do not recruit T cells, T-independent antigens fail to provoke the formation of germinal centers and therefore are unable to induce B-cell memory, class switching to IgG, IgA or IgE production, or significant amounts of affinity maturation. Thus, low-affinity IgM antibodies are produced in response to T-independent antigens and, although involving B cells, the response does not go on to exhibit the characteristics of "adaptive" immunity. The majority of antigens that stimulate B cells are, however, T-cell dependent in that the B-cell response requires help from T cells. As mentioned earlier, the BCR on the surface of the B cell internalizes bound antigen which is then processed into peptides for presentation by MHC class II molecules. Upon recognition of the peptide–MHC complex by the T cells in the secondary lymphoid tissues, the costimulatory molecule CD154 (CD40 ligand) on the T cell engages CD40 on the B cell (Calderhead et al., 2000). In addition to cell surface molecules, cytokines play a key role in the mutual activation of the T and B lymphocytes. T-cell help can also be recruited by IDCs and macrophages presenting the relevant peptide–MHC class II combination to the Th cell.

Resolution of the Immune Response

Antigen stimulates the immune response and, therefore, for foreign antigen, its clearance by the immune system will naturally lead to a waning of the response. However, additional mechanisms initially amplify and subsequently downregulate the response. Once high levels of class-switched antigen-specific IgG are produced, the antibody can inactivate the antigen-specific B cells in a manner reminiscent of classical negative feedback loops in the endocrine system. Cross-linking of the BCR to FcγRIIB on B cells by immune complexes results in the transmission of inhibitory signals into the B cell (Leibson, 2004). A number of signals from cytokines and cell-surface molecules can also be inhibitory. Ligation of the T-cell surface molecule CTLA-4 by CD80 and CD86, in contrast to ligation of CD28, provides a downregulating signal (Carreno and Collins, 2002). Some $CD4^+CD25^+$ regulatory T cells secrete IL-10 and TGF-β, which can act in an immunosuppressive capacity, while others suppress responses by a cell-contact-dependent mechanism (Taams et al., 2003).

Neuroendocrine interactions with the immune system provide a further level of regulation (Webster et al., 2002). Lymphoid tissues are richly innervated and neurotransmitters, such as epinephrine from sympathetic neurons, acetylcholine from cholinergic neurons, and substance P and calcitonin gene-related peptide (CGRP) from pain fibers have immunomodulatory roles (Steinman, 2004). Among the many other interactions between these systems is the inactivation of chemokine receptors by vasoactive intestinal peptide (Grimm et al., 2003) and an influence on the Th1/Th2 balance by pituitary adenylate cyclase-activating polypeptide (Steinman, 2004).

References

Appay, V., Zaunders, J.J., Papagno, L., Sutton, J., Jaramillo, A., Waters, A., Easterbrook, P., Grey, P., Smith, D., McMichael, A.J., Cooper, D.A, Rowland-Jones, S.L., and Kelleher, A.D. (2002). Characterization of $CD4^+$ CTLs ex vivo. *J. Immunol. 168*, 5954–5958.

Basler, C.F. and Garcia-Sastre, A. (2002). Viruses and the type I interferon antiviral system: induction and evasion. *Int. Rev. Immunol. 21*, 305–337.

Benedict, C.L., Gilfillan, S., Thai, T.H., and Kearney J.F. (2000). Terminal deoxynucleotidyl transferase and repertoire development. *Immunol. Rev. 175*, 150–157.

Berland, R. and Wortis, H.H. (2002). Origins and functions of B-1 cells with notes on the role of CD5. *Annu. Rev. Immunol. 20*, 253–300.

Birmingham, D.J. (1995). Erythrocyte complement receptors. *Crit. Rev. Immunol. 15*, 133–154.

Bradl, H., Wittmann, J., Milius, D., Vettermann, C., and Jack, H.M. (2003). Interaction of murine precursor B cell receptor with stroma cells is controlled by the unique tail of lambda 5 and stroma cell-associated heparan sulfate. *J. Immunol. 171*, 2338–2348.

Brandtzaeg, P. (1999). Regionalized immune function of tonsils and adenoids. *Immunol. Today 20*, 383–384.

Brigl, M. and Brenner, M.B. (2004). CD1: Antigen presentation and T cell function. *Annu. Rev. Immunol. 22*, 817–890.

Calderhead, D.M., Kosaka, Y., Manning, E.M., and Noelle, R.J. (2000). CD40–CD154 interactions in B-cell signaling. *Curr. Top. Microbiol. Immunol. 245*, 73–99.

Carding, S.R. and Egan, P.J. (2002). Gamma delta T cells: functional plasticity and heterogeneity. *Nat. Rev. Immunol. 2*, 336–345.

Carreno, B.M. and Collins, M. (2002). The B7 family of ligands and its receptors: New pathways for costimulation and inhibition of immune responses. *Ann. Rev. Immunol 20*, 29–53.

Chen, Y., Chou, K., Fuchs, E., Havran, W.L., and Boismenu, R. (2002). Protection of the intestinal mucosa by intraepithelial gamma delta T cells. *Proc. Natl. Acad. Sci. USA 99*, 14338–14343.

Clark, M.R., Massenburg, D., Siemasko, K., Hou, P., and Zhang, M. (2004). B-cell antigen receptor signaling requirements for targeting antigen to the MHC class II presentation pathway. *Curr. Opin. Immunol. 16*, 382–387.

Davis, D.M. and Dustin, M.L. (2004). What is the importance of the immunological synapse? *Trends Immunol. 25*, 323–327.

Dykstra, M., Cherukuri, A., Sohn, H.W., Tzeng, S.-J., and Pierce, S.K. (2003). Location is everything: Lipid rafts and immune cell signaling. *Annu. Rev. Immunol. 21*, 457–481.

Fagarasan, S. and Honjo, T. (2000). T-independent immune response: new aspects of B cell biology. *Science 290*, 89–92.

Fitzgerald, K.A., O'Neill, L.A.J., Gearing, A.J.H., and Callard, R.E. (2001). *The Cytokine Factsbook*, 2nd ed. London: Academic Press.

Frossi, B., De Carli, M., and Pucillo, C. (2004). The mast cell: an antenna of the microenvironment that directs the immune response. *J. Leukoc. Biol. 75*, 579–585.

Gabay, C. and Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med. 340*, 448–454.

Ghetie, V. and Ward, E.S. (2000). Multiple roles for the major histocompatibility complex class I-related receptor FcRn. *Annu. Rev. Immunol. 18*, 739–766.

Grimm, M.C., Newman, R., Hassim, Z., Cuan, N., Connor, S.J., Le, Y., Wang, J.M., Oppenheim, J.J., and Lloyd, A.R. (2003). Vasoactive intestinal peptide acts as a potent suppressor of inflammation *in vivo* by trans-deactivating chemokine receptors. *J. Immunol. 171*, 4990–4994.

Guermonprez, P., Valladeau, J., Zitvogel, L., Théry, C., and Amigorena, S. (2002). Antigen presentation and T cell stimulation by dendritic cells. *Annu. Rev. Immunol. 20*, 621–667.

Hayday, A., Theodoridis, E., Ramsburg, E., and Shires, J. (2001). Intraepithelial lymphocytes: exploring the Third Way in immunology. *Nat. Immunol. 2*, 997–1003.

Henson, P.M., Bratton, D.L., and Fadok, V.A. (2001). The phosphatidylserine receptor: a crucial molecular switch? *Nat. Rev. Mol. Cell. Biol. 2*, 627–633.

Hermiston, M.L., Xu, Z., and Weiss, A. (2003). CD45: A critical regulator of signaling thresholds in immune cells. *Annu. Rev. Immunol. 21*, 107–137.

Jamieson, B.D., Douek, D.C., Killian, S., Hultin, L.E., Scripture-Adams, D.D., Giorgi, J.V., Marelli, D., Koup, R.A., and Zack, J.A. (1999). Generation of functional thymocytes in the human adult. *Immunity 10*, 569–575.

Janeway, C.A., Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol. 54*, 1–13.

Janeway, C.A., Jr. and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol. 20*, 197–216.

Jefferis, R. and Lund, J. (1997). Glycosylation of antibody molecules: structural and functional significance. *Chem. Immunol. 65*, 111–128.

Johansen, F.E., Braathen, R., and Brandtzaeg, P. (2000). Role of J chain in secretory immunoglobulin formation. *Scand. J. Immunol. 52*, 240–248.

Johansen, F.E. and Brandtzaeg, P. (2004). Transcriptional regulation of the mucosal IgA system. *Trends Immunol. 25*, 150–157.

Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O. A., Malolepsyzy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., and Akdis, C.A. (2001). Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature 413*, 420–425.

Kraehenbuhl, J.P. and Neutra, M.R. (2000). Epithelial M cells: differentiation and function. *Ann. Rev. Cell. Dev. Biol. 16*, 301–332.

Krieg, A.M. (2002). CpG motifs in bacterial DNA and their immune effects. *Annu. Rev. Immunol. 20*, 709–760.

Kroczek, R.A., Mages, H.W., and Hutloff, A. (2004). Emerging paradigms of T-cell co-stimulation. *Curr. Opin. Immunol. 16*, 321–327.

Kronenberg, M. and Gapin, L. (2002).The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol. 2*, 557–568.

Leibson, P.J. (2004). The regulation of lymphocyte activation by inhibitory receptors. *Curr. Opin. Immunol. 16*, 328–336.

Ley, K. (2002). Integration of inflammatory signals by rolling neutrophils. *Immunol. Rev. 186*, 8–18.

Ley, K. (2003). The role of selectins in inflammation and disease. *Trends Mol. Med. 9*, 263–268.

Livak, F. and Petrie, H.T. (2002). Access roads for RAG-ged terrains: control of T cell receptor gene rearrangement at multiple levels. *Semin. Immunol. 14*, 297–309.

Lucas, A.H. (1999). Antibody function. In *Encyclopedia of Life Sciences*. John Wiley & Sons Ltd: Chicester. http://www.els.net.

McHeyzer-Williams, L.J., Driver, D.J., and McHeyzer-Williams, M.G. (2001). Germinal center reaction. *Curr. Opin. Hematol. 8*, 52–59.

Mellor, A.L. and Munn, D.H. (2003). Tryptophan catabolism and regulation of adaptive immunity. *J. Immunol. 170*, 5809–5813.

Morgan, P.B. and Harris, C.L. (1999). *Complement Regulatory Proteins*. London: Academic Press.

Moser, M. and Murphy, K.M. (2000). Dendritic cell regulation of TH1-TH2 development. *Nat. Immunol.* 1, 199–205.

Mosmann, T.R. and Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today 17*, 138–146.

Muller, C.P. (2000). Epitopes. In *Encyclopedia of Life Sciences*. John Wiley & Sons Ltd: Chicester. http://www.els.net.

Pear, W.S., Tu, L., and Stein, P.L. (2004). Lineage choices in the developing thymus: choosing the T and NKT pathways. *Curr. Opin. Immunol. 16*, 167–173.

Ramirez, M.C. and Sigal, L.J. (2004). The multiple routes of MHC-I cross-presentation. *Trends Microbiol. 12*, 204–207.

Rot, A. and von Andrian, U.H. (2004). Chemokines in innate and adaptive host defense: Basic chemokinese grammar for immune cells. *Annu. Rev. Immunol. 22*, 891–928.

Russell, J.H. and Ley, T.J. (2002). Lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol. 20*, 323–370.

Sawchuk, D.J., Mansilla-Soto, J., Alarcon, C., Singha, N.C., Langen, H., Bianchi, M.E., Lees-Miller, S.P., Nussenszweig, N.P., and Cortes, P. (2004). Ku70/Ku80 and DNA-PKcs modulate RAG-mediated cleavage. Implications for the enforcement of the 12/23 rule. *J. Biol. Chem. 279*, 29821–29831.

Schwartz, R.H. (2003). T cell anergy. *Ann. Rev. Immunol. 21*, 305–334.

Shastri, N., Schwab, S., and Serwold, T. (2002). Producing nature's gene chips: The generation of peptides for display by MHC class I molecules. *Annu. Rev. Immunol. 20*, 463–493.

Simister, N.E. (2003). Placental transport of immunoglobulin G. *Vaccine 21*, 3365–3369.

Slifka, M.K. and Ahmed, R. (1998). Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Curr. Opin. Immunol. 10*, 252–258.

Starr, T.K., Jameson, S.C., and Hogquist, K.A. (2003). Positive and negative selection of T cells. *Annu. Rev. Immunol. 21*, 139–176.

Steinman, L. (2004). Elaborate interactions between the immune and nervous systems. *Nat. Immunol. 5*, 575–581.

Taams, L., Vukmanovic-Stejic, M., Salmon, M., and Akbar, A. (2003). Immune regulation by $CD4^+CD25^+$ regulatory T cells: implications for transplantation tolerance. *Transpl. Immunol. 11*, 277–285.

Underhill, D.M. and Ozinsky, A. (2002). Phagocytosis of microbes: Complexity in action. Annu. Rev. Immunol. 20, 825–852.

van Nierop, K. and de Groot, C. (2002). Human follicular dendritic cells: function, origin and development. *Semin. Immunol. 14*, 251–257.

Venanzi, E.S., Benoist, C., and Mathis, D. (2004). Good riddance: Thymocyte clonal deletion prevents autoimmunity. *Curr. Opin. Immunol. 16*, 197–202.

Wang, L.D. and Clark, M.R. (2003). B-cell antigen-receptor signaling in lymphocyte development. *Immunology 110*, 411–420.

Wardlaw, A.J., Moqbel, R., and Kay, A.B. (1995). Eosinophils: biology and role in disease. *Adv. Immunol. 60*, 151–266.

Webster, J.I., Tonelli, L., and Sternberg, E.M. (2002). Neuroendocrine regulation of immunity. *Annu. Rev. Immunol. 20*, 125–163.

Woodland, D.L. and Dutton, R.W. (2003). Heterogeneity of $CD4^+$ and $CD8^+$ T cells. *Curr. Opin. Immunol. 15*, 336–342.

Yokoyama, W.M., Kim, S., and French, A.R. (2004). The dynamic life of natural killer cells. *Annu. Rev. Immunol. 22*, 405–429.

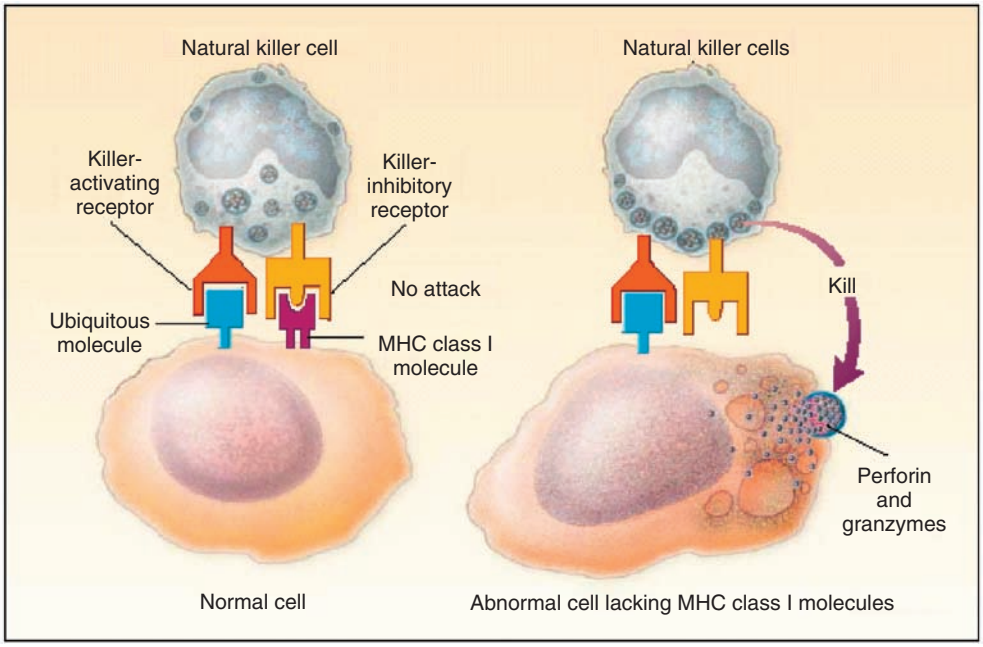


FIGURE 2.1 Natural killer (NK) cells can attack cells lacking MHC class I expression following recognition by killer-activating receptors in the absence of a downregulatory signal from killer-inhibitory receptors. The cytotoxic granules of the NK cells, which contain perforin and granzymes, become polarized to the interface between the cells and are then released into the target cell.

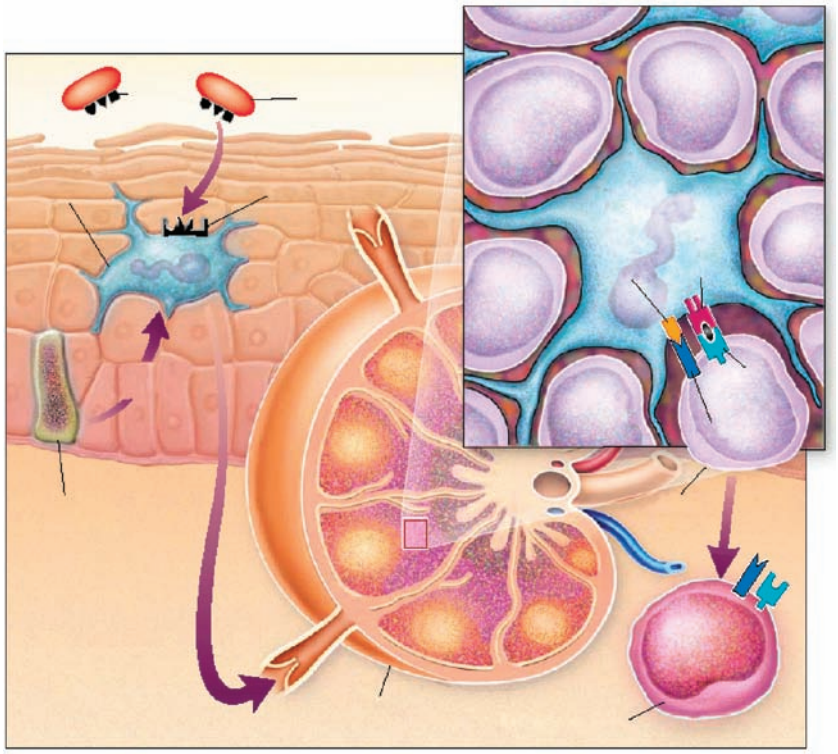


FIGURE 2.2 Pathogen-associated molecular patterns (PAMPs) allow the pattern-recognition receptors (PRR) on the interdigitating dendritic cells (IDCs) to differentiate between potentially harmful foreign microorganisms and self constituents. IDCs are also stimulated by endogenous activators such as interferon-α and heat-shock proteins released from infected or necrotic cells. The activated IDCs process the antigen to generate peptides that are presented by MHC molecules to the T-cell receptor (TCR) on T-cells in the local draining lymph nodes.

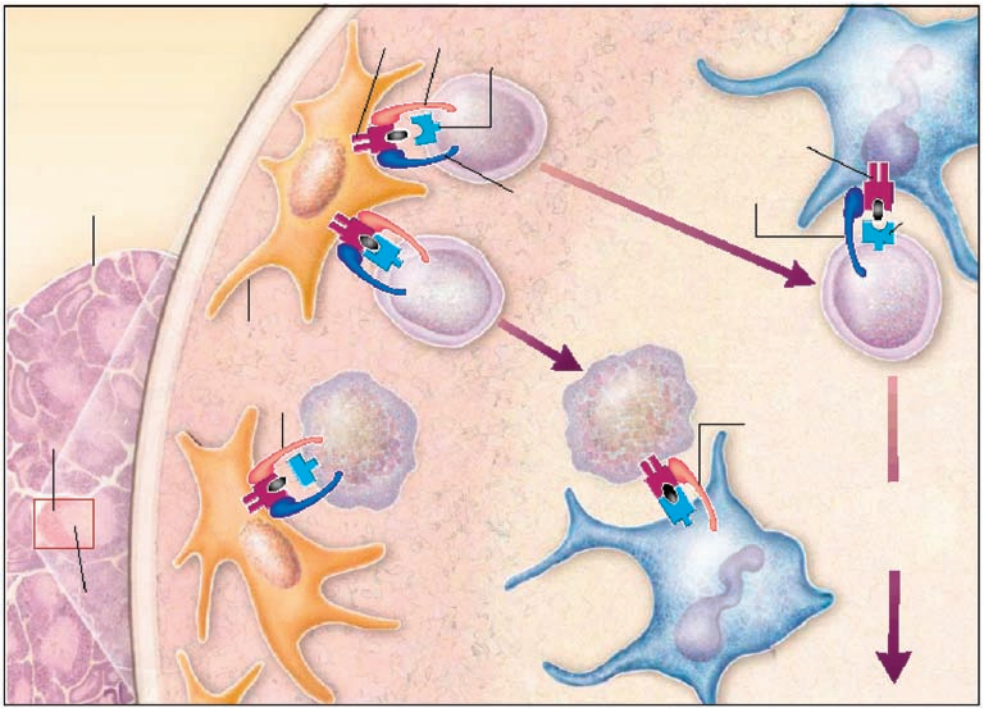


FIGURE 2.4 Positive and negative selection in the thymus. Cells with T-cell receptors (TCRs) of various affinities for self-MHC are positively selected on cortical epithelial cells. Any of these cells that bear a TCR with high affinity for self-peptide plus self-MHC (or even just MHC irrespective of the peptide contained) are subsequently eliminated by induction of apoptosis when they interact with dendritic cells, macrophages or epithelial cells in the thymic medulla (negative selection). This leaves T-cells with only a weak affinity for self-MHC. These cells form the pool of T-cells that are exported from the thymus as single $CD4^+$ or $CD8^+$ cells. In the periphery they have the potential to recognize foreign peptide plus self-MHC, and become activated if the affinity of the interaction is above a certain threshold and the recognition occurs in the presence of costimulatory signals.

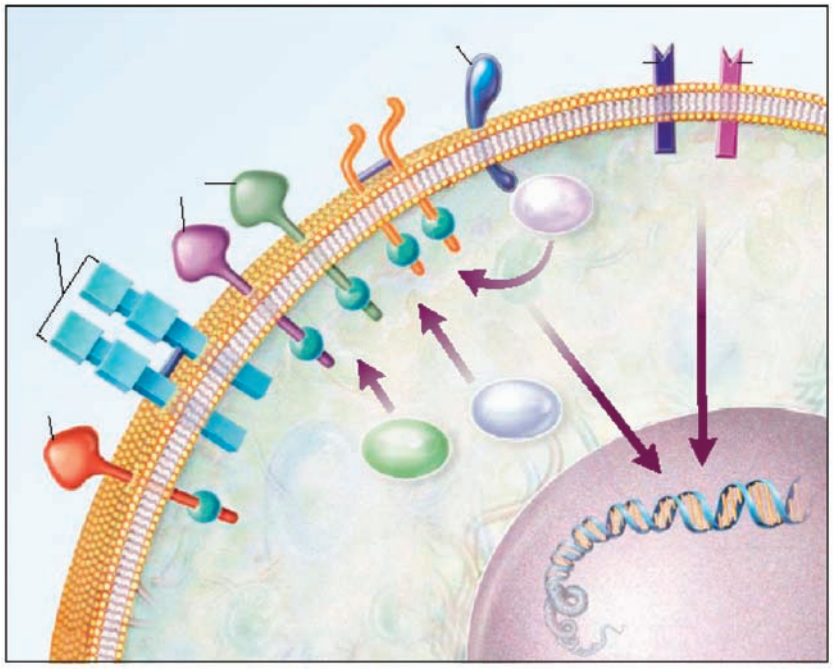


FIGURE 2.5 Lymphocyte activation involves a highly complex series of integrated events resulting from cross-linking of the antigen receptor on the cell surface. Because the antigen receptors have extremely short cytoplasmic tails they are associated with cell-surface molecules bearing cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs), which are subject to phosphorylation by protein kinases. These events lead to downstream signaling involving a number of different biochemical pathways that result in the transcriptional activation of genes involved in cellular proliferation and differentiation. The presence of signals from costimulatory molecules, such as CD28 and CD154 (CD40 ligand), is obligatory if the lymphocyte is to be activated; signals from the antigen receptor signal-transducing molecules alone lead to anergy or apoptosis. TCR, T-cell receptor.

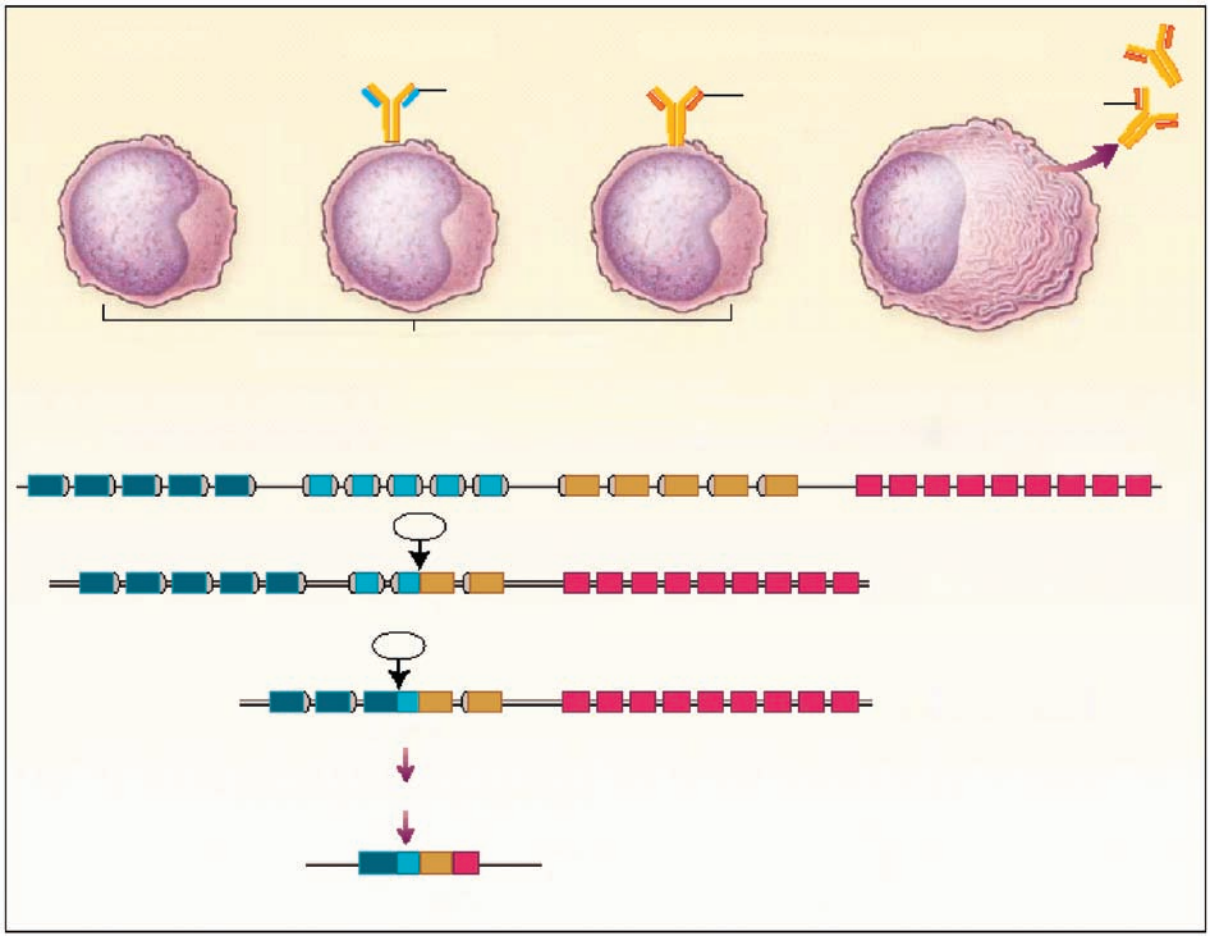


FIGURE 2.6 Diversity of antigen receptors. Early in B-cell development, pro-B-cells mature into pre-B-cells, at which stage they express the recombination activating genes RAG-1 and RAG-2. Random rearrangement of any one of 25 diversity (D) gene segments next to any one of six joining (J) gene segments is followed by rearrangement of any one of approximately 50 variable (V) gene segments next to the already rearranged D–J segment. Different B-cells will rearrange a different segment out of each pool, thereby creating one level of diversity. Additional diversity is brought about by junctional diversity due to splicing inaccuracies and by the incorporation of nucleotides mediated by the enzyme terminal deoxynucleotidyl transferase (TdT). The heavy chain primary RNA transcript is processed into messenger RNA (mRNA), with splicing of the rearranged V–D–J next to the Cμ constant region gene. This mRNA will encode a μ heavy chain which is placed on the surface of the pre-B-cell together with the surrogate light chain V pre-B λ5. As the pre-B-cell undergoes further maturation the immunoglobulin light chain V and J gene segments (for simplicity not shown) rearrange to produce a κ or λ light chain. This light chain replaces the surrogate light chain in order to produce a mature IgM B-cell receptor (BCR) on the cell surface. The BCR at this stage also usually comprises IgD antibodies of the same specificity, produced by alternative splicing of the rearranged V–D–J to either the Cμ or Cδ constant region genes. Expression of RAG-1 and RAG-2 is now switched off. Following encounter with antigen, and in the presence of co-stimulatory signals, the B-cell further differentiates into either a plasma cell, which secretes high levels of the specific antibody, or into a memory B-cell. The same general principles regarding the rearrangement process applies to the generation of αβ and γδ T-cell receptor.

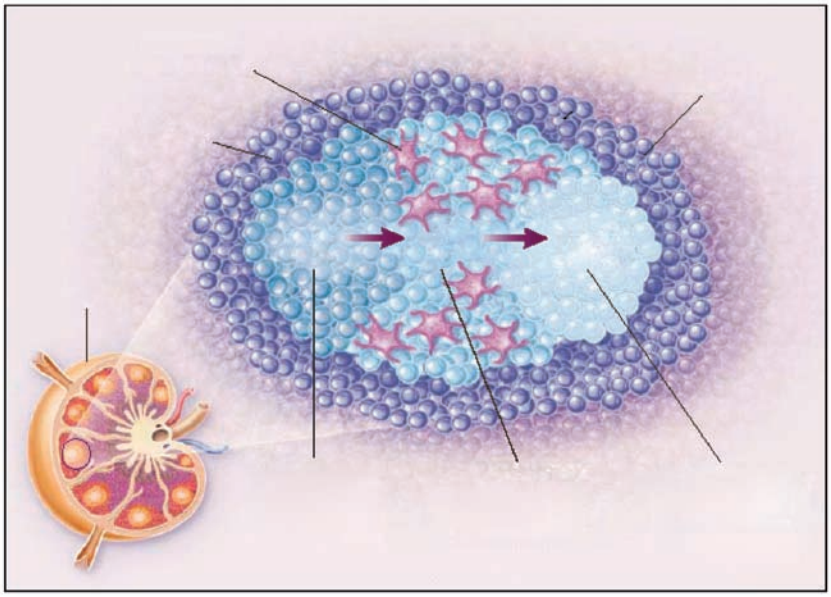


FIGURE 2.7 Germinal center. During the initiation of the acquired immune response, these structures form in the secondary lymphoid tissues in order to generate a microenvironment where all the necessary antigen-specific and innate antigen-presenting cells can interact. Antigen-stimulated B-cell proliferation occurs in the dark zone and is accompanied by affinity maturation due to somatic hypermutation of the immunoglobulin variable region genes. Upon passage into the basal light zone, high-affinity antigen-specific B-cells are positively selected by interaction with antigen, which is present in the form of immune complexes on the surface of follicular dendrite cells. B-cells which fail to be positively selected undergo apoptosis and are phagocytosed by tingible-body macrophages. The positively selected cells migrate to the apical light zone where proliferation continues, class switching occurs, and memory cells and plasma cell precursors are generated.

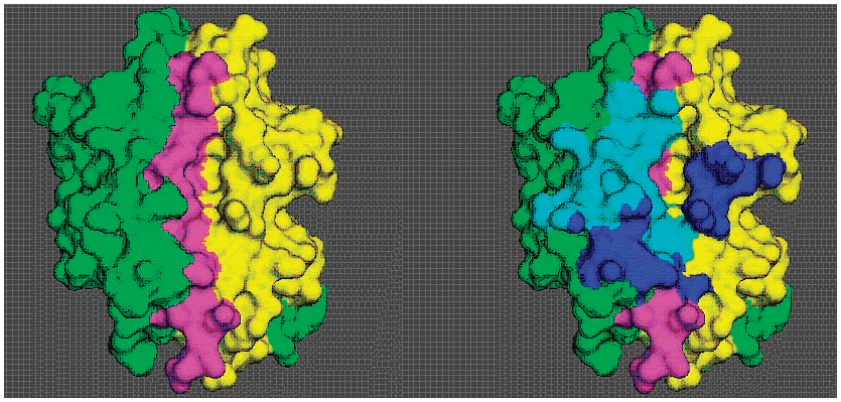


FIGURE 5.2 Trimolecular interaction between MHC, peptide, and T-cell receptor (TCR). The exposed molecular surface of an MHC class II molecule, containing a bound antigen peptide, is shown in the left panel. The MHC α chain is yellow, the β chain is green, and the peptide is shown in pink. Amino acid side chains on the underlying side of the peptide lie buried in the MHC groove, anchoring the binding interaction. This exposed surface forms the contact structure for TCR recognition; the image on the right is colored to show examples of the areas which contact the TCR, with the TCR α chain contacts shown in dark blue and the TCR β chain in light blue. Both peptide and MHC residues are involved in TCR recognition, and both contribute to the overall avidity of the interaction.

Adapted from Koopman, et al., *Arthritis and Allied Conditions*, 15th edition.

CHAPTER

3

General Features of Autoimmune Disease

ANNE DAVIDSON AND BETTY DIAMOND

Departments of Medicine and Microbiology, Columbia University Medical Center, New York, New York, USA

A host of diseases are characterized by activation of the immune system in the absence of an external threat to the organism. In these diseases, inflammation and tissue damage occur in the absence of infection, toxin exposure or tumor growth. These diseases can be characterized as those that display activation of the innate immune system and an excess of inflammatory mediators, but no evidence of an antigen-specific immune response; familial Mediterranean fever, Behçet disease, even atherosclerosis, can be considered to fall within this category. Alternatively, there are diseases characterized by an activation of the adaptive immune response with T and B lymphocytes responding to self-antigen in the absence of any detectable microbial assault or tumor invasion. These diseases constitute the vast majority of diseases considered to be autoimmune in origin, and they will be the focus of this chapter.

CELLS OF THE ADAPTIVE IMMUNE SYSTEM

There is a coordinated interplay among the cells of the adaptive immune system with dendritic cells (DCs), T cells, and B cells interacting to generate the effector response of the immune system. DCs activate T cells (Banchereau and Steinman, 1998; Granucci et al., 2004) and B cells (MacLennan and Vinuesa, 2002; Craxton et al., 2003). T cells activate DCs and B cells (Bishop and Hostager, 2003; O'Sullivan and Thomas, 2003). B cells activate T cells (Shlomchik et al., 2001). This cascade leads to an immune response that recognizes a broad spectrum of epitopes of microbial pathogens and enlists multiple effector mechanisms.

DCs are antigen-presenting cells (APCs) that are critical in initiating an immune response. Like essentially all cells, they display surface expression of class I major histocompatibility complex (MHC) molecules, which permit the presentation of intracellular antigens to T cells. DCs also express class II MHC molecules, which are present on a much more restricted set of cells and permit the presentation of extracellular antigens (Banchereau and Steinman, 1998). Multiple alleles of class I and II molecules exist and thus each individual has a unique set of MHC molecules (Beck and Trowsdale, 2000). DCs also express an array of nonpolymorphic receptors, termed Toll-like receptors (TLRs), and pattern-recognition receptors that bind microbial antigens (Geijtenbeek et al., 2004; Netea et al., 2004).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Engagement of these receptors causes the DCs to upregulate expression of costimulatory molecules and to deliver an obligatory second signal for activation. It is important to note that each DC can recognize and respond to a broad spectrum of microbial antigens.

Each T cell and each B cell express a single receptor for antigen. These antigen receptors are acquired by gene rearrangements that occur in somatic cells (Gellert, 2002); thus, there is no inheritance of the T- or B-cell repertoire. T cells mature in the thymus. Each T cell expresses a unique receptor (TCR) that recognizes a molecular complex on the surface of an APC consisting of a class I or class II MHC molecule associated with a small peptide derived from an intra- or extra-cellular protein antigen, respectively. Signaling through both the TCR and costimulatory molecules is needed to effect activation of mature T cells (Bommhardt et al., 2004; Vallejo et al., 2004). B cells also express a single receptor for antigen, but the B-cell receptor (BCR) recognizes native antigen rather than processed antigen. B-cell activation also requires signaling through both the BCR and costimulatory molecules (Crow, 2004). Activated B cells not only secrete antibody, but can also function as APCs to engage a greater number of T cells in the immune response (Lipsky, 2001; Shlomchik et al., 2001).

A critical feature of both T and B cells is that they proliferate in response to antigenic stimulation to create clonal expansions of cells with a unique antigenic specificity and to develop cells with a memory phenotype (Bishop et al., 2003; Swain, 2003; Grossman et al., 2004). Memory cells have an accelerated and enhanced response following re-exposure to antigen. B cells have the added feature of undergoing random somatic mutation of the BCR and class switching of the immunoglobulin heavy chain gene (Li et al., 2004). Thus, there is a progression from low-affinity IgM antibodies to high-affinity IgG antibodies during the course of an immune response. The memory response reactivates the high-affinity IgG-producing B cells, whose repertoire is unique to each individual.

For the immune system to function effectively, there must be a sufficient number in both the naïve and memory repertoires of T and B cells that can respond to an enormous diversity of microbial antigens, and few, if any, cells that respond to self-antigen.

DEFINING AUTOIMMUNE DISEASE

An autoimmune disease is a condition in which tissue injury is caused by T-cell or antibody reactivity to self. The immune activation may be initiated by infection, but must persist in the absence of any detectable microbial antigen (Davidson and Diamond, 2001). It is important to state that although many diseases considered to be autoimmune diseases display antiself reactivity, evidence may still be lacking that the self-reactivity is, in fact, responsible for tissue damage. It is sometimes possible to determine whether autoantibodies are pathogenic by transferring them to a rodent host; however, T-cell reactivity is not transferable from humans to rodents because T-cell activation and T-cell effector function occur only in the context of self-MHC molecules. Thus, demonstrating the pathogenicity of the autoimmune response has not been accomplished in all autoimmune diseases. In some instances, a disease is presumed to be of autoimmune origin only because B- and T cells are present in affected tissue.

Animal models of autoimmune disease have been enormously useful in aiding our understanding of both disease inception and disease pathogenesis (Peutz-Kootstra et al., 2001; Howell, 2002; Lam-Tse et al., 2002; Hanninen et al., 2003; Wooley, 2004). Some models develop spontaneous disease. Others represent genetically modified mice that target a particular pathway in the immune response. Finally, some autoimmune diseases can be triggered in animals by immunization with self-antigen. While all these animal models have been very important in informing our understanding of autoimmunity, it is important to recognize that we do not know how closely they reflect human disease (Birmingham et al., 2001). Some of these models may be more similar to human disease in the effector mechanisms of tissue injury than in the mechanisms of induction of autoreactivity. Indeed, autoantibody-mediated tissue damage is probably most alike in human disease and animal models (Monach et al., 2004). It is also important to consider that there may be extensive heterogeneity in human disease and that the animal models we study intensively may reflect only a subset of individuals with a given disease. A challenge that confronts us is to understand which animal models are most similar to human disease, and can teach us most about the genetic predisposition to disease, disease pathogenesis, and effective therapy.

PREVALENCE OF AUTOIMMUNITY

It is striking that while each autoimmune disease individually affects only a small number of people, the prevalence of all autoimmune diseases is approximately 5% (Jacobson et al., 1997). Two critical facts about autoimmune disease are important in understanding the high frequency of these diseases. First, autoreactivity is an aspect of every normal immune system. In fact, the repertoire of immunocompetent lymphocytes that provides protective immunity is selected based on autoreactivity (Gu et al., 1991; Nobrega et al., 2002; Vallejo et al., 2004). Regulation of the autoreactivity helps shape the immune system (see below), so that it does not become the pathogenic autoreactivity associated with tissue damage, requires constant vigilance. The immune system maintains a precarious balance between the

two: too little response leads to potential neglect of danger, while an over-exuberant response can potentially lead to autoreactivity. How this balance is maintained is discussed below. Second, a genetic predisposition to autoimmunity exists in all individuals with an autoimmune disease and aspects of this predisposition may be similar for many different autoimmune diseases (Wakeland et al., 2001; Gregersen, 2003; Melanitou et al., 2003; Winchester, 2004). Autoimmune disease requires not just autoreactivity, but also target-organ vulnerability (Liao et al., 1995). Some of the recent studies of the genetic basis of autoimmunity show that the genetic factors governing specific organ vulnerability are distinct from those governing autoreactivity. Thus, individuals may share pathways promoting autoreactivity, yet present with different autoimmune diseases (Shamim and Miller, 2000; Henderson et al., 2000; Prahalad et al., 2002).

GENETICS OF AUTOIMMUNITY

It is clear from epidemiologic studies and studies of animal models of autoimmune disease that there is a genetic component to essentially every autoimmune disease. A few autoimmune diseases appear to be monogenic diseases. The human disease autoimmune-polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED), an autoimmune disease of multiple endocrine organs, is a consequence of a deletion in the AIRE gene that encodes a protein that causes tissue-specific genes to be expressed in medullary epithelial cells in the thymus (Pitkanen and Peterson, 2003; Gorman et al., 2004). These cells mediate negative selection of T cells reactive with peptides that derive from tissue-specific proteins. In the absence of AIRE expression, a spectrum of autoreactive T cells fails to be deleted; these cells mature to immunocompetence and mediate an immune attack on various organs. The absence of the AIRE gene appears sufficient for autoimmunity, although the phenotype of the disease that emerges, even within a single family, can be quite variable. Similarly, a defect in the Fas gene can also lead to autoimmunity. The Fas protein is expressed on activated lymphocytes. Engagement of Fas by Fas ligand leads to the death of the Fas-expressing cell, a process critical for downregulating the immune response. Individuals deficient in Fas expression have a disease called autoimmune lymphoproliferative syndrome (ALPS) characterized by an excess of T and B cells and by autoantibody production (Fleisher et al., 2001; Grodzicky and Elkon, 2002). Of note, not all individuals with deficient Fas expression display ALPS; thus, even in this disease other genes must modulate disease phenotype.

For most autoimmune diseases, multiple susceptibility loci must coexist for a disease phenotype to be apparent. Each locus is a region mapped by linkage analysis to the disease phenotype. Studies from mouse models of autoimmune disease have also revealed the presence of loci that suppress the autoimmune phenotype (Wakeland et al., 2001). Thus, an individual's risk of developing an autoimmune disease depends on a summation of susceptibility and resistance loci. It is possible that susceptibility to a particular autoimmune disease may be mediated by entirely distinct sets of genetic loci, although within the different loci there may be genes involved in the same or convergent cellular pathways. These considerations enormously complicate the genetic analysis of autoimmune disease.

Often the genetic basis of an autoimmune disease includes the expression of a particular HLA haplotype. For example, rheumatoid arthritis in the white population is highly associated with the expression of a set of DR4 alleles that have a particular structural motif, called the "shared epitope" (Winchester, 2004). Of note, other ethnic populations do not display this same association of DR4 alleles with rheumatoid arthritis (Gorman et al., 2004). A similar symptom complex can, therefore, arise from more than one etiopathogenic mechanism. Reactive arthritis occurs in individuals expressing B27 or, less commonly, B7 class I MHC molecules. Multiple sclerosis, systemic lupus, and diabetes display particular HLA associations, as do many other autoimmune diseases (Tomlinson and Bodmer, 1995; Wong and Wen, 2003; Winchester, 2004). While it is interesting to speculate that these HLA associations with autoimmune disease determine the antigens and antigenic peptides targeted by the autoimmune response, it is also clear that a number of non-MHC genes within or in linkage disequilibrium with the MHC locus help regulate the immune response (Djilali-Saiah et al., 1996; Gebe et al., 2002). These non-MHC genes also contribute to a genetic predisposition to autoimmunity.

Most susceptibility loci appear to represent extended disease-related haplotypes with a number of genes within each susceptibility locus contributing to the predisposition to autoimmunity (Djilali-Saiah et al., 1996; Morel et al., 2001). The identification of several loci and genes involved in autoimmunity has now been accomplished (Ueda et al., 2003; Russell et al., 2004). Evidence is emerging that genetic susceptibility can be a consequence of combinations of genes within each gene locus, and not the consequence of a single gene in each locus. In murine models, as susceptibility loci are analyzed, it is apparent that many genes within each locus affect pathways that promote autoimmunity. For example, a region on chromosome 1 is implicated in autoimmunity in systemic lupus erythematosus (Morel et al., 2001). Studies of murine lupus would suggest that certain alleles of Fc receptor genes, as well as non-Fc receptor genes in this locus, constitute a risk factor for disease (Tsao, 2003; Tarzi and Cook, 2003). Thus, while some epidemiologic studies have suggested that particular alleles of at least three genes contribute to the autoimmune diathesis in each individual, it is probable that many more genes are

in fact involved, but are positioned in a smaller number of distinct loci. Genes involved in lymphocyte survival, activation and downregulation have all been identified as risk factors for autoimmunity (Ravirajan and Isenberg, 2002). Thus, autoimmunity can result from a defect in almost any pathway of lymphocyte homeostasis, and many probably affect thresholds for negative selection of autoreactive lymphocytes.

Clinically, it has long been appreciated that autoimmune diseases cluster in families. The biologic basis for this observation is now clear; the same susceptibility genes can influence many different autoimmune diseases. For example, a polymorphism of CTLA-4, an inhibitory costimulatory molecule present on activated T cells, conveys risk for insulin-dependent diabetes, autoimmune hemolytic anemia, and Graves' disease (Ueda et al., 2003), while the CARD15 (NOD-2) gene is associated with both inflammatory bowel disease and psoriasis (Rahman et al., 2003; Russell et al., 2004). The differences in disease phenotype may lie in associated genes, those governing target organ susceptibility or those that modulate disease severity (Russell et al., 2004).

HORMONES AND AUTOIMMUNITY

Since many autoimmune diseases occur more commonly in women than in men, and autoimmunity in general is almost three times more common in women than in men, there have been several investigations of the role of sex hormones in autoimmune disease. It has been difficult to draw simple conclusions from these studies. Not all autoimmune diseases are more common in women. Some, such as ankylosing spondylitis, have a much higher incidence in men. Furthermore, the predisposition to autoimmunity can be sex determined or hormonally modulated; thus, the higher incidence of disease in women may not always reflect the influence of female hormones on the immune system. The evidence also shows that the effects of sex hormones differ in different diseases. While there is significant evidence that estrogen can exacerbate systemic lupus, estrogen seems to protect against rheumatoid arthritis. Additionally, estrogen or other sex hormones might affect target-organ antigen display or target-organ susceptibility to immune-mediated damage. Thus, there is no simple paradigm to explain the relationship between sex and autoimmunity (Grimaldi et al., 2005).

AUTOIMMUNITY AND CENTRAL TOLERANCE

The hallmark of autoimmune disease is the activation of self-reactive T and B lymphocytes. A major mechanism of self-tolerance is the elimination of self-reactive immature lymphocytes by antigen ligation of the TCR or BCR at critical stages of development. For autoimmunity to develop there must be a lack of stringency in the elimination of autoreactive cells. Because TCRs and BCRs are generated by random gene rearrangements that occur within the nucleus of the cell and are not determined by knowledge of the world of self or foreign antigen, autoreactive T and B cells arise routinely. To eliminate autoreactive cells and maintain self-tolerance, T and B cells routinely undergo a selection process during their maturation in primary lymphoid organs, the thymus and bone marrow, respectively (Rajewsky, 1996; Vallejo et al., 2004). B cells again undergo a second process of selection after somatic mutation of immunoglobulin genes, as somatic mutation routinely generates autoreactivity.

T cells that mature in the thymus and enter peripheral lymphoid organs must display TCRs with some affinity for self-peptide–self-MHC complexes in order to receive the necessary signals for survival, termed positive selection. T cells arising in the thymus that express TCRs lacking any affinity for the self-peptide–self-MHC complexes fail to undergo positive selection and die. T cells that are strongly reactive to self-peptide–self-MHC complexes are eliminated in a process termed negative selection. The threshold for both positive and negative selection represents a continuum. As the peptide–MHC complexes present in the thymus differ in each individual and the threshold for negative selection varies from individual to individual, each individual releases a different repertoire of antimicrobial and antiself reactive T cells to the periphery, each reflecting a different spectrum of foreign and self-peptide specificities (Werlen et al., 2003; Bommhardt et al., 2004; Vallejo et al., 2004). It is also probable that certain stimuli can rescue T cells.

B cells similarly undergo a process of negative selection prior to achieving immunocompetence. This process occurs in the bone marrow and continues in the spleen where B cells migrate as transitional cells after exiting the bone marrow (Davidson et al., 2002). Whether B cells require positive selection on self-antigen for survival and need to display some degree of autoreactivity remains an area of active investigation. It is clear, however, that highly autoreactive B cells are negatively selected on self-antigens encountered during early maturation, and again, the threshold for deletion is different for each individual (Monroe et al., 2003). Deletion occurs with the highest extent of BCR cross-linking; anergy occurs with less cross-linking. Thus, the degree of autoreactivity in the B-cell repertoire is also variable. Autoreactive B cells that have been signaled to undergo deletion can be rescued in a proinflammatory setting by engagement of costimulatory molecules on the B-cell membrane or by signaling through TLRs. Thus, the repertoire of naïve B cells will vary over time within an individual, with higher-affinity autoreactive B cells present

during times of infection or inflammation, and fewer, lower-affinity autoreactive cells present during times of immunologic quiescence.

AUTOIMMUNITY AND PERIPHERAL TOLERANCE

Negative selection of T and B cells occurs in the periphery as well as in primary lymphoid organs, permitting the removal of autoreactive cells that do not encounter autoantigen in the thymus or bone marrow. This process of negative selection is termed peripheral tolerance. Like central tolerance, it is mediated by engagement of the TCR or BCR in a noninflammatory setting (Defrance et al., 2002; Walker and Abbas, 2002). Although it has been traditional to debate whether autoimmunity results from a defect in central tolerance in the thymus or bone marrow, or in peripheral tolerance in secondary lymphoid organs, current knowledge of tolerance induction suggests that this may be an artificial distinction. Critical to both central and peripheral tolerance is engagement of the antigen receptor. While central and peripheral tolerance appear to differ in some features, it is likely that individuals with stringent negative selection have stringent negative selection in both central and peripheral lymphoid organs. Similarly, individuals with lax selection do not delete autoreactive cells effectively in either central or peripheral lymphoid organs. The idea that an autoimmune-prone individual has a general lack of stringency in tolerance might help explain why such individuals often have more than one autoimmune disease. In animal models, it is clear that poor negative selection in the naïve B- or T-cell repertoire is a condition for autoimmune disease (Ridgway et al., 1996; Lang and Bellgrau, 2002; Liossis and Zouali, 2004), and that laxity in negative selection can predispose to organ-specific autoimmunity of multiple organs. In summary, the thresholds for survival and deletion need to be set within appropriate limits. Too little deletion and autoreactivity ensues; too much deletion and the protective repertoire may be compromised. Any genetic change that reduces deletion may be a risk factor for autoimmunity.

ROLE OF ENVIRONMENTAL FACTORS

Environmental factors are also important triggers for expression of autoimmunity. Smoking, drug exposure, diet, chemical exposure, and sunlight have all been implicated as risk factors for particular diseases (Price and Venables, 1995; George et al., 1997; Knip and Akerblom, 1999; Steen, 1999; Cantorna, 2000; D'Cruz, 2000; Fournie et al., 2001; Moriyama and Eisenbarth, 2002; Debandt et al., 2003). Clearly, infection or antigen exposure can precipitate autoimmune disease. It has even been suggested that most autoimmune diseases represent the late sequelae of an infectious process (Pender, 2003; Varela-Calvino and Peakman, 2003). Proving this hypothesis has, however, been difficult. For some diseases, such as rheumatic fever, the causal connection between microbial infection, the antimicrobial response, and autoimmune disease is clearly established (Cunningham, 2003; Guilherme and Kalil, 2004). For other diseases, there is suggestive epidemiologic evidence in humans or evidence from animal models that autoimmunity can follow microbial infection, or T-cell or antibody cross-reactivity with both microbial and self-antigen has been identified (James et al., 2001; Kuon and Sieper, 2003; Strassburg et al., 2003). In general, researchers have sought to implicate particular infections in the pathogenesis of particular autoimmune diseases, but it is possible that for some autoimmune diseases there is more than one possible microbial trigger.

ACTIVATION OF THE IMMUNE SYSTEM

Activation of both T and B cells in the periphery requires that the cells receive two signals, one derived by ligation of the antigen receptor and the other by engagement of a co-stimulatory receptor. In general, when antigen enters the system, there is an activation of DCs, the critical APC in a primary immune response. This occurs because microbes express molecules that bind to pattern-recognition receptors or TLRs on the DC. The consequence of this binding is upregulation of the costimulatory molecules CD80 (B7.1) and CD86 (B7.2) on DCs, and transformation of the DC from resting, or tolerogenic (Steinman et al., 2003), to activated, or immunogenic (Hackstein and Thomson, 2004). T cells recognizing microbial peptide in either class I or class II MHC molecules on the immunogenic DC will be activated. Because some degree of autoreactivity is present in all T cells, each time a T cell is activated by a foreign-peptide–self-MHC complex, that activated T cell may also recognize a self-peptide–self-MHC complex. It is a feature of memory T cells that they can be activated by a lower-affinity interaction with the TCR than is required to activate primary T cells (Welsh et al., 2004). Thus, a T-cell that is not activated by a self-peptide–self-MHC complex while still a naïve cell may be activated by that complex once it becomes a memory T cell. Memory T cells may, therefore, be autoreactive in a proinflammatory setting.

There are many examples in the literature of a T-cell derived from an individual with autoimmune disease that recognizes both a microbial peptide and a self-peptide. This cross-reactivity is termed molecular mimicry, and represents a mechanism by which autoimmunity can be triggered by

infection (Albert and Inman, 1999; Wekerle and Hohlfeld, 2003). The hypothesis that molecular mimicry predisposes to autoimmunity clearly has validity in rodent models of autoimmune disease, and suggests that laxity in selection of the naïve T-cell repertoire can be a major contributor to autoimmunity. Those individuals with less stringent negative selection will have multiple T cells that can be activated by foreign antigen and will also display pathogenic autoreactivity.

The activated T cell provides T-cell help or costimulatory signals to B cells that are encountering microbial antigen. B cells that bind both microbial antigen and self-antigen will ingest, process, and present epitopes of self-antigen, which can then be recognized by T cells. Because B cells often process antigen to different peptides than do DCs, the B cells can present novel epitopes of self-antigen and activate T cells with novel autospecificities (Sercarz et al., 1993; Bockenstedt et al., 1995; Yan and Mamula, 2002). These cross-reactive B cells will, therefore, contribute to a cascade of autoreactivity, as they activate an expanded repertoire of T cells. The B-cell repertoire, therefore, critically influences the T-cell repertoire. The fewer autoreactive B cells present, the less presentation of self-antigen to T cells.

DOWNREGULATION OF AN IMMUNE RESPONSE

The induction of an immune response needs to be followed by a downregulation or elimination of many of the cells that have undergone clonal expansion. A major observation of recent studies of autoimmune disease is that a defect in the restoration of immune homeostasis, or in downregulation of an immune response, can be a risk factor for autoimmunity. Since all reactivity with foreign antigen includes reactivity to self-antigen, antiself responses are routinely generated in the process of mounting an immune response to foreign antigen. The potential pathogenicity of the autoimmune response will vary from individual to individual. In general, however, the mechanisms that exist to dampen the immune response also diminish the autoreactivity, and even potentially pathogenic autoreactivity is downregulated. B and T cells are routinely downregulated as soon as they are activated. For the B cell, this occurs, in part, by cross-linking of the BCR and FcRIIB by antigen–antibody complexes. When FcRIIB is absent or deficient, autoantibody production is poorly controlled (Ravetch and Bolland, 2001; Samuelsson et al., 2001). Multiple coinhibitory molecules are expressed on activated T cells. Interaction with their receptors transduces an inhibitory signal to the T cells, signaling them to downmodulate their response. There is also some evidence that engagement of CD80 (B7) on the APC by the T-cell coinhibitory molecule CTLA-4 transduces an inhibitory signal to the APCs. Mutations in two coinhibitory molecules, PD1 and CTLA-4, are associated with several autoimmune diseases (Chen, 2004; Khoury and Sayegh, 2004). Both B and T cells are also susceptible to activation-induced cell death mediated through Fas–Fas ligand interactions (Brunner et al., 2003; Li-Weber and Krammer, 2003). Defects in this process can lead to autoimmunity.

Thus, controlling the immune response is critical to normal homeostasis of the immune system and is mediated by multiple inhibitory pathways. A major component of autoimmune disease in some individuals may be a defect in the suppression of immune activation.

REGULATORY T CELLS

Another area of intensive study is the characterization and mechanisms of action of populations of regulatory cells. Many years ago, any discussion of autoimmune disease necessarily included a discussion of suppressor T cells. The study of suppressor T cells fell into temporary disrepute, but has been recently reinvigorated and several populations of regulatory T cells have now been identified, some of which arise during thymic development and others are induced by antigen exposure in the periphery (Cottrez and Groux, 2004). Natural killer (NK) T cells recognize lipid antigens in CD1 molecules (Brigl and Brenner, 2004). There is evidence in some diseases that a decrease in this population precedes the onset of autoimmunity. There is also evidence that a change in cytokine profile of this subset, particularly a loss of interleukin (IL)-4 production, associates with disease onset (Poulton and Baxter, 2001). In the nonobese diabetic (NOD) mouse model, it has been shown that activation of NK T cells with a synthetic ligand can prevent the progression to diabetes (Wang et al., 2001). There is also a population of $CD4^+/CD25^+$ (γ chain of the IL-2 receptor) T cells that mature in the thymus and appear to be critical in suppression of autoimmunity. These cells express the transcription factor Foxp3 and the cell surface receptor GITR, which distinguishes them from activated T cells that also express CD25. They suppress effector T cells in a cell contact-dependent but non-antigen-specific manner; it is possible that they also suppress APCs (Fontenot and Rudensky, 2004; Sakaguchi, 2004). They have been demonstrated to decline in number prior to overt diabetes in NOD mice and diabetes can be delayed by an infusion of these cells (Tang et al., 2004; Tarbell et al., 2004).

Other studies have identified a population of CD8 suppressor cells that may directly lyse autoreactive cells or may secrete immunosuppressive cytokines (Cortesini et al., 2001; Filaci and Suciu-Foca, 2002). Cells expressing the immunosuppressive cytokines transforming growth factor (TGF)-β (Th3 cells) (Weiner, 2001) or IL-10 (TR1 cells) (Roncarolo et al., 2001) arise under particular condi-

tions of antigen exposure and once activated, mediate non-antigen-specific suppression.

It is now apparent that a bias to the development of Th1 cells is present in most autoimmune diseases. Because Th2 cells control the activation of Th1 cells, the activation of Th2 cells can diminish autoimmunity in murine models of disease, regardless of whether the tissue damage is mediated by antibodies or by T cells (Dong and Flavell, 2001; Hill and Sarvetnick, 2002; Sugimoto et al., 2002; Szabo et al., 2003; Ghoreschi and Rocken, 2004; Gomez et al., 2004; Milani et al., 2003; Wurtz et al., 2004). There is some evidence that low doses of antigen preferentially activate Th2 cells (Boonstra et al., 2003). It is interesting to speculate that self-antigen is often presented in limiting amounts and leads preferentially to the activation of self-reactive nonpathogenic Th2 cells.

There are multiple populations of regulatory cells, any one of which may be deficient in autoimmune disease. The balance between effector cells and regulatory cells may determine whether an autoreactive response that arises in the course of microbial exposure is terminated or perpetuated. Since intense immunosuppression may inactivate both effector and regulatory T cells, new approaches to the therapy of autoimmunity may be directed at finding ways to inhibit effector cells, while allowing regulatory cells to expand.

ROLE OF ANTIGEN AS A DRIVER OF AUTOIMMUNITY

A major question in autoimmune disease is whether the process is autonomous or driven by antigen, and if the latter whether the antigen is self-antigen or foreign antigen. Animal models of disease definitively show that molecular mimicry following activation by microbial antigen can initiate autoreactivity (Cunningham, 2003; Kuwabara, 2004; Olson et al., 2004).

There are also data suggesting that self-antigen drives the autoimmune response. First, in animal models of systemic lupus, it appears that an excess of apoptotic cells or a problem in their clearance can result in a lupus-like serology with antichromatin reactivity (Liu et al., 2004). Current understanding would suggest that apoptotic debris can activate TLRs and transform tolerogenic DCs into immunogenic APCs, as well as activate B cells (Leadbetter et al., 2002; Lovgren et al., 2004).

Second, extensive tissue damage can lead to the presentation of normally sequestered self-antigen in a proinflammatory setting (Horwitz et al., 2002; Liu et al., 2002; Vezys and Lefrancois, 2002). The proinflammatory setting may be enhanced by apoptosis of cells following tissue insult. This can clearly lead to an autoimmune response. Whether in some individuals this response is perpetuated because of a lack of appropriate restoration to homeostasis is an important question.

Finally, polymorphisms in autoantigens may also constitute risk factors for autoimmune disease (Suzuki et al., 2003; Pauza et al., 2004). As the genetic susceptibility to autoimmune disease is further explored, it will become more apparent the degree to which molecular mimicry, aberrant expression of autoantigens, or exposure to previously sequestered antigen in an immunogenic setting contributes to disease.

MECHANISMS OF TISSUE DAMAGE

Studies over the past decade have clearly demonstrated that the mechanisms that incite autoimmune disease may differ substantially from the mechanisms that propagate tissue damage. Autoreactive T and B cells that are activated in secondary lymphoid organs and initiate disease may have a different cytokine profile from the effector cells that migrate into target organs and cause tissue fibrosis (Campbell et al., 2001). Thus, it is clearer and clearer that at each stage of autoimmune disease, induction of autoreactivity and tissue destruction need to be separately explored, and that the previous characterization of certain cytokines as proinflammatory and others as anti-inflammatory may be misleading. While TGF-β may dampen the induction of autoreactivity, it may hasten tissue fibrosis (Letterio and Roberts, 1998). Similarly, IL-10 is anti-inflammatory during disease initiation through its inhibitory effects on APCs, but can drive T-cell proliferation, immunoglobulin class switching, and antibody production later in disease (Mocellin et al., 2004). Even the proinflammatory cytokine interferon-γ can have anti-inflammatory properties in the early stages of some autoimmune diseases (Billiau, 1996; Grohmann and Puccetti, 2002; Rosloniec et al., 2002). It is perhaps important, as we move forward in studies of autoimmune disease, to consider the mechanism of both immune activation and tissue destruction, and to be aware that cytokines, hormones or other mediators may exhibit differential effects in each process. Studies of animal models have now clearly shown that it is possible to intervene in disease progression to protect organs from immune-mediated destruction, even while autoreactivity continues unabated (Clynes et al., 1998; Schiffer et al., 2002).

FLARES AND REMISSIONS DURING DISEASE

The vast majority of animal models of autoimmune disease represent chronic progressive disease activity. Once the autoimmune disease becomes manifest, it progresses to organ failure or death. Much human autoimmune disease, in

contrast, is characterized by periods of disease remission and flare. Little is known in human disease about the cellular events that lead to disease remission. It is also true that little is known regarding the cause of disease flares. A major area of ignorance concerns the cell type responsible for disease flares. It is not known whether flares represent a de-novo activation of naïve autoreactive cells or a reactivation of quiescent memory cells. Our ignorance in this regard derives largely from the difficulty of sampling a large repertoire of autoreactive T or B cells. Often, these cells are poorly represented in peripheral blood (Newman et al., 2003; Reddy et al., 2003; Reijonen et al., 2003, Bischof et al., 2004). Even when present in blood, and therefore accessible to analysis, they may be so infrequent that their analysis represents a major challenge.

THERAPEUTIC IMPLICATIONS

Current global immunosuppressive therapies remain overly toxic, and do not reflect our new understanding of autoimmune disease. Autoimmunity can result from defects either in repertoire selection or in immune regulation. The idea that autoimmune disease may be caused by excessive laxity in tolerance induction, either centrally or peripherally, or by inadequate downregulation of an immune response that has been engendered by microbial invasion or by tissue injury has therapeutic implications. The current therapies of autoimmune disease are based on a perceived need to institute immunosuppression and anti-inflammatory therapy at the time of autoimmune tissue destruction. Our most updated understanding of autoimmunity would suggest that it might be more appropriate to consider treating disease during times of disease quiescence. The goal of this therapeutic approach would be to alter T- and B-cell repertoire selection. One pathway to target for modulation of the repertoire is the TCR or BCR signaling pathway; increasing the signal delivered to naïve T and B cells may lead to greater stringency of negative selection with lower-affinity autoreactive cells maturing to immunocompetence. This might decrease autoimmunity without causing global immunosuppression. Currently, antigen-specific therapies remain a dream.

Multiple pathways of immune activation, including costimulatory pathways, and TLR and pattern-recognition receptor signaling pathways can be targeted to reduce activation of the immune system. Altering pathways involved in downregulation of the immune response is also an attractive therapeutic strategy. During periods of disease quiescence it may be possible to drive the expansion of regulatory cells. Maintaining an ample number and function of regulatory T-cell subsets either *in vivo* or through *ex vivo* manipulations of cells is a therapeutic approach poised to move soon from the realm of fantasy to that of reality.

Protecting target organs and preventing irreversible tissue damage will require different therapeutic strategies from blocking the induction of autoreactivity. These new therapeutic approaches offer the hope of maintaining immunocompetence while eliminating the consequences of pathogenic autoreactivity. The era of a uniform approach to therapy with intense immune ablation to treat autoimmunity may soon be over.

GOALS FOR THE FUTURE

Over the past several years, new technologies have been developed that will substantially increase our understanding of autoimmune disease. The development of microarray technology, which makes it possible to determine the level of expression of a very large number of genes or proteins in defined populations of cells, will provide new insights into disease pathogenesis and new ways to phenotype patients with autoimmune disease. These technologies are highly likely to provide sets of biomarkers that will help determine risk for developing a particular disease, characterize current activity of the disease and disease prognosis, and predict response to therapy. Already, data from microarray analyses of gene expression in peripheral blood cells suggest that systemic lupus and Sjögren syndrome share an "interferon signature" characterized by high expression of a number of interferon-inducible genes (Baechler et al., 2003; Bennett et al., 2003); rheumatoid arthritis, in contrast, is characterized by a different pattern of gene expression (van der Pouw Kraan et al., 2003). It is reasonable to predict that patterns of gene and protein expression will reveal differences and similarities among autoimmune disease. We can also look forward to the identification of subsets of patients within a given disease. Distinct patterns may distinguish patients with impending flares and may be an early marker of response to therapy.

The development of biomarkers will, undoubtedly, improve the therapy of autoimmune disease. It may be possible to identify early those patients whose disease is likely to be severe and to monitor disease activity without waiting for clinical symptomatology. Ultimately, it may be possible to customize therapy for each patient, thereby enhancing therapy and avoiding unnecessary toxicities.

CONCLUDING REMARKS

The past several years have witnessed a change in our understanding of autoimmunity and a clear new direction in our approach to the study of autoimmunity. It now seems most useful to consider autoimmunity as a failure in T- and B-cell repertoire selection or a failure in the regulation of activated T and B cells. It is also clear that autoimmunity

needs to be coupled to target-organ vulnerability to immune attack for autoimmune disease to be present. This understanding suggests new therapeutic targets, and new therapeutic strategies.

The focus on new technologies to provide biomarkers of immune function represents an exciting opportunity to treat disease prior to tissue damage and to customize therapy for each patient. Furthermore, studies of gene and protein expression will help elucidate those mechanisms of immune dysfunction that are shared among multiple autoimmune diseases and those that are unique to a particular disease. Thus, there are reasons to be optimistic, but acquiring the necessary new knowledge and translating that knowledge to therapy will take many years.

Acknowledgments

Supported by grants from the NIH to AD (AI47291 and AI31229) and BD.

References

Albert, L.J. and Inman, R.D. (1999). Molecular mimicry and autoimmunity. *N. Engl. J. Med. 341*, 2068–2074.

Baechler, E.C., Batliwalla, F.M., Karypis, G., Gaffney, P.M., Ortmann, W.A., Espe, K.J., Shark, K.B., Grande, W.J., Hughes, K.M., Kapur, V., Gregersen, P.K., and Behrens, T.W. (2003). Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. USA 100*, 2610–2615.

Banchereau, J. and Steinman, R.M. (1998). Dendritic cells and the control of immunity. *Nature 392*, 245–252.

Beck, S. and Trowsdale, J. (2000). The human major histocompatability complex: lessons from the DNA sequence. *Annu. Rev. Genomics Hum. Genet. 1*, 117–137.

Bennett, L., Palucka, A.K., Arce, E., Cantrell, V., Borvak, J., Banchereau, J., and Pascual, V. (2003). Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med. 197*, 711–723.

Billiau, A. (1996). Interferon-gamma in autoimmunity. *Cytokine Growth Factor Rev. 7*, 25–34.

Birmingham, D.J., Rovin, B.H., Yu, C.Y., and Hebert, L.A. (2001). Of mice and men: the relevance of the mouse to the study of human SLE. *Immunol. Res. 24*, 211–224.

Bischof, F., Hofmann, M., Schumacher, T.N., Vyth-Dreese, F.A., Weissert, R., Schild, H., Kruisbeek, A.M., and Melms, A. (2004). Analysis of autoreactive CD4 T cells in experimental autoimmune encephalomyelitis after primary and secondary challenge using MHC class II tetramers. *J. Immunol. 172*, 2878–2884.

Bishop, G.A. and Hostager, B.S. (2003). The CD40-CD154 interaction in B cell–T cell liaisons. *Cytokine Growth Factor Rev. 14*, 297–309.

Bishop, G.A., Haxhinasto, S.A., Stunz, L.L., and Hostager, B.S. (2003). Antigen-specific B-lymphocyte activation. *Crit. Rev. Immunol. 23*, 149–197.

Bockenstedt, L.K., Gee, R.J., and Mamula, M.J. (1995). Self-peptides in the initiation of lupus autoimmunity. *J. Immunol. 154*, 3516–3524.

Bommhardt, U., Beyer, M., Hunig, T., and Reichardt, H.M. (2004). Molecular and cellular mechanisms of T cell development. *Cell. Mol. Life. Sci. 61*, 263–280.

Boonstra, A., Asselin-Paturel, C., Gilliet, M., Crain, C., Trinchieri, G., Liu, Y.J., and O'Garra, A. (2003). Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential Toll-like receptor ligation. *J. Exp. Med. 197*, 101–109.

Brigl, M. and Brenner, M.B. (2004). CD1: antigen presentation and T cell function. *Annu. Rev. Immunol. 22*, 817–890.

Brunner, T., Wasem, C., Torgler, R., Cima, I., Jakob, S., and Corazza, N. (2003). Fas (CD95/Apo-1) ligand regulation in T cell homeostasis, cell-mediated cytotoxicity and immune pathology. *Semin. Immunol. 15*, 167–176.

Campbell, D.J., Kim, C.H., and Butcher, E.C. (2001). Separable effector T cell populations specialized for B cell help or tissue inflammation. *Nat. Immunol. 2*, 876–881.

Cantorna, M.T. (2000). Vitamin D and autoimmunity: is vitamin D status an environmental factor affecting autoimmune disease prevalence? *Proc. Soc. Exp. Biol. Med. 223*, 230–233.

Chen, L. (2004). Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat. Rev. Immunol. 4*, 336–347.

Clynes, R., Dumitru, C., and Ravetch, J.V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science 279*, 1052–1054.

Cortesini, R., LeMaoult, J., Ciubotariu, R., and Cortesini, N.S. (2001). CD8+CD28– T suppressor cells and the induction of antigen-specific, antigen-presenting cell-mediated suppression of Th reactivity. *Immunol. Rev. 182*, 201–206.

Cottrez, F. and Groux, H. (2004). Specialization in tolerance: innate CD(4+)CD(25+) versus acquired TR1 and TH3 regulatory T cells. *Transplantation 77*, S12–15.

Craxton, A., Magaletti, D., Ryan, E.J., and Clark, E.A. (2003). Macrophage- and dendritic-cell-dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. *Blood 101*, 4464–4471.

Crow, M.K. (2004). Costimulatory molecules and T-cell–B-cell interactions. *Rheum. Dis. Clin. North Am. 30*, 175–191, vii–viii.

Cunningham, M. W. (2003). Autoimmunity and molecular mimicry in the pathogenesis of post-streptococcal heart disease. *Front. Biosci. 8*, s533–543.

Davidson, A. and Diamond, B. (2001). Autoimmune diseases. *N. Engl. J. Med. 345*, 340–350.

Davidson, A., Konstantinov, K., and Diamond, B. (2002). In J. Smolen and P. Lipsky (eds), *Biological Therapy in Rheumatology*. London: Martin Dunitz.

D'Cruz, D. (2000). Autoimmune diseases associated with drugs, chemicals and environmental factors. *Toxicol. Lett. 112–113*, 421–432.

Debandt, M., Vittecoq, O., Descamps, V., Le Loet, X., and Meyer, O. (2003). Anti-TNF-alpha-induced systemic lupus syndrome. *Clin. Rheumatol. 22*, 56–61.

Defrance, T., Casamayor-Palleja, M., and Krammer, P.H. (2002). The life and death of a B cell. *Adv. Cancer Res. 86*, 195–225.

Djilali-Saiah, I., Benini, V., Daniel, S., Assan, R., Bach, J.F., and Caillat-Zucman, S. (1996). Linkage disequilibrium between HLA class II (DR, DQ, DP) and antigen processing (LMP, TAP, DM) genes of the major histocompatibility complex. *Tissue Antigens 48*, 87–92.

Dong, C. and Flavell, R.A. (2001). Th1 and Th2 cells. *Curr. Opin. Hematol. 8*, 47–51.

Filaci, G. and Suciu-Foca, N. (2002). CD8+ T suppressor cells are back to the game: are they players in autoimmunity? *Autoimmun. Rev. 1*, 279–283.

Fleisher, T.A., Straus, S.E., and Bleesing, J.J. (2001). A genetic disorder of lymphocyte apoptosis involving the Fas pathway: the autoimmune lymphoproliferative syndrome. *Curr. Allergy Asthma Rep. 1*, 534–540.

Fontenot, J.D. and Rudensky, A.Y. (2004). Molecular aspects of regulatory T cell development. *Semin. Immunol. 16*, 73–80.

Fournie, G.J., Mas, M., Cautain, B., Savignac, M., Subra, J.F., Pelletier, L., Saoudi, A., Lagrange, D., Calise, M., and Druet, P. (2001). Induction of autoimmunity through bystander effects. Lessons from immunological disorders induced by heavy metals. *J. Autoimmun. 16*, 319–326.

Gebe, J.A., Swanson, E., and Kwok, W.W. (2002). HLA class II peptide-binding and autoimmunity. *Tissue Antigens 59*, 78–87.

Geijtenbeek, T.B., Van Vliet, S.J., Engering, A., 't Hart, B.A., and Van Kooyk, Y. (2004). Self- and nonself-recognition by C-type lectins on dendritic cells. *Annu. Rev. Immunol. 22*, 33–54.

Gellert, M. (2002). V(D)J recombination: RAG proteins, repair factors, and regulation. *Annu. Rev. Biochem. 71*, 101–132.

George, J., Levy, Y., and Shoenfeld, Y. (1997) Smoking and immunity: an additional player in the mosaic of autoimmunity. *Scand. J. Immunol. 45*, 1–6.

Ghoreschi, K. and Rocken, M. (2004). Immune deviation strategies in the therapy of psoriasis. *Curr. Drug. Targets Inflamm. Allergy 3*, 193–198.

Gomez, D., Correa, P.A., Gomez, L.M., Cadena, J., Molina, J.F., and Anaya, J.M. (2004). Th1/Th2 cytokines in patients with systemic lupus erythematosus: is tumor necrosis factor alpha protective? *Semin. Arthritis Rheum. 33*, 404–13.

Gorman, J.D., Lum, R.F., Chen, J.J., Suarez-Almazor, M.E., Thomson, G., and Criswell, L.A. (2004). Impact of shared epitope genotype and ethnicity on erosive disease: a meta-analysis of 3,240 rheumatoid arthritis patients. *Arthritis Rheum. 50*, 400–412.

Granucci, F., Zanoni, I., Feau, S., Capuano, G., and Ricciardi-Castagnoli, P. (2004). The regulatory role of dendritic cells in the immune response. *Int. Arch. Allergy Immunol. 134*, 179–185.

Gregersen, P.K. (2003). Teasing apart the complex genetics of human autoimmunity: lessons from rheumatoid arthritis. *Clin. Immunol. 107*, 1–9.

Grimaldi, C.M., Hill, L., Xu, X., Peeva, E., and Diamond, B. (2005). Hormonal modulation of B cell development and repertoire selection. *Mol. Immunol. 42*, 811–820.

Grodzicky, T. and Elkon, K.B. (2002). Apoptosis: a case where too much or too little can lead to autoimmunity. *Mt. Sinai J. Med. 69*, 208–219.

Grohmann, U. and Puccetti, P. (2002). The immunosuppressive activity of proinflammatory cytokines in experimental models: potential for therapeutic intervention in autoimmunity. *Curr. Drug Targets Inflamm. Allergy 1*, 77–87.

Grossman, Z., Min, B., Meier-Schellersheim, M., and Paul, W.E. (2004). Concomitant regulation of T-cell activation and homeostasis. *Nat. Rev. Immunol. 4*, 387–395.

Gu, H., Tarlinton, D., Muller, W., Rajewsky, K., and Forster, I. (1991). Most peripheral B cells in mice are ligand selected. *J. Exp. Med. 173*, 1357–1371.

Guilherme, L. and Kalil, J. (2004). Rheumatic fever: from sore throat to autoimmune heart lesions. *Int. Arch. Allergy Immunol. 134*, 56–64.

Hackstein, H. and Thomson, A.W. (2004). Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat. Rev. Immunol. 4*, 24–34.

Hanninen, A., Hamilton-Williams, E., and Kurts, C. (2003). Development of new strategies to prevent type 1 diabetes: the role of animal models. *Ann. Med. 35*, 546–563.

Henderson, R.D., Bain, C.J., and Pender, M.P. (2000). The occurrence of autoimmune diseases in patients with multiple sclerosis and their families. *J. Clin. Neurosci. 7*, 434–437.

Hill, N. and Sarvetnick, N. (2002). Cytokines: promoters and dampeners of autoimmunity. *Curr. Opin. Immunol. 14*, 791–797.

Horwitz, M.S., Ilic, A., Fine, C., Rodriguez, E., and Sarvetnick, N. (2002). Presented antigen from damaged pancreatic beta cells activates autoreactive T cells in virus-mediated autoimmune diabetes. *J. Clin. Invest. 109*, 79–87.

Howell, C.D. (2002). Animal models of autoimmunity. *Clin. Liver Dis. 6*, 487–495.

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*, 223–243.

James, J.A., Neas, B.R., Moser, K.L., Hall, T., Bruner, G.R., Sestak, A.L., and Harley, J.B. (2001). Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum. 44*, 1122–1126.

Khoury, S.J. and Sayegh, M.H. (2004). The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity 20*, 529–538.

Knip, M. and Akerblom, H.K. (1999). Environmental factors in the pathogenesis of type 1 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes 107(Suppl 3)*, S93–100.

Kuon, W. and Sieper, J. (2003). Identification of HLA-B27-restricted peptides in reactive arthritis and other spondyloarthropathies: computer algorithms and fluorescent activated cell sorting analysis as tools for hunting of HLA-B27-restricted chlamydial and autologous crossreactive peptides involved in reactive arthritis and ankylosing spondylitis. *Rheum. Dis. Clin. North Am. 29*, 595–611.

Kuwabara, S. (2004). Guillain-Barré syndrome: epidemiology, pathophysiology and management. *Drugs 64*, 597–610.

Lam-Tse, W.K., Lernmark, A., and Drexhage, H.A. (2002). Animal models of endocrine/organ-specific autoimmune diseases: do they really help us to understand human autoimmunity? *Springer Semin. Immunopathol. 24*, 297–321.

Lang, J. and Bellgrau, D. (2002). A T-cell functional phenotype common among autoimmune-prone rodent strains. *Scand. J. Immunol. 55*, 546–559.

Leadbetter, E., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature 416*, 603–607.

Letterio, J.J. and Roberts, A.B. (1998). Regulation of immune responses by TGF-beta. *Annu. Rev. Immunol. 16*, 137–161.

Li, Z., Woo, C.J., Iglesias-Ussel, M.D., Ronai, D., and Scharff, M.D. (2004). The generation of antibody diversity through somatic hypermutation and class switch recombination. *Genes Dev. 18*, 1–11.

Liao, L., Sindhwani, R., Rojkind, M., Factor, S., Leinwand, L., and Diamond, B. (1995). Antibody-mediated autoimmune myocarditis depends on genetically determined target organ sensitivity. *J. Exp. Med. 181*, 1123–1131.

Liossis, S.N. and Zouali, M. (2004). B lymphocyte selection and survival in systemic lupus. *Int. Arch. Allergy Immunol. 133*, 72–83.

Lipsky, P.E. (2001). Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. *Nat. Immunol. 2*, 764–766.

Liu, C.C., Navratil, J.S., Sabatine, J.M., and Ahearn, J.M. (2004). Apoptosis, complement and systemic lupus erythematosus: a mechanistic view. *Curr. Dir. Autoimmun. 7*, 49–86.

Liu, H.R., Zhao, R.R., Jiao, X.Y., Wang, Y.Y., and Fu, M. (2002). Relationship of myocardial remodeling to the genesis of serum autoantibodies to cardiac beta(1)-adrenoceptors and muscarinic type 2 acetylcholine receptors in rats. *J. Am. Coll. Cardiol. 39*, 1866–1873.

Li-Weber, M. and Krammer, P.H. (2003). Function and regulation of the CD95 (APO–1/Fas) ligand in the immune system. *Semin. Immunol. 15*, 145–157.

Lovgren, T., Eloranta, M.L., Bave, U., Alm, G.V., and Ronnblom, L. (2004). Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum. 50*, 1861–1872.

MacLennan, I. and Vinuesa, C. (2002). Dendritic cells, BAFF, and APRIL: innate players in adaptive antibody responses. *Immunity 17*, 235–238.

Melanitou, E., Fain, P., and Eisenbarth, G.S. (2003). Genetics of type 1A (immune mediated) diabetes. *J. Autoimmun. 21*, 93–98.

Milani, M., Ostlie, N., Wang, W., and Conti-Fine, B.M. (2003). T cells and cytokines in the pathogenesis of acquired myasthenia gravis. *Ann. N. Y. Acad. Sci. 998*, 284–307.

Mocellin, S., Marincola, F., Rossi, C.R., Nitti, D., and Lise, M. (2004). The multifaceted relationship between IL–10 and adaptive immunity:

putting together the pieces of a puzzle. *Cytokine Growth Factor Rev. 15*, 61–76.

Monach, P.A., Benoist, C., and Mathis, D. (2004). The role of antibodies in mouse models of rheumatoid arthritis, and relevance to human disease. *Adv. Immunol. 82*, 217–248.

Monroe, J.G., Bannish, G., Fuentes-Panana, E.M., King, L.B., Sandel, P.C., Chung, J., and Sater, R. (2003). Positive and negative selection during B lymphocyte development. *Immunol. Res. 27*, 427–442.

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*, 1787–1792.

Moriyama, H. and Eisenbarth, G.S. (2002). Genetics and environmental factors in endocrine/organ-specific autoimmunity: have there been any major advances? *Springer Semin. Immunopathol. 24*, 231–42.

Netea, M.G., van der Graaf, C., Van der Meer, J.W., and Kullberg, B.J. (2004). Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. *J. Leukoc. Biol. 75*, 749–755.

Newman, J., Rice, J.S., Wang, C., Harris, S.L., and Diamond, B. (2003). Identification of an antigen-specific B cell population. *J. Immunol. Methods 272*, 177–187.

Nobrega, A., Stransky, B., Nicolas, N., and Coutinho, A. (2002). Regeneration of natural antibody repertoire after massive ablation of lymphoid system: robust selection mechanisms preserve antigen binding specificities. *J. Immunol. 169*, 2971–2978.

Olson, J.K., Ludovic Croxford, J., and Miller, S.D. (2004). Innate and adaptive immune requirements for induction of autoimmune demyelinating disease by molecular mimicry. *Mol. Immunol. 40*, 1103–1108.

O'Sullivan, B. and Thomas, R. (2003). CD40 and dendritic cell function. *Crit. Rev. Immunol. 23*, 83–107.

Pauza, M.E., Dobbs, C.M., He, J., Patterson, T., Wagner, S., Anobile, B.S., Bradley, B.J., Lo, D., and Haskins, K. (2004). T-cell receptor transgenic response to an endogenous polymorphic autoantigen determines susceptibility to diabetes. *Diabetes 53*, 978–988.

Pender, M.P. (2003). Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. *Trends Immunol. 24*, 584–588.

Peutz-Kootstra, C.J., de Heer, E., Hoedemaeker, P.J., Abrass, C.K., and Bruijn, J.A. (2001). Lupus nephritis: lessons from experimental animal models. *J. Lab. Clin. Med. 137*, 244–260.

Pitkanen, J. and Peterson, P. (2003). Autoimmune regulator: from loss of function to autoimmunity. *Genes Immun. 4*, 12–21.

Poulton, L.D. and Baxter, A.G. (2001). Clinical application of NKT cell assays to the prediction of type 1 diabetes. *Diabetes Metab. Res. Rev. 17*, 429–435.

Prahalad, S., Shear, E.S., Thompson, S.D., Giannini, E.H., and Glass, D.N. (2002). Increased prevalence of familial autoimmunity in simplex and multiplex families with juvenile rheumatoid arthritis. *Arthritis Rheum. 46*, 1851–1856.

Price, E.J. and Venables, P.J. (1995). Drug-induced lupus. *Drug Saf. 12*, 283–290.

Rahman, P., Bartlett, S., Siannis, F., Pellet, F.J., Farewell, V.T., Peddle, L., Schentag, C.T., Alderdice, C.A., Hamilton, S., Khraishi, M., Tobin, Y., Hefferton, D., and Gladman, D.D. (2003). CARD15: a pleiotropic autoimmune gene that confers susceptibility to psoriatic arthritis. *Am. J. Hum. Genet. 73*, 677–681.

Rajewsky, K. (1996). Clonal selection and learning in the antibody system. *Nature 381*, 751–758.

Ravetch, J.V. and Bolland, S. (2001). IgG Fc receptors. *Annu. Rev. Immunol. 19*, 275–290.

Ravirajan, C.T. and Isenberg, D.A. (2002). Transgenic models of tolerance and autoimmunity: with special reference to systemic lupus erythematosus. *Lupus 11*, 843–849.

Reddy, J., Bettelli, E., Nicholson, L., Waldner, H., Jang, M.H., Wucherpfennig, K.W., and Kuchroo, V.K. (2003). Detection of autoreactive myelin proteolipid protein 139–151-specific T cells by using MHC II (IAs) tetramers. *J. Immunol. 170*, 870–877.

Reijonen, H., Kwok, W.W., and Nepom, G.T. (2003). Detection of CD4+ autoreactive T cells in T1D using HLA class II tetramers. *Ann. N. Y. Acad. Sci. 1005*, 82–87.

Ridgway, W.M., Fasso, M., Lanctot, A., Garvey, C., and Fathman, C.G. (1996). Breaking self-tolerance in nonobese diabetic mice. *J. Exp. Med. 183*, 1657–1662.

Roncarolo, M.G., Bacchetta, R., Bordignon, C., Narula, S., and Levings, M.K. (2001). Type 1 T regulatory cells. *Immunol. Rev. 182*, 68–79.

Rosloniec, E.F., Latham, K., and Guedez, Y.B. (2002). Paradoxical roles of IFN-gamma in models of Th1-mediated autoimmunity. *Arthritis Res. 4*, 333–336.

Russell, R.K., Nimmo, E.R., and Satsangi, J. (2004). Molecular genetics of Crohn's disease. *Curr. Opin. Genet. Dev. 14*, 264–270.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.

Samuelsson, A., Towers, T.L., and Ravetch, J.V. (2001). Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science 291*, 484–486.

Schiffer, L., Sinha, J., Wang, X., and Davidson., A. (2002). *SLE Therapeutics Meeting,* Washington, DC.

Sercarz, E.E., Lehmann, P.V., Ametani, A., Benichou, G., Miller, A., and Moudgil, K. (1993). Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol. 11*, 729–766.

Shamim, E.A. and Miller, F.W. (2000). Familial autoimmunity and the idiopathic inflammatory myopathies. *Curr. Rheumatol. Rep. 2*, 201–211.

Shlomchik, M.J., Craft, J.E., and Mamula, M.J. (2001). From T to B and back again: positive feedback in systemic autoimmune disease. *Nat. Rev. Immunol. 1*, 147–153.

Steen, V.D. (1999). Occupational scleroderma. *Curr. Opin. Rheumatol. 11*, 490–494.

Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. (2003). Tolerogenic dendritic cells. *Annu. Rev. Immunol. 21*, 685–711.

Strassburg, C.P., Vogel, A., and Manns, M.P. (2003). Autoimmunity and hepatitis C. *Autoimmun. Rev. 2*, 322–331.

Sugimoto, K., Morimoto, S., Kaneko, H., Nozawa, K., Tokano, Y., Takasaki, Y., and Hashimoto, H. (2002). Decreased IL-4 producing CD4+ T cells in patients with active systemic lupus erythematosus relation to IL-12R expression. *Autoimmunity 35*, 381–387.

Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukuwa, H., Yoshino, S., Yokioka, M., Tohama, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y., Yamamota, K. (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet. 34*, 395–402.

Swain, S.L. (2003). Regulation of the generation and maintenance of T-cell memory: a direct, default pathway from effectors to memory cells. *Microbes. Infect. 5*, 213–219.

Szabo, S.J., Sullivan, B.M., Peng, S.L., and Glimcher, L.H. (2003). Molecular mechanisms regulating Th1 immune responses. *Annu. Rev. Immunol. 21*, 713–758.

Tang, Q., Henriksen, K.J., Bi, M., Finger, E. B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med. 199*, 1455–1465.

Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., and Steinman, R.M. (2004). CD25+ CD4+ T cells, expanded with dendritic cells presenting a single

autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med. 199*, 1467–1477.

Tarzi, R.M. and Cook, H.T. (2003). Role of Fcgamma receptors in glomerulonephritis. *Nephron Exp. Nephrol. 95*, e7–12.

Tomlinson, I.P. and Bodmer, W.F. (1995). The HLA system and the analysis of multifactorial genetic disease. *Trends Genet. 11*, 493–498.

Tsao, B.P. (2003). The genetics of human systemic lupus erythematosus. *Trends Immunol. 24*, 595–602.

Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., Rainbow, D.B., Hunter, K.M., Smith, A.N., Di Genova, G., Herr, M.H., Dhalman, I., Payne, F., Smyth, D., Lowe, C., Twells, R.C., Howlett, S., Healy, B., Nutland, S., Rance, H.E., Everett, V., Smink, L.J., Lam, A.C., Cordell, H.J., Walker, N.M., Bordin, C., Hulme, J., Motzo, C., Cucca, F., Hess, J.F., Metzker, M.L., Rogers, J., Gregory, S., Allahabadia, A., Nithiyananthan, R., Tuomilehto-Wolf, E., Tuomilehto, J., Bingley, P., Gillespie, K.M., Undlien, D.E., Ronningen, K.S., Guja, C., Ionescu-Tirgoviste, C., Savage, D.A., Maxwell, A.P., Carson, D.J., Patterson, C.C., Franklyn, J.A., Clayton, D.G., Peterson, L.B., Wicker, L.S., Todd, J.A., and Gough, S.C. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature 423*, 506–511.

Vallejo, A.N., Davila, E., Weyand, C.M., and Goronzy, J.J. (2004). Biology of T lymphocytes. *Rheum. Dis. Clin. North Am. 30*, 135–157.

van der Pouw Kraan, T.C., van Gaalen, F.A., Huizinga, T.W., Pieterman, E., Breedveld, F.C., and Verweij, C.L. (2003). Discovery of distinctive gene expression profiles in rheumatoid synovium using cDNA microarray technology: evidence for the existence of multiple pathways of tissue destruction and repair. *Genes Immun. 4*, 187–196.

Varela-Calvino, R. and Peakman, M. (2003). Enteroviruses and type 1 diabetes. *Diabetes Metab. Res. Rev. 19*, 431–441.

Vezys, V. and Lefrancois, L. (2002). Cutting edge: inflammatory signals drive organ-specific autoimmunity to normally cross-tolerizing endogenous antigen. *J. Immunol. 169*, 6677–6680.

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.

Walker, L.S. and Abbas, A.K. (2002). The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat. Rev. Immunol. 2*, 11–19.

Wang, B., Geng, Y.B., and Wang, C.R. (2001). CD1-restricted NK T cells protect nonobese diabetic mice from developing diabetes. *J. Exp. Med. 194*, 313–320.

Weiner, H.L. (2001). Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes. Infect. 3*, 947–954.

Wekerle, H. and Hohlfeld, R. (2003). Molecular mimicry in multiple sclerosis. *N. Engl. J. Med. 349*, 185–186.

Welsh, R.M., Selin, L.K., and Szomolanyi-Tsuda, E. (2004). Immunological memory to viral infections. *Annu. Rev. Immunol. 22*, 711–743.

Werlen, G., Hausmann, B., Naeher, D., and Palmer, E. (2003). Signaling life and death in the thymus: timing is everything. *Science 299*, 1859–1863.

Winchester, R. (2004). The genetics of autoimmune-mediated rheumatic diseases: clinical and biologic implications. *Rheum. Dis. Clin. North Am. 30*, 213–227, viii.

Wong, F.S. and Wen, L. (2003). The study of HLA class II and autoimmune diabetes. *Curr. Mol. Med. 3*, 1–15.

Wooley, P.H. (2004). The usefulness and the limitations of animal models in identifying targets for therapy in arthritis. *Best Pract. Res. Clin. Rheumatol. 18*, 47–58.

Wurtz, O., Bajenoff, M., and Guerder, S. (2004). IL-4-mediated inhibition of IFN-gamma production by CD4+ T cells proceeds by several developmentally regulated mechanisms. *Int. Immunol. 16*, 501–508.

Yan, J. and Mamula, M.J. (2002). Autoreactive T cells revealed in the normal repertoire: escape from negative selection and peripheral tolerance. *J. Immunol. 168*, 3188–3194.

CHAPTER

4

Antigen Presentation, Dendritic Cells, and Autoimmunity

MURIEL MOSER

Laboratoire de Physiologie Animale, Institut de Biologie et Médecine Moléculaires, Université de Bruxelles, Gosselies, Belgium

Dendritic cells (DCs) were identified in the spleens of mice in 1973 and named after their branch-like projections (from δενδρεον, tree) (Steinman and Cohn, 1973). They were subsequently shown to be the most potent stimulators of the primary mixed leukocyte reaction. Their unique capacity to sensitize naïve T cells *in vitro* and *in vivo* was amply documented in the following years.

Although cells with a dendritic structure were visualized in the skin in 1868 by a young medical student, Paul Langerhans, it took more than a century before they were identified as cells from the dendritic family. The relationship between epidermal Langerhans cells and DCs was demonstrated when they were shown to mature into potent immunostimulatory DCs *in vitro*. Schuler and Steinman (1985) showed that Langerhans cells resemble spleen DCs when cultured for 2–3 days, and undergo a progressive increase in stimulatory capacity, becoming 3–10 times more stimulatory than spleen DCs.

The DC family includes many members which reside in most tissues of the body and display unique properties that have not been described for other antigen-presenting cells (APCs): 1) dendritic morphology on activation; 2) motility; 3) specialization of function over time—during a phenomenon called maturation, they shift from an antigen-capturing mode to a T-cell sensitizing mode; 4) elevated expression of major histocompatibility complex (MHC) molecules and intermediate-to-high expression of costimulatory molecules; and 5) production of cytokines.

DENDRITIC CELLS IN IMMUNITY

Immature DCs are ideally equipped to sensitize T lymphocytes specific for dangerous antigens encountered earlier in the periphery. They act as sentinels for infections in peripheral tissues and then migrate to the lymphoid organs where they transmit information regarding the nature of the pathogens and the lymphoid tissues, and activate lymphocytes specific for those microbial antigens. At the immature stage, DCs exhibit potent endocytic activity: they constitutively macropinocytose extracellular fluids, and express various receptors specific for non-self-antigens. DCs do not present antigen immediately after uptake but express the

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

immunogenic class II MHC–peptide complexes only after maturation. This delay in antigen presentation confers to DCs the capacity to display, in the lymphoid organs, antigens encountered earlier in peripheral tissues (Mellman and Steinman, 2001). Interestingly, DCs display some "fidelity" to the antigen encountered in inflammatory conditions (inducing their maturation) as they lose their capacity to capture and process antigens during this process.

The potent accessory properties of DCs appear to develop sequentially. In particular, the ability to process antigens and to sensitize naïve T cells develops in sequence during their maturation. This process is associated with upregulation of MHC restriction elements and costimulatory molecules, as well as production of stimulatory cytokines. The maturation of DCs is usually coupled to their movement to the zone where T cells are located in the lymphoid organs. The colocalization of fully competent DCs with lymphocytes is required for the engagement of membrane-bound ligand–receptor pairs required for optimal T-cell priming and is likely to represent the first step in the immune response.

It is noteworthy that the maturation of DCs is often induced by microbial constituents and inflammatory cytokines, thereby favoring the activation of T cells specific for non-self-infectious antigens. In particular, DCs express several receptors, including Toll-like receptors (TLRs), which recognize conserved pathogen-associated molecular patterns. It is interesting that TLRs control activation of the adaptive immune responses by inducing DC maturation (Schnare et al., 2001). However, it is likely that those mature DCs not only express antigens taken up at the site of infection but also self-antigens that are continuously processed and presented in the context of MHC class I and II molecules. This would imply that fully competent DCs may sensitize autoimmune T cells that have escaped negative selection in the thymus. Recent reports suggest that DCs trigger several lifeguard mechanisms that may prevent the onset of autoimmunity in peripheral tissues, including regulation of antigen presentation (DC lifespan), peripheral deletion or anergy of autoreactive T cells, induction/maintenance of regulatory T cells, and a novel mechanism which implies tuning of activation threshold (see below).

REGULATION OF DENDRITIC CELL LIFESPAN

The lifespan of new migrant dendritics in the lymphoid organs is a key issue in the induction of immunity. The imaging of T-cell–DC interactions in lymph nodes (Stoll et al., 2002) revealed that T cells form long-lived associations with a single APC and show evidence of activation only after 36–48 h. In agreement with these observations, the migration of DCs has been shown to strongly impact the magnitude and quality of $CD4^+$ T-cell responses, which are proportional to the number of antigen-carrying DCs that reach the lymph node (Martin-Fontecha et al., 2003). High numbers of DCs would increase the probability of a DC–T-cell encounter, favor a sustained stimulation through presumably monogamous interactions, and reduce competition among T cells.

The outcome of mature DCs in lymph nodes has long remained elusive. Injection of lipopolysaccharide (LPS) has been shown to induce the phenotypic and functional maturation of splenic DCs and their rapid (6 h) migration in the T-cell zone. Twenty-four hours after LPS injection, very few $CD11c^+$ cells remained in the spleen in contact with T-lymphocytes (De Smedt et al., 1996). The selective loss of $CD11c^+$ cells was found to correlate with the presence of apoptotic cells, as assessed by TUNEL staining on spleen sections. Injection of ovalbumin (OVA) peptide in OVA-specific, T-cell receptor (TCR)-transgenic mice strongly delayed the LPS-induced programmed cell death *in situ*, suggesting that T lymphocytes may rescue DCs presenting their own antigen (De Smedt et al., 1998).

The programmed cell death could be an autonomous mechanism or could be triggered by other cell populations. The hypothesis that DCs have discrete stages of life and that mature DCs are programmed to die is supported by the observation that growth-dependent long-term cultures of DCs display three stages of maturation: immature, mature, and apoptotic (Rescigno et al., 1998). Interestingly, a recent report (Hou and Van Parijs, 2004) indicates that mature DCs have a shorter lifespan than immature ones. Thus, TLRs and T-cell costimulatory molecules both triggered a DC survival pathway that was dependent on Bcl-x_L. However, TLR engagement later triggered DC apoptosis through upregulation of the proapoptotic Bcl-2 family member Bim. The ratio of Bim/Bcl-2 expressed in DCs treated with LPS or lipoteichoic acid (LTA) increased between day 1 and 4 in culture, resulting in an inversion of the ratio of antiapoptotic-to-proapoptotic proteins expressed by DCs stimulated through TLRs. The existence of a "molecular timer" set by TLR ligands shows that the apoptosis of mature DCs is an active process. Of note, DCs cultured with LPS ± agonistic anti-CD40 monoclonal antibodies similarly undergo apoptosis, suggesting that the timer is dominant over the expression of antiapoptotic molecule Bcl-x_L increased by T-cell signals. Which (if any) signal(s) from the adaptive immune response may rescue DCs from premature death induced by the innate system remains to be determined (Moser, 2004).

DENDRITIC CELLS IN TOLERANCE

T-cell tolerance to self-antigens is considered to reflect a combination of central and peripheral tolerance. Central tolerance leads to deletion of immature T cells in the thymus and is largely responsible for eliminating autoreactive T cells. However, the limited expression of tissue-specific antigens in the thymus suggests that additional mechanisms

may exist in the periphery that silence autoreactive T cells.

Thymic Dendritic Cells and Negative Selection

To evaluate the relative capacities of epithelial or bone marrow-derived cells in inducing apoptosis or positive selection, Brocker et al. (1997) generated a transgenic mouse model in which they targeted gene expression specifically to DCs. They used the CD11c promoter to express MHC class II I-E molecules specifically on DCs of all tissues (these mice will be referred to as B6CD11c-Eα^d). The selective expression of I-E on thymic DCs, but not cortical or medullary epithelial cells, B cells, or macrophages, allowed it to be tested whether thymic DCs *in vivo* would be capable of inducing clonal deletion. The frequency was measured of I-E reactive V$\beta5^+$ and V$\beta11^+$ T cells in C57BL/6 control mice, B6-Eα^d mice (a transgenic line expressing I-Eα^d under the control of a segment of the I-E MHC class II promoter) or B6CD11c-Eα^d mice. In B6-Eα^d mice, 65% of CD4$^+$ V$\beta5^+$ T cells and more than 95% of V$\beta11^+$ CD4$^+$ T cells were deleted. Of note, a similar degree of deletion was observed in the B6 CD11c-E_α^d strain, suggesting that the thymic DCs were the most potent cell type in eliminating self-reactive T cells, and that MHC class II expression on the epithelium or other bone marrow-derived cells was not necessary for complete clonal elimination of I-E-reactive T cells in the CD4$^+$ compartment. In addition, the same group demonstrated that thymic DCs *in vivo* were not able to induce MHC restriction of CD4$^+$ T cells, i.e., positive selection.

It remains to be determined, however, how thymic DCs express autoantigens, including tissue-restricted antigens. Although the transcription factor AIRE has been shown to promote ectopic expression of peripheral tissue-restricted antigens in the thymus, there is evidence that AIRE must be expressed in the stromal cells of the thymus (rather than in hematopoietic cells) in order to control autoimmunity in the periphery (Anderson et al., 2002). An interesting possibility would be that thymic epithelial cells may focus their self-antigen display on peripheral self, whereas DCs may be specialized to induce tolerance to hematopoietic self (Derbinski et al., 2001).

Peripheral Tolerance

For 25 years, immunologists have focused their attention on the role of DCs as adjuvant, and it was generally agreed that the principal function of DCs was to initiate T- and B-cell-mediated immunity. However, more recent observations convincingly suggest that DCs may play an active role in peripheral tolerance.

Heath and colleagues studied the outcome of constitutive class-I restricted exogenous presentation of self-antigens *in vivo* (Kurts et al., 1996). They used the rat insulin promoter (RIP)-mOVA transgenic mouse model, where a membrane-bound form of OVA was expressed by pancreatic β cells, kidney proximal tubular cells, the thymus, and the testis of male mice. Transfer of OVA-specific CD8$^+$ T cells led to the activation of these cells in the draining lymph nodes. This was due to class I-restricted cross-presentation of exogenous OVA on a bone marrow-derived APC population. To identify the cross-presenting cell, the authors created transgenic mice where MHC class I expression was driven selectively in DCs, and demonstrated that DCs were sufficient to cross-present self-antigens *in vivo* (Kurts et al., 2001). Interestingly, CD8$^+$ T cells activated by cross-presentation were deleted from the peripheral pool of recirculating lymphocytes. These observations suggested that APCs may be tolerogenic, and provided evidence for an extrathymic mechanism capable of inducing the loss of CD8$^+$ T-cells responding to self-antigens expressed in tissues outside the lymphoid compartment.

Although the nature of the tolerogenic DC population remains elusive, the role of potential candidates has been highlighted, including immature DCs, DCs maturing under noninflammatory conditions, specialized subsets of DCs, and DCs expressing indoleamine 2,3-dioxygenase.

Steady-State Dendritic Cells

A number of observations suggest that steady-state DCs may play a role in peripheral tolerance. First, several studies in mice and humans reported that injection of DCs exposed *ex vivo* to antigen but not to full maturational stimuli induces a state of hyporesponsiveness. In humans, Jonuleit et al. (2000) used immature DCs derived from monocytes cultured in granulocyte-macrophage colony-stimulating factor (GM-CSF) plus interleukin (IL)-4, which were induced to mature by providing a defined cytokine cocktail. As expected, mature DCs induced the expansion of T cells with a polarization towards Th1. In contrast, repetitive stimulation of naïve cord blood-derived T cells with allogeneic immature DCs resulted in the development of a population of non-expanding and IL-10-producing T cells. These regulatory T cells displayed suppressor function, in an antigen-nonspecific manner and independently of CTLA-4, IL-10, and transforming growth factor (TGF)-β. *In vivo*, Dhopdakar et al. (2001) analyzed the immune response induced after injection of immature DCs pulsed with influenza matrix peptide and keyhole limpet hemocyanin in two healthy subjects. A decline in matrix-specific interferon (IFN)-γ-producing T cells was observed, whereas such cells were detected before immunization as expected, because most adults have been exposed to the influenza virus. The decline in IFN-γ production was associated with the development of IL-10-producing cells specific for the same antigen.

Second, studies using inducible expression and presentation of lymphocytic choriomeningitis virus-derived epitopes

by resting or activated DCs *in vivo* confirmed that DCs may induce immunity and tolerance depending solely on their activation status. Probst et al. (2003) have used a Cre/LoxP-based system that allows inducible antigen presentation by DCs *in vivo* to study the immunologic consequences of antigen presentation by resting versus mature DCs without adoptively transferring DCs, and with physiologic numbers of endogenous, naïve responder T cells. The authors found that presentation of lymphocytic choriomeningitis virus (LCMV)-derived cytotoxic T lymphocyte (CTL) epitopes by steady-state DCs resulted in antigen-specific tolerance, whereas antigen presentation by activated DCs led to priming of endogenous CTL with expansion and development of protective effector function.

Third, antibodies directed against DEC-205 have been used to target an antigen on immature DCs. DEC-205 is an endocytic receptor highly expressed by DCs and which carries antigens into intracellular antigen-processing compartments. Early studies have shown that targeting hen-egg lysozyme (HEL) and OVA antigens to steady-state DCs *in vivo* (peptides were engineered into the heavy chain of the anti-DEC-205 antibody) resulted in tolerance (Hawiger et al., 2001). Injection of engineered anti-DEC-205 resulted in transient activation followed by a severe reduction in the number of antigen-specific T cells within 7 days, and the residual T cells became unresponsive to challenge with OVA in complete Freund adjuvant. By contrast, coinjection of the DC-targeted antigen and anti-CD40-activating antibody led to prolonged activation and immunity. Mahnke et al. (2003) subsequently reported that OVA protein coupled to anti-DEC antibodies and injected into mice led to antigen presentation restricted to CD11c cells and initial expansion of antigen-specific T cells. However, these cells were subsequently anergized and expressed CD25 and CTLA-4 antigen, the phenotypic hallmark of regulatory T cells. Functional analysis of this cell population showed that $CD25^+$ T cells could suppress proliferation and IL-2 production of conventional T lymphocytes in a cell–cell contact-dependent way.

Targeting DCs in the steady-state (at least through DEC-205) can induce peripheral tolerance not only for $CD4^+$ but also for $CD8^+$ T cells. Bonifaz et al. (2002) reported that anti-DEC-205 chemically coupled to full-length OVA delivered the protein selectively to DCs *in vivo* and mediated presentation of protein antigen via the exogenous but TAP-dependent MHC I pathway. Subcutaneous injection of anti-DEC-205:OVA induced four to seven cycles of division of transferred TCR transgenic $CD8^+$ cells, but the cells were then deleted, leading to a state of antigen-specific tolerance.

Using a similar antigen-delivery system for an autoantigen, Hawiger et al. (2004) described a novel mechanism whereby DCs induce unresponsiveness associated with dynamic inhibition of secondary rather than primary responses. Targeting a peptide of myelin oligodendrocyte glycoprotein (MOG) through anti-DEC antibodies (engineered to express MOG) induced profound T-cell tolerance to MOG and prevented induction of experimental autoimmune encephalomyelitis (EAE). Of note, a novel mechanism of tolerance has been shown to account for the lack of responsiveness: indeed, the authors found that T cells tolerized by steady-state DCs remained highly responsive to TCR stimulation *in vitro* but failed to respond to antigen *in vivo*. Also of note, expression of CD5 was found to be enhanced on antigen-specific peripheral T cells in these mice and the tolerance appeared to be CD5 dependent. CD5 is a negative regulator of TCR signaling, which directly influences the threshold of activation of thymocytes, suggesting that tolerance may result from a "tuning" of signaling thresholds in the periphery. Therefore, autoimmune T cells may lose their reactivity following exposure to persistent self-antigens. $CD5^{hi}$ T cells did not inhibit activation of naïve T cells to the same antigen *in vivo* and could not regulate naïve cells in cotransfer experiments.

The physiologic role of immature DCs in peripheral tolerance *in vivo* (through T-cell anergy or differentiation of regulatory T cells) is challenged by the general acceptance that DC migration to lymphoid organs is necessarily coupled to their maturation. The chemokine receptors CCR6 and CCR7 are inversely regulated during maturation, and CCR7 has been shown to direct maturing DCs to the T-cell zone of lymphoid follicles which expresses MIP3β (Dieu-Nosjean et al., 2000). However, there is increased evidence that DCs capture and transport tissue-specific self-antigens even in the steady-state. Veiled cells transport tissue-specific antigens to the lymph nodes (Bujdoso et al., 1989), migrating Langerhans cells contain melanosomes (Mishima, 1966), and DCs of the gastric lymph nodes constitutively present parietal cell-restricted H^+/K^+-ATPase in healthy mice (Scheinecker et al., 2002). Huang et al. (2000) have identified a DC subset that constitutively transports apoptotic intestinal epithelial cell remnants to T-cell areas of mesenteric lymph nodes *in vivo*. These cells are weak APCs and contain cytoplasmic apoptotic DNA and epithelial cell-restricted cytokeratins. In the lung, Vermaelen et al. (2001) reported a steady-state flux of DCs that transport macromolecules from the airways to the thoracic lymph nodes. Collectively, these observations suggest that immature DCs are good candidates as tolerogenic DCs capable of inducing peripheral T-cell unresponsiveness.

(Semi)-Mature Dendritic Cells

Although the tolerogenic potential of DCs was first proposed to correlate with their immature state, several reports have described populations of (semi)-mature DCs that induce tolerance *in vivo*. Menges et al. (2002) showed that

repetitive injections of semi-mature DCs, pulsed with an autoantigenic MOG peptide, could induce protection from EAE in mice. Maturation by TNF-α induced high levels of MHC class II and costimulatory molecules on DCs, which remained weak producers of proinflammatory cytokines. The protection was antigen specific and partially dependent on IL-10. Of note, immature DCs (generated in the presence of IL-10) did not influence the score of the disease under the same conditions. Similarly, mature pulmonary DCs induce the development of regulatory T cells in the bronchial lymph nodes of mice exposed to respiratory allergen. This process required T-cell costimulation via the inducible costimulator (ICOS)-ligand pathway and regulatory T cells produced IL-10 and blocked the development of allergen-induced airway hyperreactivity. Similarly, McGuirk et al. (2002) reported that the filamentous hemagglutinin (FHA) of *Bordetella pertussis* modified cytokine production and costimulatory molecules (increased CD86 and moderately enhanced CD40) expression on DCs drove DCs into a phenotype that could selectively enhance the induction of type 1 regulatory T (Tr1) cells *in vitro* (see below). Tr1 cells specific for FHA and pertactin were induced at the mucosal surface in the lungs of mice during acute infection with *Bordetella pertussis* (McGuirk et al., 2002). These Tr1 cells secreted high levels of IL-10 and inhibited protective type 1 helper T (Th1) cell responses against *Bordetella pertussis in vitro* and *in vivo*. Of note, the authors reported that FHA interacted directly with DCs to induce IL-10, and inhibit IL-12 and inflammatory chemokine production.

Lutz and Schuler (2002) have proposed that the semi-mature DCs correspond to the steady-state DCs which constitutively migrate to the lymphoid organs. This developmental stage would be considered as mature according to their surface marker analysis (high expression of MHC II and costimulatory molecules), but immature since they do not release high levels of proinflammatory cytokines. In some cases, the production of IL-10 may participate in their tolerogenic properties. This hypothesis postulates that tissue-resident DCs (immature) and steady-state migrating DCs are different, and that some degree of maturation would be required for mediating homeostatic DC migration. The respective role of immature and partially mature DCs in peripheral tolerance remains unclear and deserves further attention.

Indoleamin 2,3-Dioxygenase-Expressing Dendritic Cells

The role of indoleamin 2,3-dioxygenase (IDO) in the regulation of immune responses has been highlighted by Munn et al. (1998) who demonstrated that IDO expression at the maternal–fetal interface was necessary to prevent immunologic rejection. Thus, treatment of pregnant mice with a pharmacologic inhibitor of IDO resulted in rapid T-cell-induced rejection of all allogeneic concepti, suggesting that the fetus suppresses maternal T-cell immunity by catabolizing tryptophan.

It was subsequently reported that one mechanism by which APCs may regulate T-cell responses is through the expression of IDO. In particular, a subset of human monocyte-derived DCs has been shown to use IDO to inhibit T-cell proliferation *in vitro* (Munn et al., 2002). Although maturation has no effect on the basal expression of IDO protein, activation of mature DCs with IFN-γ resulted in downregulation of IDO, which could be prevented by the presence of IL-10 during maturation. Of note, a few IDO^+ cells were detected in normal lymphoid tissue, whereas accumulation of IDO^+ cells was found in lymph nodes from patients with melanoma, breast, colon, lung, and pancreatic cancers.

Additional studies showed that mouse $CD4^+CD25^+$ regulatory T cells (see below) could initiate tryptophan catabolism in DCs, through a CTLA-4-dependent mechanism. Long-term survival of pancreatic islet allografts was induced by the soluble fusion protein CTLA4-Ig and depended upon effective tryptophan catabolism (Grohmann et al., 2002). This process required CD80 (B7) expression and cytokine production by the DCs. Various types of APCs, and in particular the $CD8\alpha^+$ fraction in the spleen, have been shown to inhibit the generation of T-cell responses *in vivo* by the expression of IDO, through the generation of specific tryptophan catabolites in the kynurenine metabolic pathway, which act as apoptotic agents in T cells. To study the mechanism of action of IDO, the human IDO gene was inserted into an adenoviral vector and expressed in DCs. Kynurenine, 3-hydroxykynurenine, and 3-hydroxyanthralinic acid, but no other IDO-induced tryptophan metabolites, were found to suppress the T-cell response, the suppressive effects being cumulative (Terness et al., 2002). The cytotoxic action on T cells preferentially affected activated T cells. Of note, in addition to T cells, B cells and NK cells were also killed, whereas DCs were not affected. The activity of IDO appears tightly regulated in DCs that express IDO constitutively: in particular, triggering of functional IDO required ligation of CD80 (B7) molecules on the DCs. The ability to trigger active IDO is confined to the $CD4^+$ T-cell subset through the engagement of CTLA4/B7 molecules (Munn et al., 2004).

Collectively, these observations suggest that localized control of tryptophan catabolism in specific tissue microenvironments may contribute to the induction and maintenance of peripheral tolerance.

A Specialized Dendritic Cell Subset?

A few studies have suggested that tolerogenic DCs may represent a specialized lineage.

$CD8\alpha^+$ Dendritic Cell Subset

Initial studies by Shortman's group demonstrated that splenic $CD8\alpha^-$ DCs induced a vigorous proliferative response in $CD4^+$ T cells, whereas $CD8\alpha^+$ DCs induced a weaker response that was associated with T-cell apoptosis (Suss and Shortman, 1996). This programmed cell death appeared to result from interaction of Fas on T cells with FasL on $CD8\alpha^+$ DCs. This observation was the first evidence for a tolerogenic function of DCs expressing a $CD8\alpha\alpha$ homodimer. This notion was consistent with the reduced phagocytic capacity of $CD8\alpha^+$ DCs, their localization in T-cell zones of lymphoid organs, as well as their expression of high levels of self-peptides–MHC complexes expressed by DCs in the T-cell area. The tolerogenic capacity of $CD8\alpha^+$ DCs was demonstrated *in vivo* using a model of tumor/self-peptide (P815AB) presentation for induction of class I-restricted skin test reactivity. It should be noted that the negative regulatory effect was found to be restricted to P815AB and was not observed with other antigens. More recent studies suggest, however, that the $CD8\alpha^+$ DC subset may produce elevated levels of IDO and display tolerogenic properties (see above).

$CD8\alpha^-CD4^+$ Dendritic Cell Subset

Legge et al. (2002) have shown that an immunoglobulin-chimera carrying the encephalitogenic myelin proteolipid PLP1 peptide was presented to T cells 100-fold better than free PLP1. Of note, aggregation of immunoglobulin–PLP1 facilitated cross-linking of Fcγ receptors on APCs and induced IL-10 by macrophages and DCs without upregulating costimulatory molecules. Aggregated immunoglobulin–PLP1 induced a strong reduction in paralytic severity and promoted full recovery from EAE in mice with ongoing disease. IL-10 production on triggering of the Fcγ receptor was confined to the $CD8\alpha^-CD4^+$ subset, which supported suppression of autoreactive T cells and reversal of EAE in the FcγR-deficient mice (Legge et al., 2002).

$CD11c^{low}$ $CD45Rb^{high}$ Dendritic Cells

A population of DCs with specific expression of CD45RB was identified that induced tolerance and Tr1 cell differentiation *in vivo*. $CD11c^{low}$ $CD45Rb^{high}$ DCs could be generated *in vitro* from bone marrow cells in the presence of IL-10 and were detected in the spleen and lymph nodes of normal mice (Wakkach et al., 2003). This $CD11c^{low}$ $CD45Rb^{high}$ DC subset displayed plasmacytoid morphology and an immature phenotype even after activation, and secreted high levels of IL-10. Transfer of $CD11c^{low}$ $CD45Rb^{high}$ DCs, pulsed with antigen *in vitro*, induced the differentiation of specific type 1-regulatory cells leading to antigen-specific tolerance *in vivo*. A single injection of 3×10^5 OVA peptide-pulsed cells was sufficient to strongly decrease the ability of the treated mouse to mount a T- and B-cell-mediated immune response directed against the whole OVA protein in the presence of different adjuvants.

DENDRITIC CELLS AND REGULATORY T CELLS

In the late 1970s, Gershon and his collaborators provided evidence for the existence of "suppressor cells" (Calkins et al., 1976). The authors showed that spleen cells could be educated *in vitro* to induce thymus-dependent help or suppression of the immune response to sheep red blood cells. After a long eclipse, suppressor cells have taken center stage in studies of immune responses and were renamed regulatory cells.

Essentially, three populations of cells exhibiting suppressive mechanisms have been described: naturally occurring $CD4^+CD25^+$ T cells and induced Tr1 and Th3 cells. The $CD4^+CD25^+$ regulatory T (Treg) cells arise spontaneously during ontogeny, are present in the periphery of normal mice, and are associated with protection from a number of autoimmune processes. They express CD25 and Foxp3 constitutively and suppress immune responses via direct cell–cell interactions, although IL-10 and/or TGF-β seem(s) to be partially involved in suppression *in vivo*. By contrast, Tr1 cells differentiate in the presence of IL-10, produce high levels of IL-10 and TGF-β, and suppress via the production of these immunosuppressive cytokines (Levings et al., 2002). Th3 cells were identified in studies of oral tolerance and produce TGF-β as well as variable amounts of IL-10 and IL-4. IL-4 was shown to be a key factor for the differentiation of Th3 cells, whereas TGF-β seems to mediate the immunosuppressive effect of Th3 cells.

There is some evidence that DCs, at some stage of maturation or restricted to a specialized lineage, may induce peripheral tolerance indirectly, through the induction and/or activation of regulatory T cells.

Dendritic Cells as Inducers of $CD4^+CD25^+$ Regulatory T Cells

Although it was initially believed that these $CD25^+$ Treg cells inhibited immune responses against self-antigens, subsequent reports clearly demonstrated that these cells also suppressed immune responses against microbes and innocuous foreign antigens. During infection by *Leishmania major*, $CD4^+CD25^+$ T cells have been shown to accumulate in the dermis, where they suppress by both IL-10-dependent and -independent mechanisms and so inhibit the ability of effector T cells to eliminate the parasite from the site (Belkaid et al., 2002). Several reports have shown that Treg cells

proliferate in response to immunization with antigen in adjuvant (Walker et al., 2003) or loaded on DCs (Oldenhove et al., 2003;Yamazaki et al., 2003). In particular, development of Th1-type responses was enhanced in mice depleted of $CD25^+$ T cells and immunized by injection of antigen-pulsed mature DCs, suggesting that Treg cells naturally exert a negative feedback effect on Th1 responses.

The essential role of $CD25^+$ Treg cells in the maintenance of peripheral tolerance has been further documented by a few reports showing alterations of $CD4^+CD25^+$ Treg function in patients suffering from autoimmune diseases. Viglietta et al. (2004) reported a significant decrease in the effector function of $CD4^+CD25^{hi}$ T cells from peripheral blood of patients with multiple sclerosis. These cells poorly inhibited responder T-cell proliferation as well as IFN-γ production. Similarly, $CD25^+$ Treg cells were found to be defective in their suppressive capacity in patients suffering from autoimmune polyglandular syndrome type II (Kriegel et al., 2004).

Of note, a recent report demonstrates that $CD4^+CD25^-$ T cells can convert into $CD4^+CD25^+$ T cells *in vivo* (see Chapters 9 and 10). The converted $CD25^+$ T cells exhibit characteristics of the naturally occurring $CD25^+$ T cells, and in particular were anergic *in vitro*, suppressed proliferation of responder cells, and expressed high levels of the transcription factor Foxp3 mRNA (Liang et al., 2005). CD80 (B7) costimulation was found to be an absolute requirement for this conversion, suggesting that DCs, which express CD80 (B7) *in vivo* in the absence of intentional stimulation, may be involved in this phenomenon.

Dendritic Cells as Targets of $CD4^+CD25^+$ Regulatory T Cells

DCs may not only be inducers but also targets of Treg activity. Indeed, one report suggests that $CD4^+CD25^+$ cells could alter the phenotype and function of DCs *in vitro*. Thus, DCs exposed to $CD4^+CD25^+$ cells expressed low levels of MHC and costimulatory molecules, and were less efficient at eliciting IFN-γ secretion by $CD8^+$ cells than were DCs cultured alone (Serra et al., 2003). Mature DCs are refractory to suppressive activity of Treg cells, suggesting that Treg cells may serve to keep DCs in the off-mode in steady-state conditions.

Another mechanism of action of Treg cells involves the regulation of immunosuppressive tryptophan catabolism in DCs, as discussed above.

Dendritic Cells as Inducers of Type 1 Regulatory T Cells

As stated above, several reports have demonstrated that DCs may induce a state of tolerance by triggering the IL-10-dependent development and function of Tr1 cells. Although there is ample evidence that immature or semi-mature DCs may trigger Tr1 cells, this activity has also been reported for mature DCs (see above).

MECHANISMS OF TOLERANCE

Direct and indirect mechanisms may be involved in the induction of peripheral tolerance by DCs. In particular, DCs may induce deletion of autoreactive T cells, through the production of tryptophan metabolites or signaling through CD95. Tolerogenic DCs may also act indirectly through the activation of Treg cells, a mechanism that would be more effective in promoting a memory for tolerance and a long-term, antigen-specific hyporesponsiveness. Although the respective role of DC subclasses and of regulatory T-cell populations remains unclear, we believe that immature and mature DCs act through distinct regulatory T-cell subsets. Steady-state DCs (semi-mature or immature) would present mainly self-antigens and either themselves produce, or induce the production of, IL-10 and thereby drive the differentiation of IL-10-producing Tr1 cells. By contrast, DCs that have matured in an inflammatory/infectious environment would undergo maturation and present self- as well as microbial antigens. These mature DCs would promote the survival/activation of naturally occurring $CD4^+CD25^+$ T cells by expressing costimulatory molecules and inducing IL-2 production. These Treg cells would therefore control the immune responses directed to microbial, self- and environmental antigens presented by mature DCs.

If this is true, the repertoire to self-antigens would be regulated at three checkpoints: 1) by thymic DCs through negative selection; 2) by immature DCs through Tr1; and 3) by mature DCs through activation of $CD4^+CD25^+$. By contrast, the repertoire to foreign antigens would be under the control only of $CD4^+CD25^+$ Treg cells, which would prevent an excessive immune response which can be deleterious to the organism.

A report by Pasare and Medzhitov (2003) suggests, however, that microbial induction of the Toll pathway may block the suppressive effect of $CD4^+CD25^+$ Treg cells, allowing activation of pathogen-specific adaptive immune responses. This would suggest that TLR-mediated recognition of pathogen signatures controls the induction of T-cell responses at two levels: through the induction of costimulation and through the release of suppressive activity by naturally occurring T cells. Production of IL-6 by DCs in response to TLR engagement was found to be critical to overcome the inhibition by Treg cells. Of note, the authors postulate that during chronic infection, blockade of suppression of self-reactive T cells may occur, which would explain the link between infection and some autoimmune diseases.

Kurts and colleagues (Hamilton-Williams et al., 2005) recently addressed the question whether TLR ligands would break tolerance to self-antigens. They mimicked a potent temporary infection by injecting a single large dose of TLR ligands into transgenic RIP-mOVA mice, which express the model antigen OVA in pancreatic islet β cells and in kidney proximal tubules. They showed that administration of TLR ligands resulted in DC activation and increased proliferation of cytokine production by T cells. However, coinjection of TLR ligand and OVA did not break tolerance in the absence of specific help, suggesting that cross-tolerance can prevent autoimmunity even when DCs are activated by microbial products.

DENDRITIC CELLS AND AUTOIMMUNITY

Nonobese Diabetic Mouse Model

Studies of autoimmune diabetes are facilitated by mouse models such as nonobese diabetic (NOD) mice which develop diabetes spontaneously. During the first stage of insulitis in this biphasic disease, the islets are invaded by DCs, macrophages, and B and T cells. This insulitis remains essentially static, and progression to diabetes occurs only at a later stage, in a proportion of mice, when the islet infiltrate converts to a destructive process, resulting in destruction of β cells and eventually loss of insulin and glucose homeostasis (see Chapter 36). Turley et al. (2003) have underscored the role of DCs in this process: they have shown that the stimulus initiating insulitis appears to be a ripple of β-cell death and that APCs that ferry cell debris from the pancreas to the pancreatic lymph nodes is a DC of $CD11c^+CD8\alpha^-$ phenotype.

There is evidence that the predisposition of NOD mice to develop autoimmune disease can be attributed to defects in central and peripheral tolerance mechanisms. Kishimoto and Sprent (2001) presented evidence that NOD mice display a defect in negative selection of thymocytes, an effect associated with upregulation of the antiapoptotic molecule cFLIP. Similarly, Lesage et al. (2002) tracked the fate of high-avidity $CD4^+$ T cells that recognize a self-antigen expressed in pancreatic islet β cells on autoimmune-susceptible or -resistant genetic backgrounds, and showed that these cells escaped clonal deletion in the thymus in NOD mice.

Unexpectedly, it was found that spontaneous diabetes was exacerbated in CD80/CD86-deficient and CD28-deficient NOD mice compared with control animals (Salomon et al., 2000). These data contradicted previous studies showing that antigen-induced models of autoimmunity could be reduced when CD28 costimulation was blocked during the priming with the relevant self-antigen. The CD80/CD86- and CD28-deficient mice were found to present a profound decrease in function of the $CD4^+CD25^+$ Treg cell subset and the transfer of this subset from control NOD animals into CD28-deficient mice delays/prevents diabetes, suggesting that Treg cells control autoimmune diabetes (Salomon et al., 2000).

Regulatory T cells appear to coexist in balance with aggressive effector cells directly in the autoimmune infiltrate during the stage of static insulitis (Herman et al., 2004). Loss of this balance leads to rapid onset of diabetes, suggesting that effector T cells capable of transferring disease are actively suppressed by regulatory T cells during the nondestructive period of static insulitis. These $CD25^+CD69^-$ cells express high levels of IL-10, glucocorticoid-induced TNFR family-related protein (GITR), and inducible costimulator (ICOS). Of note, blockade of costimulatory signals, such as CTLA-4 or ICOS, led to a rapid onset of diabetes and to a change of the ratio of effector-to-regulatory T cells in the pancreatic infiltrate.

Of note, these $CD25^+$ Treg cells essentially expressed the clonotypic TCR BDC2.5 (specific for a diabetogenic $CD4^+$ T-cell clone), implying that TCR specificity for antigens in the target organ may be important for their effectiveness. In agreement with these studies, Tang et al. (2004) reported that small numbers of antigen-specific Treg cells, expanded *in vitro* from autoimmune NOD mice, could reverse diabetes after disease onset. The islet autoantigen-specific BDC2.5 Treg cells were more efficient than polyclonal NOD Treg cells in regulating autoimmune responses *in vivo*. Steinman and colleagues (Tarbell et al., 2004) have shown that DCs from NOD mice could expand $CD4^+CD25^+$ suppressor T cells from transgenic mice specific for a natural pancreatic islet β cell antigen, and that these expanded T cells exhibited increased suppressive activity as compared to nontransgenic cells and suppressed autoimmune disease *in vivo*.

Dendritic Cells in Human Diseases

The role of DCs in the induction of autoimmune diseases in humans is still poorly defined. A few reports suggest that members of the DC family may determine reactivity to self-antigens. Banchereau and colleagues (Jego et al., 2003) have underscored the role of two DC subsets in patients suffering from systemic lupus erythematosus. They have shown that the plasmacytoid DCs release large amounts of IFN-α which induce the maturation of classical DCs. These mature DCs, which have captured autoantigens from apoptotic cells (see Chapter 15), activate autoreactive T and B cells. The same authors have reported a reciprocal regulation between TNF and IFN-α, two cytokines regarded as essential elements of autoimmunity and importantly as regulators of DC function (Palucka et al., 2005). A recent report suggests that DCs may be involved in the early steps of autoimmune uveitis (Howard et al., 2005). The authors showed that two retinal autoantigens, S-antigen and interphotoreceptor retinoid-

binding protein, which are associated with uveitis in humans and rodents (see Chapter 49), can attract immature DCs and lymphocytes expressing CXCR3 and CXCR5.

CONCLUDING REMARKS: REGULATION OF IMMUNITY TO SELF AND NONSELF

DCs express several receptors that enable them to recognize self from nonself. They also display a unique phenotypic and functional maturation process, often associated with their migration. Of note, this specialization of function over time and location helps to focus the immune response on antigens likely to be delivered under inflammatory conditions.

In addition to their immunostimulatory properties, DCs have the capacity to induce T-cell deletion and/or anergy, to trigger expansion of naturally occurring $CD4^+CD25^+$ regulatory T cells, and to activate the development of Tr1.

Collectively, these observations suggest that DCs favor immunity to nonself antigens by orchestrating the positive and negative regulation of immune responses. The mechanisms by which DCs display the opposite functions, i.e., induction of immunity and tolerance, are still unclear and will be an essential focus for future studies.

References

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the AIRE protein. *Science 298*, 1395–1401.

Belkaid, Y., Piccirillo, C.A., Mendez, S., Shevach, E.M., and Sacks, D.L. (2002). $CD4^+CD25^+$ regulatory T cells control *Leishmania major* persistence and immunity. *Nature 420*, 502–507.

Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M.C., and Steinman, R.M. (2002). Efficient targeting of protein antigen to the dendritic cell receptor DEC–205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral $CD8^+$ T cell tolerance. *J. Exp. Med. 196*, 1627–1638.

Brocker, T., Riedinger, M., and Karjalainen, K. (1997). Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes *in vivo*. *J. Exp. Med. 185*, 541–550.

Bujdoso, R., Hopkins, J., Dutia, B.M., Young, P., and McConnell, I. (1989). Characterization of sheep afferent lymph dendritic cells and their role in antigen carriage. *J. Exp. Med. 170*, 1285–1301.

Calkins, C.E., Orbach-Arbouys, S., Stutman, O., and Gershon, R.K. (1976). Cell interactions in the suppression of *in vitro* antibody responses. *J. Exp. Med. 143*, 1421–1428.

De Smedt, T., Pajak, B., Klaus, G.G., Noelle, R.J., Urbain, J., Leo, O., and Moser, M. (1998). Antigen-specific T lymphocytes regulate lipopolysaccharide-induced apoptosis of dendritic cells *in vivo*. *J. Immunol. 161*, 4476–4479.

De Smedt, T., Pajak, B., Muraille, E., Lespagnard, L., Heinen, E., De Baetselier, P., Urbain, J., Leo, O., and Moser, M. (1996). Regulation of dendritic cell numbers and maturation by lipopolysaccharide *in vivo*. *J. Exp. Med. 184*, 1413–1424.

Derbinski, J., Schulte, A., Kyewski, B., and Klein, L. (2001). Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat. Immunol. 2*, 1032–1039.

Dhodapkar, M.V., Steinman, R.M., Krasovsky, J., Munz, C., and Bhardwaj, N. (2001). Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J. Exp. Med. 193*, 233–238.

Dieu-Nosjean, M.C., Massacrier, C., Homey, B., Vanbervliet, B., Pin, J.J., Vicari, A., Lebecque, S., Dezutter-Dambuyant, C., Schmitt, D., Zlotnik, A., and Caux, C. (2000). Macrophage inflammatory protein 3alpha is expressed at inflamed epithelial surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. *J. Exp. Med. 192*, 705–718.

Grohmann, U., Orabona, C., Fallarino, F., Vacca, C., Calcinaro, F., Falorni, A., Candeloro, P., Belladonna, M.L., Bianchi, R., Fioretti, M.C., and Puccetti, P. (2002). CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol. 3*, 1097–1101.

Hamilton-Williams, E.E., Lang, A., Benke, D., Davey, G.M., Wiesmuller, K.H., and Kurts, C. (2005). Cutting edge: TLR ligands are not sufficient to break cross-tolerance to self-antigens. *J. Immunol. 174*, 1159–1163.

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.

Hawiger, D., Masilamani, R.F., Bettelli, E., Kuchroo, V.K., and Nussenzweig, M.C. (2004). Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells *in vivo*. *Immunity 20*, 695–705.

Herman, A.E., Freeman, G.J., Mathis, D., and Benoist, C. (2004). $CD4^+CD25^+$ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. *J. Exp. Med. 199*, 1479–1489.

Hou, W.S., and Van Parijs, L. (2004). A Bcl-2-dependent molecular timer regulates the lifespan and immunogenicity of dendritic cells. *Nat. Immunol. 5*, 583–589.

Howard, O.M., Dong, H.F., Su, S.B., Caspi, R.R., Chen, X., Plotz, P., and Oppenheim, J.J. (2005). Autoantigens signal through chemokine receptors: uveitis antigens induce CXCR3- and CXCR5-expressing lymphocytes and immature dendritic cells to migrate. *Blood 105*, 4207–4214.

Huang, F.P., Platt, N., Wykes, M., Major, J. R., Powell, T.J., Jenkins, C.D., and MacPherson, G.G. (2000). A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J. Exp. Med. 191*, 435–444.

Jego, G., Palucka, A.K., Blanck, J.P., Chalouni, C., Pascual, V., and Banchereau, J. (2003). Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity 19*, 225–234.

Jonuleit, H., Schmitt, E., Schuler, G., Knop, J., and Enk, A.H. (2000). Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med. 192*, 1213–1222.

Kishimoto, H. and Sprent, J. (2001). A defect in central tolerance in NOD mice. *Nat. Immunol. 2*, 1025–1031.

Kriegel, M.A., Lohmann, T., Gabler, C., Blank, N., Kalden, J.R., and Lorenz, H.M. (2004). Defective suppressor function of human $CD4^+$ $CD25^+$ regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med. 199*, 1285–1291.

Kurts, C., Cannarile, M., Klebba, I., and Brocker, T. (2001). Dendritic cells are sufficient to cross-present self-antigens to CD8 T cells *in vivo*. *J. Immunol. 166*, 1439–1442.

Kurts, C., Heath, W.R., Carbone, F.R., Allison, J., Miller, J.F., and Kosaka, H. (1996). Constitutive class I-restricted exogenous presentation of self antigens *in vivo*. *J. Exp. Med. 184*, 923–930.

Legge, K.L., Gregg, R.K., Maldonado-Lopez, R., Li, L., Caprio, J.C., Moser, M., and Zaghouani, H. (2002). On the role of dendritic cells in peripheral T cell tolerance and modulation of autoimmunity. *J. Exp. Med. 196*, 217–227.

Lesage, S., Hartley, S.B., Akkaraju, S., Wilson, J., Townsend, M., and Goodnow, C.C. (2002). Failure to censor forbidden clones of CD4 T cells in autoimmune diabetes. *J. Exp. Med. 196*, 1175–1188.

Levings, M.K., Sangregorio, R., Sartirana, C., Moschin, A.L., Battaglia, M., Orban, P.C., and Roncarolo, M.G. (2002). Human $CD25^+CD4^+$ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J. Exp. Med. 196*, 1335–1346.

Liang, S., Alard, P., Zhao, Y., Parnell, S., Clark, S.L., and Kosiewicz, M.M. (2005). Conversion of $CD4^+CD25^-$ cells into $CD4^+CD25^+$ regulatory T cells *in vivo* requires B7 costimulation, but not the thymus. *J. Exp. Med. 201*, 127.

Lutz, M.B., and Schuler, G. (2002). Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol. 23*, 445–449.

Mahnke, K., Qian, Y., Knop, J., and Enk, A.H. (2003). Induction of $CD4^+/CD25^+$ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood 101*, 4862–4869.

Martin-Fontecha, A., Sebastiani, S., Hopken, U.E., Uguccioni, M., Lipp, M., Lanzavecchia, A., and Sallusto, F. (2003). Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. *J. Exp. Med. 198*, 615–621.

McGuirk, P., McCann, C., and Mills, K.H. (2002). Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. *J. Exp. Med. 195*, 221–231.

Mellman, I. and Steinman, R.M. (2001). Dendritic cells: specialized and regulated antigen processing machines. *Cell 106*, 255–258.

Menges, M., Rossner, S., Voigtlander, C., Schindler, H., Kukutsch, N.A., Bogdan, C., Erb, K., Schuler, G., and Lutz, M.B. (2002). Repetitive injections of dendritic cells matured with tumor necrosis factor alpha induce antigen-specific protection of mice from autoimmunity. *J. Exp. Med.* 195**,** 15–21.

Mishima, Y. (1966). Melanosomes in phagocytic vacuoles in Langerhans cells. Electron microscopy of keratin-stripped human epidermis. *J. Cell. Biol. 30*, 417–423.

Moser, M. (2004). Balancing life and death. *Nat. Immunol. 5*, 559–560.

Munn, D.H., Sharma, M.D., Lee, J. R., Jhaver, K.G., Johnson, T.S., Keskin, D.B., Marshall, B., Chandler, P., Antonia, S.J., Burgess, R., Slingluff, C.L., Jr., and Mellor, A.L. (2002). Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science 297*, 1867–1870.

Munn, D.H., Sharma, M.D., and Mellor, A.L. (2004). Ligation of B7–1/B7–2 by human $CD4^+$ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. *J. Immunol. 172*, 4100–4110.

Munn, D.H., Zhou, M., Attwood, J.T., Bondarev, I., Conway, S.J., Marshall, B., Brown, C., and Mellor, A.L. (1998). Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science 281*, 1191–1193.

Oldenhove, G., de Heusch, M., Urbain-Vansanten, G., Urbain, G., Maliszewski, C., Leo, O., and Moser, M. (2003). $CD4^+$ $CD25^+$ regulatory T cells control T helper cell type 1 responses to foreign antigens induced by mature dendritic cells *in vivo*. *J. Exp. Med. 198*, 259–262.

Palucka, A.K., Blanck, J.P., Bennett, L., Pascual, V., and Banchereau, J. (2005). Cross-regulation of TNF and IFN-alpha in autoimmune diseases. *Proc Natl Acad Sci USA 102*, 3372–3377.

Pasare, C. and Medzhitov, R. (2003). Toll pathway-dependent blockade of $CD4^+CD25^+$ T cell-mediated suppression by dendritic cells. *Science 299*, 1033–1036.

Probst, H.C., Lagnel, J., Kollias, G., and van den Broek, M. (2003). Inducible transgenic mice reveal resting dendritic cells as potent inducers of $CD8^+$ T cell tolerance. *Immunity 18*, 713–720.

Rescigno, M., Martino, M., Sutherland, C.L., Gold, M.R., and Ricciardi-Castagnoli, P. (1998). Dendritic cell survival and maturation are regulated by different signaling pathways. *J. Exp. Med. 188*, 2175–2180.

Salomon, B., Lenschow, D.J., Rhee, L., Ashourian, N., Singh, B., Sharpe, A., and Bluestone, J.A. (2000). B7/CD28 costimulation is essential for the homeostasis of the $CD4^+CD25^+$ immunoregulatory T cells that control autoimmune diabetes. *Immunity 12*, 431–440.

Scheinecker, C., McHugh, R., Shevach, E.M., and Germain, R.N. (2002). Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J. Exp. Med. 196*, 1079–1090.

Schnare, M., Barton, G.M., Holt, A.C., Takeda, K., Akira, S., and Medzhitov, R. (2001). Toll-like receptors control activation of adaptive immune responses. *Nat. Immunol. 2*, 947–950.

Schuler, G. and Steinman, R.M. (1985). Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells *in vitro*. *J. Exp. Med. 161*, 526–546.

Serra, P., Amrani, A., Yamanouchi, J., Han, B., Thiessen, S., Utsugi, T., Verdaguer, J., and Santamaria, P. (2003). CD40 ligation releases immature dendritic cells from the control of regulatory $CD4^+CD25^+$ T cells. *Immunity 19*, 877–889.

Steinman, R.M. and Cohn, Z.A. (1973). Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 137, 1142–1162.

Stoll, S., Delon, J., Brotz, T.M., and Germain, R.N. (2002). Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science 296*, 1873–1876.

Suss, G. and Shortman, K. (1996). A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. *J. Exp. Med. 183*, 1789–1796.

Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). *in vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med. 199*, 1455.

Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., and Steinman, R.M. (2004). $CD25^+$ $CD4^+$ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med. 199*, 1467–1477.

Terness, P., Bauer, T.M., Rose, L., Dufter, C., Watzlik, A., Simon, H., and Opelz, G. (2002). Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J. Exp. Med. 196*, 447–457.

Turley, S., Poirot, L., Hattori, M., Benoist, C., and Mathis, D. (2003). Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. *J. Exp. Med. 198*, 1527–1537.

Vermaelen, K.Y., Carro-Muino, I., Lambrecht, B.N., and Pauwels, R.A. (2001). Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J. Exp. Med. 193*, 51–60.

Viglietta, V., Baecher-Allan, C., Weiner, H.L., and Hafler, D.A. (2004). Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med. 199*, 971–979.

Wakkach, A., Fournier, N., Brun, V., Breittmayer, J.P., Cottrez, F., and Groux, H. (2003). Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation *in vivo*. *Immunity 18*, 605–617.

Walker, L.S., Chodos, A., Eggena, M., Dooms, H., and Abbas, A.K. (2003). Antigen-dependent proliferation of $CD4^+$ $CD25^+$ regulatory T cells *in vivo*. *J. Exp. Med. 198*, 249–258.

Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K., and Steinman, R. M. (2003). Direct expansion of functional $CD25^+$ $CD4^+$ regulatory T cells by antigen-processing dendritic cells. *J. Exp. Med. 198*, 235–247.

CHAPTER

5

Major Histocompatibility Complex and Autoimmunity

MINDI R. WALKER AND GERALD T. NEPOM

Benaroya Research Institute at Virginia Mason, Seattle, Washington, USA

Discrimination between self- and nonself-recognition is a fundamental role of the adaptive immune system, and is accomplished through mechanisms linked to the major histocompatibility complex (MHC), known in humans as the human leukocyte antigen (HLA) genes. Immune responses to pathogens take many forms, broadly categorized as including innate pattern recognition and adaptive antigen recognition. In the latter mechanism, T and B lymphocytes utilize different strategies: B-cell recognition is mediated by immunoglobulins, which bind conformational epitopes exposed on pathogen-associated molecules, while the T-cell response pathogens are not recognized directly, but are first processed into peptide antigens and then presented to T cells by specialized antigen-presenting cells (APCs). This antigen-presentation pathway generates thousands of peptide fragments from pathogen targets and displays these peptide fragments bound to MHC molecules, offering them to T cells for recognition by antigen-specific receptors.

This MHC-associated immune recognition mechanism is unfortunately error prone. In fact, not only are pathogen-associated peptide fragments presented by MHC molecules, but most of the time the same MHC molecules are occupied by presentation of self-peptides, products of normal cellular function and degradation. Thus, when not directing immunity to pathogens, MHC molecules on APCs are engaged in an active form of self-antigen expression, which appears to play a key role in homeostatic mechanisms, such as maintenance of pools of circulating memory cells capable of rapid recall immune responses and of fairly fixed numbers of circulating lymphocytes under normal, healthy conditions.

These two diverse functions—protection from pathogens and maintenance of homeostasis—are accomplished by closely related immune recognition pathways, using many of the same MHC genes and molecules. The evolution of the vertebrate immune system has been an ongoing effort to strike a balance between these functions, which poses a fundamental issue in our understanding of the MHC in autoimmunity: e.g., genetic mechanisms for the expansion and diversification of the T-cell receptor (TCR) repertoire allow for extremely broad potential reactivity. This is advantageous in the context of immune responses to rapidly changing infectious pathogens, but is potentially harmful in the context of recognizing molecules associated with normal tissues. Similarly, potent regulatory mechanisms are necessary to control T-cell expansion and inappropriate activation, but these regulatory mechanisms run the risk of being utilized during pathogen responses, with deleterious outcome.

Thus, the need for sophisticated sensing mechanisms and regulatory mechanisms is paramount, and a large number of immunologically-related genes are devoted to this task. In this chapter we will discuss these genes, and the functional consequences of structural diversity within the MHC gene products that participate in this pathway.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

MAJOR HISTOCOMPATIBILITY COMPLEX DIVERSITY

The principle function of MHC molecules is to bind and present peptides to the antigen-specific TCR on T lymphocytes, and to sets of receptors on natural killer T (NKT) cells which also control activation and inhibition of this effector lymphocyte population. There are two types of MHC molecules responsible for presentation of antigen to T cells; MHC class I and class II, which direct antigen recognition to the $CD8^+$ and $CD4^+$ subsets of T lymphocytes, respectively. An illustration of the evolutionary pressures to diversify the immune system comes from a comparison of T-cell and NKT-cell recognition of MHC molecules. Several pathogens invoke molecular mechanisms to inactivate MHC class I molecules, apparently to aid escape from immune attack by T cells. NKT receptors also recognize MHC class I molecules, but by utilizing inhibitory receptors for this recognition provide an immune response to target cells which lack MHC expression, thereby countering this pathogen escape strategy.

The genes for MHC molecules in humans are located on chromosome 6 in the HLA region, and are known as HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G. When complexed with another molecule, β2 microglobulin, products of these genes form the MHC class I molecules. The MHC class II gene is divided into three loci, HLA-DR, HLA-DQ, and HLA-DP, and each locus contains a class II α gene and one or more β genes, which together encode the class II αβ heterodimer molecules. The most important functional property of the MHC genes is an extremely high degree of polymorphism in the human population. Hundreds of alleles exist, and there is tremendous variation both within and between different ethnic backgrounds.

The MHC class I and class II genes are the most polymorphic genes in the human genome. Within the MHC class II gene most of the allelic diversity is localized to the second exon. In the MHC class I gene, the sequence diversity is located within exon 2 or 3. These regions of each gene are responsible for coding the peptide-binding grove for each molecule. The allelic diversity at these regions is generated by recombinational events, such as gene conversions or reciprocal recombination (Erlich and Gyllensten, 1991), and by numerous point mutations which cluster within the peptide-binding regions.

Other genes important for immune system function are also found within the MHC. Located between the HLA-DR region (class II) and the HLA-B locus (class I), the class III region contains genes that encode members of the complement family, C2 and C4, members of the tumor necrosis factor (TNF) family (TNF-α and -β), the MHC class I-associated chain A (MICA) and MICB loci, and several others. Examples linking these genes to autoimmunity include deficiencies in C4 which are associated with systemic lupus erythematosus, and overproduction of TNF-α, which has been associated with the development of several autoimmune diseases. Elsewhere in the MHC, the transporter associated with antigen-processing (TAP) genes encode transporter proteins which facilitate the loading of peptides into newly synthesized MHC class I molecules, and the nearby LMP2 and LMP7 genes, which encode interferon (IFN)-γ-inducible subunits of the proteosome complex. HLA-E and HLA-G are MHC class I loci, with a more limited tissue distribution and polymorphism than the HLA-A, -B, and -C genes, and are important in NKT-cell recognition.

EVOLUTION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

Evidence from allelic frequency distribution, patterns of nucleotide substitution in coding exons, phylogenetic analysis of sequences, and differences in the evolutionary pattern between exons and introns of MHC genes all suggest that natural selection is responsible for generating and maintaining MHC polymorphism. In addition to the large number of alleles present in the MHC, MHC genes have several features that distinguish selection from random mutation, including the presence of rare alleles found at similar frequencies in different populations, and the distribution of regions of variation closely tied to the functions exhibited by those regions. Hughes and Nei (1988) noted that if MHC molecules were undergoing selection, then the selection or amino-acid altering substitutions would be greatest in the peptide-binding region. This is indeed the case and these substitutions preferentially lead to coding changes, as opposed to changes that are silent, and merely reflect a higher mutation rate in this area. The genetic variation also results in frequent heterozygosity at the sites involved in peptide presentation, providing a link between functional diversification and population variation.

Pathogens may be the major source of selective pressure to drive diversification of MHC alleles. The way pathogens drive selection of MHC diversity has been explained in three major hypotheses. In the first, individuals with heterozygous MHC alleles would have an advantage over those with homozygous MHC alleles because they would have the ability to present a wider range of peptides. The range of peptides an individual could present would depend on the degree of overlap between the ranges of peptides the two MHC alleles could present. This method of selection would therefore favor individuals with the greatest divergent range of peptide-binding specificities. This hypothesis is consistent with observations that MHC class I homozygous autoimmune deficiency syndrome (AIDS) patients have a faster progression to late-stage AIDS (Carrington et al., 1999; Tang et al., 1999), and MHC class II heterozygosity

has been advantageous for hepatitis B virus infection (Thursz et al., 1997).

The second hypothesis is based on a presumption of rare allele advantage. In this hypothesis individuals with rare alleles would have an advantage over those with common alleles because of the coevolution of pathogen diversity to target common alleles. An example comes from studies of malaria, associated with an increase in the frequency of HLA-B*53 in the Gambian population (Hill et al., 1991).

In the third hypothesis, HLA variation would fluctuate with pathogen variation. As pathogens change, MHC alleles would change in response, albeit at a much slower rate. This model differs from the second one in that allelic differences change with the frequency of the pathogen, whereas in the second model the frequency of the allele dictates how well a given individual will respond to a particular pathogen.

MAJOR HISTOCOMPATIBILITY–PEPTIDE INTERACTIONS

The specificity of the MHC–peptide interaction relies on structural complementarity between the antigenic peptides and the binding groove of the MHC molecule. The binding groove is bordered by α-helical loops that form a canyon, inside which binding pockets are formed by different combinations of amino acids, which determine the types of peptides able to bind. These pocket variations in amino acid sequence in MHC molecules are due to allelic variations in the MHC gene. Subtle differences in the peptide-binding groove correspond to varying abilities of different peptides to bind different MHC molecules, and in turn result in the differences in ability of individuals to respond to different peptides. Atoms of the bound peptide backbone form hydrogen bonds with the nonpolymorphic MHC residues, while bound peptide side chains interact with the side chains of the polymorphic MHC.

MHC molecular interactions with both peptide main chain and side chains are critical for maintaining the structure and stability of the MHC–peptide complex. Thus, the overall strength of binding is also determined directly by the nature of specific HLA genetic variation. As discussed below, the strength of binding controls the duration of signals transduced between the MHC–peptide complex and the antigen-specific TCR, a variable with important ramifications for T-cell selection and autoimmunity. In some cases, multiple binding registers may occur within the peptide-binding groove, which would greatly increase the opportunities to recognize specific antigens, but which could possibly also contribute to the masking of autoantigens during thymic selection (Goverman, 1999; He et al., 2002).

MHC class I molecules are loaded when proteins within the cytosol are degraded into small peptides by a proteosome. The proteosome is a multifunctional protease with several catalytic subunits. These peptides are then transported into the endoplasmic reticulum by the TAP and are then loaded into the MHC class I molecules with the help of chaperone proteins and translocated to the cell surface. On the other hand, MHC class II molecules are typically used to present extracellular peptides to $CD4^+$ helper T (Th) cells. First, extracellular proteins are endocytosed by the APC. At the same time, newly synthesized MHC class II molecules are being exported through the Golgi. Newly synthesized MHC class II molecules bind the invariant chain within the endoplasmic reticulum. The invariant chain serves two purposes: to prevent binding of endogenous peptides within the endoplasmic reticulum lumen and to direct the export of MHC molecules to endosomes. Within the endosome, proteolytic processing of the endocytosed peptide and invariant chain occurs. Proteolytic processing of the invariant chain leaves a small peptide, class II-associated invariant chain peptide (CLIP), within the MHC class II peptide groove. Dissociation of CLIP is facilitated by specialized MHC molecules, DO and DM. Removal of the CLIP peptide allows loading of the extracellular peptide into the peptide-binding groove of the MHC class II molecule. The MHC molecule is then exported to the plasma membrane where it presents the bound peptide to TCR molecules on $CD4^+$ Th cells.

T-Cell Selection

T cells develop and mature during the process of thymic selection. During this process, self-peptides within the thymus are presented on MHC molecules to T-cells. The interaction between TCR and MHC–peptide complexes involves intermolecular, and therefore intercellular, contacts. The specificity of the TCR binding is controlled by both peptide contacts and MHC contacts. Both the peptide and the MHC contribute to the overall avidity of the trimolecular interaction and, therefore, to activation of the T cell. The MHC therefore controls not only the interaction between peptide and MHC, but also the interaction between TCR and MHC. This MHC-controlled TCR recognition is termed MHC restriction and is a key checkpoint in the genetic control of a T-cell immune response. HLA genetic polymorphism therefore controls interaction with the T cell and T-cell activation through three structural mechanisms: directly through binding the antigenic peptide, directly through binding the TCR, and indirectly by altering the conformation of the bound peptide and influencing the interaction between peptide and TCR.

Positive selection of thymocytes is mediated in the thymic cortex by epithelial cells presenting antigen to immature T cells, whereas negative selection occurs predominantly in the medulla and is mediated by medullary epithelial APCs and dendritic cells (DCs). T cells that do not

recognize peptides within the thymus die from neglect. This rids the T-cell repertoire of nonreactive T cells, while T cells that recognize self-peptides during negative selection as agonists or with high affinity are also deleted from the T-cell repertoire. This form of central tolerance is a major physiologic barrier that should delete T cells that are highly reactive to self-antigens. However, this deletion mechanism is inefficient, and T cells that are autoreactive often escape negative selection and can be found in the periphery of normal healthy individuals. When these cells are unregulated and become activated, autoimmune diseases are a likely outcome. Figure 5.1 illustrates this progression: autoantigen-specific $CD4^+$ T cells are not detected in assays utilizing unfractionated peripheral blood from normal individuals; however, removal of regulatory cells unmasks the autoreactive potential, and self-responsive $CD4^+$ T cells are now evident. These cells are very similar in phenotype and TCR repertoire to activated autoreactive T cells, which are frequently found in the peripheral blood of patients with active autoimmune disease.

There are several maturation phases wherein mistakes in T-cell selection may lead to autoimmunity. This is most clearly evidenced by the fact that animals undergoing thymectomy between day 2 and 5 of life succumb to severe autoimmunity (Taguchi and Nishizuka, 1980; Sakaguchi et al., 1994). This autoimmunity can be directed against several organs and is characterized by the infiltration of T cells into the affected organs and organ-specific antibodies in the serum (Taguchi et al., 1980). Thymectomy-induced autoimmunity is typically explained by a lack of regulatory T cells (Tregs) because selection of these cells is delayed until the second week of life (Sakaguchi et al., 1994). Similarly, animals that undergo irradiation along with cyclosporine A (CsA) treatment have disrupted T-cell selection and unbalanced Th1 versus Th2 responses in the periphery, which lead to autoimmunity. This autoimmunity is also believed to be caused by a defect in negative selection resulting in an increase in the output of autoreactive cells (Sorokin et al., 1986; Beijleveld et al., 1995; Damoiseaux et al., 1997). Mice deficient in Zap-70, a signaling molecule that receives signals from the TCR, also have defects in both positive and negative selection (Negishi et al., 1995; Sakaguchi et al., 2003).These mice have autoreactive T cells in the periphery which mediate a syndrome resembling rheumatoid arthritis.

It has become clear over the past several years that thymic stromal cells, medullary epithelial cells in particular, express several peripheral tissue-specific antigens, which are important in negative selection (Kyewski et al., 2002). Expression of these tissue-specific antigens is controlled by the autoimmune regulatory (AIRE) gene. Mutations in the AIRE gene have recently been shown to result in the multiorgan autoimmune disease, autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) (Nagamine et al., 1997), which is also known as autoimmune polyglandular syndrome-type 1 (APS-1). AIRE knockout mice also have lymphocytic infiltrates and autoantibodies against several peripheral organs and tissues (Anderson et al., 2002; Ramsey et al., 2002). Studies in nonobese diabetic (NOD) mice suggest that the level of expression of tissue-specific antigens within the thymus and thus clonal deletion controls the response to these antigens in the periphery (Kishimoto and Sprent, 2001; Chentoufi and Polychronakos, 2002; Dubois-Lafforgue et al., 2002; Lesage et al., 2002; Moriyama et al., 2003; Thebault-Baumont et al., 2003; Villunger et al., 2003).

Mistakes in T-cell selection also occur when the number of peptides presented in the thymus is reduced or is not controlled. For example, mice that lack the MHC class II invariant chain have substantial loss of MHC class II expression and $CD4^+$ T-cell selection (Bikoff et al., 1993; Viville et al., 1993; Elliott et al., 1994). In addition, $DM^{-/-}$ mice have about one-quarter the number of normal circulating T cells, and the majority of the APCs within these mice present CLIP because of the lack in DM-mediated catalysis of the release

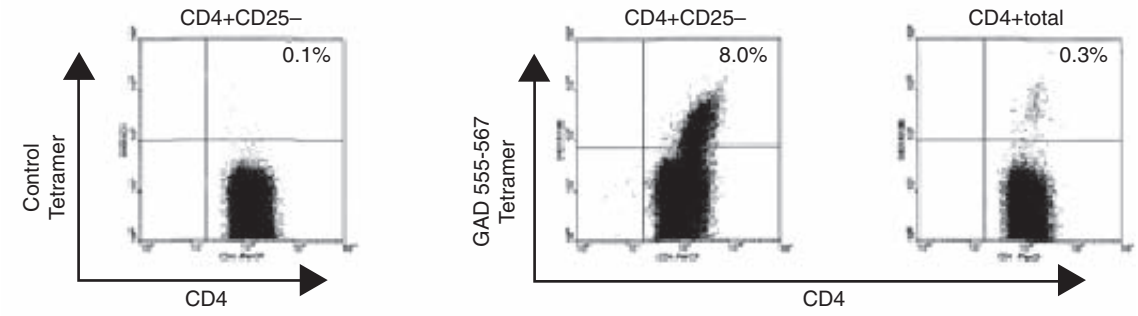


FIGURE 5.1 Autoreactive T cells are present in human peripheral blood, under regulatory control. $CD4^+$ T cells specific for GAD65, an autoantigen associated with autoimmune diabetes, are visualized by fluorescent flow cytometry using MHC tetramers. Removal of regulatory T cells (middle panel) unmasks a robust proliferative response which results in an expansion of tetramer-positive cells. The left panel shows the same cell population analyzed using an unrelated control tetramer. Adapted from Danke et al. (2004), with permission.

of this peptide (Fung-Leung et al., 1996; Martin et al., 1996; Miyazaki et al., 1996; Grubin et al., 1997; Surh et al., 1997; Tourne et al., 1997). A large percentage of T cells in these mice respond to syngeneic APCs from normal mice, a characteristic of unregulated autoreactivity. Similar observations have been made in I-A^bE_α (aa52–68) mice in which the class II I-E_α (52–68aa) peptide is covalently linked to the β chain of the I-A^b heterodimers; APCs in these animals display I-A^b where nearly all molecules contain the I-E_α (52–68) peptide (Ignatowicz et al., 1995; Fukui et al., 1997). Autoimmunity in these mice may therefore be due to the low number of self-peptides being presented within the thymus and thus a lack of negative selection.

Differences in MHC alleles may contribute to autoimmunity during thymic selection either by presenting autoantigens for positive selection or by inefficiently performing negative selection, such as by limiting the amount of self-peptides presented. T cells that undergo positive thymic selection normally react to self-peptides presented in the thymus with weak agonistic or low-affinity responses. However, there are methods to escape negative selection if T cells are too highly reactive to self; e.g., T cells have the ability to permanently downregulate the coreceptors CD4 and CD8, or T cells may mature under the influence of activation blockade or Tregs as a way of escaping negative selection. In addition, many human T cells express multiple TCR α chains, and it is conceivable that T cells selected for one specificity may acquire other specificities after maturation. Examples where certain MHC alleles contribute to susceptibility due to binding of autoantigens with poor affinity to that particular MHC, in which a lack of negative selection of the autoimmune T cells within the thymus leads to autoimmunity, are found in several animal models (Fairchild et al., 1993; Liu et al., 1995; Carrasco-Marin et al., 1996; Ridgway et al., 1998).

T-Cell Activation

The functional outcome of the TCR–MHC–peptide interaction is complex and variable. The T-cell response is strongly influenced by lineage commitment, maturation stage, cytokine environment, and the nature of the APCs and costimulators. However, a key determinant in this interaction is the TCR–MHC–peptide signal itself. Variation in the strength of signal mediated through this interaction leads to alteration of T-cell responses, both naïve and memory responses. Essentially, variation in the strength of signal through the TCR–MHC–peptide complex changes the threshold for activation responses and modulates the commitment to specific response pathways. At the molecular level, the interface between MHC–peptide complexes and the TCR occupies an approximately 10×20 Å interface with 40 or more specific intermolecular contacts (Figure 5.2). Seemingly minor variations within this interface can lead to a profound difference in functional outcome. For example, in the HLA-A2-tax system, four different crystal structures derive from single amino acid substitutions at the TCR–peptide contacts, which lead to very subtle shifts in the three-dimensional complex but correspond to a range of functions from agonist to antagonist (Ding et al., 1999).

While the affinity of an individual TCR–MHC–peptide interaction is quite low, the overall avidity of the interaction is greatly enhanced during the intercellular interaction between T cell and APC by the clustering and multimerization

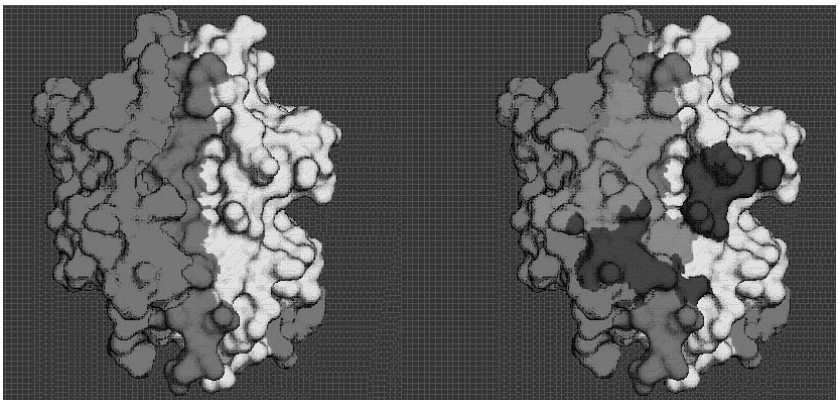


FIGURE 5.2 Trimolecular interaction between MHC, peptide, and T-cell receptor (TCR). The exposed molecular surface of an MHC class II molecule, containing a bound antigen peptide, is shown in the left panel. The MHC α chain is yellow, the β chain is green, and the peptide is shown in pink. Amino acid side chains on the underlying side of the peptide lie buried in the MHC groove, anchoring the binding interaction. This exposed surface forms the contact structure for TCR recognition; the image on the right is colored to show examples of the areas which contact the TCR, with the TCR α chain contacts shown in dark blue and the TCR β chain in light blue. Both peptide and MHC residues are involved in TCR recognition, and both contribute to the overall avidity of the interaction. See color plate section.

Adapted from Buckner and Nepom (2005), with permission.

of the complex due to multiple site interactions. Strength of signal is a function of the density and duration of these complexes at the cellular interface, in combination with accessory molecules and membrane structure effects. As summarized by van der Merwe (2001), there is no evidence for change in structural interface of TCR–MHC–peptide interactions, which correlate with the functional outcome of binding, and many data consistent with the hypothesis that signal duration or strength is the key element.

While there is still considerable uncertainty and controversy about the mechanisms by which signal duration determines outcome [i.e., the serial triggering model (Lanzavecchia and Sallusto, 2001) or oligomerization in lipid rafts acting as signal amplifiers (Drake and Braciale, 2001; Viola, 2001) or time-dependent assembly of tyrosine kinase-dependent protein interactions (Pacini et al., 2000)], regardless of mechanism, the outcome of T-cell stimulation is strongly dependent on the strength of signal. T-cell polarization (Iezzi et al., 1999), anergy induction (Appel et al., 2001), and commitment to memory or regulatory responses (Lanzavecchia and Sallusto, 2000) all differ in their "weak" and "strong" TCR–MHC peptide interactions.

Studies using murine models and soluble class I–peptide multimers to analyze CD8 responses have observed a range of functional avidities among cells with differential activation properties (Derby et al., 2001; Rosette et al., 2001). Functional avidity in such studies depends not only on the affinity of the TCR–peptide–MHC complex, but on the density of these complexes, and their assembly or "packing" on the T-cell surface, potentially as a response to activation (Hesse et al., 2001; Margulies, 2001; Slifka and Whitton, 2001). The importance of these functional avidity differences is crucial not only for determining T-cell response on a single cell basis, but also for inducing major shifts in the population of T cells elicited in a polyclonal response. Within a complex polyclonal response, T cells of different avidity will be selected for differential activation and lineage commitment for effector pathways, with profound functional consequences. For example, selection of high-avidity responses has been suggested to underlie a mechanism for sensing low-density antigen (Fahmy et al., 2001), for determining the hierarchy of peptide epitopes recognized during determinant spreading (Tian et al., 2001), and for eliminating high-avidity autoreactive cells (Anderton et al., 2001).

During the process of thymic selection, T cells that are highly reactive to self-proteins should be deleted from the T-cell repertoire; however, post-translational modifications of proteins can occur that lead to new epitopes, which can now be recognized by positively selected T cells. Evidence of the creation of neoepitopes in disease exists in celiac disease, rheumatoid arthritis, and atherosclerosis. Celiac disease is characterized by autoantibodies to tissue transglutaminase. This enzyme converts glutamine to asparagine within gluten proteins in the intestine to generate neoepitopes that preferentially bind DQ2 class II molecules for antigen presentation (Quarsten et al., 1999). In rheumatoid arthritis, the presence of citrullated proteins is highly predictive of the onset of disease with neoepitopes of matrix proteins, such as vimentin created by conversion of arginine to citrulline (van Boekel et al., 2002). Oxidized low-density lipoproteins (LDLs) are markers for coronary atherosclerosis and may similarly contribute to neoepitopes for immunologic responses in the inflammatory response associated with the progress of coronary artery disease (CAD) (Holvoet et al., 1998a; 1998b).

Other environmental influences also can change the threshold for MHC–peptide–TCR triggering. Differences in pH at the site of inflammation may cause MHC structural changes that may lead to high-affinity interaction (Hausmann et al., 1999), or differences in Toll-like receptor (TLR) molecules may cause maturation of DCs, which will provide an immune stimulatory signal even when self-peptides are presented. In addition, the level of costimulation may be different within the thymus and periphery. CD80 (B7) levels have been shown to be low on thymic cortical epithelial cells, which are the APCs that induce positive selection. Indeed, expression of CD80 (B7) molecules within the pancreas of transgenic animals is sufficient to promote diabetes in the context of susceptible MHC alleles (Wen et al., 2000). Molecular mimicry or cross-reactivity with bacterial and viral antigens have also been shown to both accelerate and protect from autoimmunity (Zhao et al., 1998; Katz-Levy et al., 1999; Brehm et al., 2002; Christen and von Herrath, 2004).

T-Cell Regulation

MHC–peptide interactions regulate T-cell homeostasis. Just as self-peptide–MHC complexes mediate positive selection in the thymus, continual recognition of self also seems to play a key role in the survival of T cells in the periphery (Janeway et al., 1984; Ernst et al., 1999; Freitas and Rocha, 1999; Goldrath and Bevan, 1999). $CD4^+$ T cells quickly die in the periphery of mice that lack MHC class II molecules (Takeda et al., 1996; Brocker, 1997), even when MHC class II molecules are expressed selectively in the thymus (Rooke et al., 1997). This requirement for MHC apparently fosters the maintenance of low-level proliferation in memory T-cell compartments and a similar function for Treg homeostasis may influence autoimmune disease predisposition.

Because T-cell selection in the thymus is an imperfect process, there are several mechanisms in place to help ensure that aberant activation of autoreactive cells that escape negative selection do not proliferate out of control. One mechanism to control autoreactive cells is through Tregs. Table 5.1 summarizes the properties of Tregs and their relationship to autoimmunity. Three types of Tregs

Table 5.1. MHC interactions in regulatory T-cell generation and function

Functional property	Immune compartment	Role in autoimmunity
Selection of Treg	During thymic development	Establishing an avidity threshold for regulatory T–cell maturation
Activation of Treg	During antigen exposure in the periphery	Treg require TCR signals for expression of their regulatory program
Homing of Treg	During an ongoing immune response	Requirement for MHC–antigen interaction directs Treg function to specific sites
Persistence of Treg	Normal physiology	Maintains a homeostatic level of Treg function, probably essential for prevention of autoimmunity
Induction of Treg	Post-thymic development	Provides for generation of Treg in the periphery, may aid in controlling autoimmunity

have been described, $CD4^+CD25^+$ (T_R), type 1 regulatory T (Tr1) cells, and Th3 cells. Studies in mice have shown that $CD4^+$ T_R, which are characterized by the expression of CD25 and Foxp3, are derived from the thymus and exert their suppressive activity in a cell-contact-dependent, cytokine-independent manner (Sakaguchi et al., 1995; Baecher-Allan et al., 2001; Dieckmann et al., 2001; Jonuleit et al., 2001). Tr1 and Th3 cells are thought to be peripherally derived and exert their suppressive activity via IL-10 and transforming growth factor (TGF)-β, respectively (Chen et al., 1994; Groux et al., 1996). Both types of regulatory T cell have to be activated through the TCR in order to be suppressive. The Tregs have recently been categorized based on their source as either "natural" thymically derived cells, such as $CD4^+CD25^+$ T_R, or "adaptive" Tregs, which can be generated in the periphery, including Tr1 and Th3 cells (Bluestone and Abbas, 2003).

The nature of the antigen specificity of Tregs is controversial. Freshly isolated unmodified $CD4^+CD25^+$ T cells have been able to suppress proliferation in assays using peptides to stimulate cells rather than polyclonal anti-CD3 stimulation (Kohm et al., 2002; Taams et al., 2002). T-cell clones that express CD25 and exhibit *in vitro* suppressive activity when given cognate antigen have recently been described (Levings et al., 2002b; Wang et al., 2004). Repeated alloantigen stimulation of $CD4^+CD25^+$ T cells (Jiang et al., 2003) or priming mice with alloantigen (Dai et al., 2004) has also resulted in antigen-specific Tregs. Most notably, two recent reports show that *in vitro* expanded antigen-specific $CD4^+CD25^+$ T cells can suppress the development of diabetes in NOD mice even after injection of diabetogenic T cells (Tang et al., 2004; Tarbell et al., 2004). These two reports also suggest that the antigen-specific regulatory T cells once activated have the ability to control responses to other antigens present at the site of inflammation, which would be particularly useful in therapy for autoimmune diseases where multiple autoantigens are present. Similar human Tregs have been described, generated *in vitro* following TCR activation (Walker et al., 2003; 2005).

Selection of Tregs may be controlled by the affinity of the TCR for a peptide–MHC complex. $CD4^+CD25^+$ Tregs have been shown to preferentially express endogenous α chains in transgenic mice and are severely decreased in transgenic mice lacking TCRα expression, whereas $CD4^+CD25^-$ cells develop normally (Suto et al., 2002). This suggests an affinity difference in selection of Tregs. It has been suggested that Tregs may be the T cells with the highest affinity for peptides presented in the thymus, just below the threshold for negative selection (Jordan et al., 2001; Apostolou et al., 2002; Kawahata et al., 2002). Transgenic animals created with a high-affinity TCR preferentially select $CD4^+CD25^+$ T cells as opposed to low-affinity TCR transgenic animals (Jordan et al., 2001). Thus, Tregs may be the cells within the thymus with the highest affinity for self-peptides, a property that could contribute to the ability of these cells to regulate aberrant responses to self in the periphery.

Regulation of immune responses in the periphery has been the objective of many new therapies. In particular, current trials involving the use of anti-CD3 antibodies aim to control peripheral T-cell responses through a combination of T-cell activation-induced cell death (AICD), Th2 polarization and secretion of IL-10, and the induction of TGF-β producing Tregs (Masteller and Bluestone, 2002; Belghith et al., 2003; Chatenoud, 2003). Alternatively, to avoid general immune suppression, several promising experimental therapies utilize the MHC–peptide complex to provide specificity. In particular, the soluble peptide–MHC class II chimeras has been used to prevent autoimmune diabetes onset and restore normal glycemia in mice (Casares et al., 2002; Masteller et al., 2003), reduce the relapse rate of experimental autoimmune encephalomyelitis (EAE) (Huan et al., 2004), or reduce the severity of and delay the onset of collagen-induced arthritis (Zuo et al., 2002). *In vitro* studies using human cells have also shown promise with MHC–peptide dimers. Soluble dimeric DR2–IgG fusion protein with a bound peptide from myelin basic protein (MBP) has been shown to cause anergy in MBP-specific

T cells (Appel et al, 2001). All these methods of inducing peripheral tolerance to self-antigens are thought to occur by induction of anergy or production of immunoregulatory cytokines. Immunoregulatory cytokines such as IL-10 and TGF-β have also been implicated in producing Tregs (Yamagiwa et al., 2001; Dieckmann et al., 2002; Jonuleit et al., 2002; Levings et al., 2002a; Zheng et al., 2002; Chen et al., 2003a; 2003b; Horwitz et al, 2003). As mentioned above, this method of preventing peripheral immune responses may be particularly promising due to the lack of antigen specificity of these cells once activated. Tregs could be activated selectively by antigen at the site of autoimmunity, but may control the response to other epitopes present at that site through bystander suppression, which is of particular importance in autoimmune diseases where pathology is directed against several antigens.

Another way to manipulate the MHC–peptide interaction with TCR for immunotherapy is through the use of altered peptides which act as TCR antagonists. T-cell epitopes can be altered by one or more amino acids to become an altered peptide ligand (APL). APLs elicit reduced or partial responses in contrast to their fully antigenic wild-type counterparts. Some APLs not only fail to elicit certain T-cell responses, but can also inhibit T-cell responses in mice (Basu et al, 1998). TCR antagonists are usually clone specific and require excess antagonist over agonist in order to suppress the response to the agonist (De Magistris et al., 1992; Jameson et al, 1993), although some general antagonists have been described (Anderton et al., 1999; Toda et al., 2000). However, the use of APLs as a therapy has led to responses to the APL itself and also to cross-reactive responses to disease-associated autoantigens (Bielekova et al., 2000; Kappos et al., 2000). This latter property makes it unlikely that APL therapies will fulfill the necessary safety profiles for most clinical applications.

From the perspective of disease pathogenesis, however, antagonistic self-peptides may play a significant role in many immune responses. The T-cell repertoire is selected from partially agonistic self-peptides and therefore, fully mature T cells in the periphery may recognize modified self-peptides as partial agonists or antagonists. The ability of certain MHC alleles to bind and present antagonistic self-peptides likely differs with different alleles, and could explain why some HLA types have inhibitory effects on autoimmune responses.

CONCLUDING REMARKS

Selection, activation, and regulation of autoreactive T cells, as outlined above, represent fundamental features which condition the immune system for pathogen responses and for homeostatic properties. The error-prone nature of these essential functions leads to altered functional thresholds, which predispose to autoreactivity and autoimmunity. Many different MHC genes are implicated in human disease; e.g., several class II MHC molecules are associated with the development of type 1 diabetes, with *DRB1*0302* being the principal risk allele (Bertrams and Baur, 1984; Nepom and Erlich, 1991; 2002; Nepom, 1993; Thomson et al., 1988). On the other hand, *DQB1*0602* is associated with protection from disease (Baisch et al., 1990). *DRB1*0401* and *DRB1*0404* are both associated with rheumatoid arthritis (Nepom et al., 1987; Nepom and Nepom, 1992; Ollier and Thomson, 1992; Winchester et al, 1992); however, a closely related allele *DRB1*0402* is associated with pemphigus vulgaris (Scharf et al., 1988). *DRB1*1501* and *DRB5*0101* are highly associated with the development of multiple sclerosis (Tiwari and Terasaki, 1985) and *DQB1*0201* has been associated with celiac disease (Sollid et al., 1989). HLA transgenic mice have been successfully designated containing DR2, DR3, DR4, DQ6, and DQ8, and have allowed the study of several autoimmune conditions, including diabetes, rheumatoid arthritis, systemic lupus erythematosus, EAE, and experimental autoimmune uveitis (EAU) (Taneja and David, 1998; Abraham et al., 2000; Das et al., 2000). In each case, it is likely that unique allelic polymorphisms confer subtle variation on peptide or TCR interactions and these establish the thresholds which ultimately determine autoreactive T-cell selection, activation, and regulation. In this sense, the MHC contribution to autoimmunity is permissive for disease susceptibility, creating an error-prone framework which, in combination with non-MHC genes and environmental variables in specific end organs, leads to the overall clinical spectrum manifest as MHC-associated autoimmunity.

References

Abraham, R.S., Kudva, Y.C., Wilson, S.B., Strominger, J.L., and David, C.S. (2000). Co-expression of HLA DR3 and DQ8 results in the development of spontaneous insulitis and loss of tolerance to GAD65 in transgenic mice. *Diabetes 49*, 548–554.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science 298*, 1395–1401.

Anderton, S.M., Kissler, S., Lamont, A.G., and Wraith, D.C. (1999). Therapeutic potential of TCR antagonists is determined by their ability to modulate a diverse repertoire of autoreactive T cells. *Eur. J. Immunol. 29*, 1850–1857.

Anderton, S.M., Radu, C.G., Lowrey, P.A., Ward, E.S., and Wraith, D.C. (2001). Negative selection during the peripheral immune response to antigen. *J. Exp. Med. 193*, 1–11.

Apostolou, I., Sarukhan, A., Klein, L., and von Boehmer, H. (2002). Origin of regulatory T cells with known specificity for antigen. *Nat. Immunol. 3*, 756–763.

Appel, H., Seth, N.P., Gauthier, L., and Wucherpfennig, K.W. (2001). Anergy induction by dimeric TCR ligands. *J. Immunol. 166*, 5279–5285.

Baecher-Allan, C., Brown, J.A., Freeman, G.J., and Hafler, D.A. (2001). CD4+CD25high regulatory cells in human peripheral blood. *J. Immunol. 167*, 1245–1253.

Baisch, J.M., Weeks, T., Giles, T., Hoover, M., Stastny, P., and Capra, J.D. (1990). Analysis of HLA-DQ genotypes and susceptibility in insulin-dependent diabetes mellitus. *N. Engl. J. Med. 322*, 1836–1841.

Basu, D., Williams, C.B., and Allen, P.M. (1998). *In vivo* antagonism of a T cell response by an endogenously expressed ligand. *Proc. Natl. Acad. Sci. USA 95*, 14332–14336.

Beijleveld, L.J., Damoiseaux, J.G., and Breda Vriesman, P.J. (1995). Differential effects of X-irradiation and cyclosporin-A administration on the thymus with respect to the generation of cyclosporin-A-induced autoimmunity. *Dev. Immunol. 4*, 127–138.

Belghith, M., Bluestone, J.A., Barriot, S., Megret, J., Bach, J.F., and Chatenoud, L. (2003). TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat. Med. 9*, 1202–1208.

Bertrams, J. and Baur, M. (1984). Insulin dependant diabetes mellitus. In E. Albert, M. Baur and W. Mayer (eds), *Histocompatibility Testing* (pp. 348–358). New York: Springer-Verlag.

Bielekova, B., Goodwin, B., Richert, N., Cortese, I., Kondo, T., Afshar, G., Gran, B., Eaton, J., Antel, J., Frank, J.A., McFarland, H.F., and Martin, R. (2000). Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: Results of a phase II clinical trial with an altered peptide ligand. *Nat. Med. 6*, 1167–1175.

Bikoff, E.K., Huang, L.Y., Episkopou, V., van Meerwijk, J., Germain, R.N., and Robertson, E.J. (1993). Defective major histocompatibility complex class II assembly, transport, peptide acquisition, and CD4+ T cell selection in mice lacking invariant chain expression. *J. Exp. Med. 177*, 1699–1712.

Bluestone, J.A. and Abbas, A.K. (2003). Natural versus adaptive regulatory T cells. *Nat. Rev. Immunol. 3*, 253–257.

Brehm, M.A., Pinto, A.K., Daniels, K.A., Schneck, J.P., Welsh, R.M., and Selin, L.K. (2002). T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. *Nat. Immunol. 3*, 627–634.

Brocker, T. (1997). Survival of mature CD4 T lymphocytes is dependent on major histocomptibility complex class II-expressing dendritic cells. *J. Exp. Med. 186*, 1223–1232.

Buckner, J. and Nepom, G.T. (2005). Structure, function, and genetics of the human leukocyte antigen complex in rheumatic disease. In W.J. Koopman and L.W. Moreland (eds), *Arthritis and Allied Conditions*, 15th edn. Philadelphia: Lippincott, Williams and Wilkins.

Carrasco-Marin, E., Shimizu, J., Kanagawa, O., and Unanue, E.R. (1996). The class II MHC I-A^{g7} molecules from non-obese diabetic mice are poor peptide binders. *J. Immunol. 156*, 450–458.

Carrington, M., Nelson, G.W., Martin, M.P., Kissner, T., Vlahov, D., Goedert, J.J., Kaslow, R., Buchbinder, S., Hoots, K., and O'Brien, S.J. (1999). HLA and HIV–1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science 283*, 1748–1752.

Casares, S., Hurtado, A., McEvoy, R., Sarukhan, A., von Boehmer, H., and Brumeanu, T.D. (2002). Down-regulation of diabetogenic CD4+ T cells by a soluble dimeric peptide-MHC class II chimera. *Nat. Immunol. 3*, 383–391.

Chatenoud, L. (2003). CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol. 3*, 123–132.

Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., and Weiner, H.L. (1994). Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science 265*, 1237–1240.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003a). Conversion of peripheral CD4+CD25− naïve T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J.Exp.Med. 198*, 1875–1886.

Chen, Z.M., O'Shaughnessy, M.J., Gramaglia, I., Panoskaltsis-Mortari, A., Murphy, W.J., Narula, S., Roncarolo, M.G., and Blazar, B.R. (2003b). IL–10 and TGF-β induce alloreactive CD4+CD25− T cells to acquire regulatory cell function. *Blood 101*, 5076–5083.

Chentoufi, A.A. and Polychronakos, C. (2002). Insulin expression levels in the thymus modulate insulin-specific autoreactive T-cell tolerance: the mechanism by which the IDDM2 locus may predispose to diabetes. *Diabetes 51*, 1383–1390.

Christen, U. and von Herrath, M.G. (2004). Induction, acceleration or prevention of autoimmunity by molecular mimicry. *Mol. Immunol. 40*, 1113–1120.

Dai, Z., Li, Q., Wang, Y., Gao, G., Diggs, L.S., Tellides, G., and Lakkis, F.G. (2004). CD4+CD25+ regulatory T cells suppress allograft rejection mediated by memory CD8+ T cells via a CD30-dependent mechanism. *J. Clin. Invest. 113*, 310–317.

Damoiseaux, J.G., Beijleveld, L.J., and Breda Vriesman, P.J. (1997). Multiple effects of cyclosporin A on the thymus in relation to T-cell development and autoimmunity. *Clin Immunol. Immunopathol. 82*, 197–202.

Danke, N.A., Koelle, D.M., Yee, C., Beberay, S., and Kwok, W.W. (2004). Autoreactive T cells in healthy individuals. J. Immunol. 172, 5967–5972.

Das, P., Drescher, K.M., Geluk, A., Bradley, D.S., Rodriguez, M., and David, C.S. (2000). Complementation between specific HLA-DR and HLA-DQ genes in transgenic mice determines susceptibility to experimental autoimmune encephalomyelitis. *Hum. Immunol. 61*, 279–289.

De Magistris, M.T., Alexander, J., Coggeshall, M., Altman, A., Gaeta, F.C. A., Grey, H.M., and Sette, A. (1992). Antigen analog–major histocompatibility complexes act as antagonists of the T cell receptor. *Cell 68*, 625–634.

Derby, M.A., Wang, J., Margulies, D.H., and Berzofsky, J.A. (2001). Two intermediate-avidity cytotoxic T lymphocyte clones with a disparity between functional avidity and MHC tetramer staining. *Int. Immunol. 13*, 817–824.

Dieckmann, D., Plottner, H., Berchtold, S., Berger, T., and Schuler, G. (2001). Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. *J. Exp. Med. 193*, 1303–1310.

Dieckmann, D., Bruett, C.H., Ploettner, H., Lutz, M.B., and Schuler, G. (2002). Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J. Exp. Med. 196*, 247–253.

Ding, Y.H., Baker, B.M., Garboczi, D.N., Biddison, W.E., and Wiley, D.C. (1999). Four A6-TCR/peptide/HLA-A2 structures that generate very different T cell signals are nearly identical. *Immunity 11*, 45–56.

Drake, D.R., III and Braciale, T.J. (2001). Cutting edge: lipid raft integrity affects the efficiency of MHC class I tetramer binding and cell surface TCR arrangement on CD8+ T cells. *J Immunol 166*, 7009–7013.

Dubois-Lafforgue, D., Mogenet, L., Thebault, K., Jami, J., Krief, P., Boitard, C. (2002). Proinsulin 2 knockout NOD mice: a model for genetic variation of insulin gene expression in type 1 diabetes. *Diabetes 51* (Suppl 3), S489–S493.

Elliott, E.A., Drake, J.R., Amigorena, S., Elsemore, J., Webster, P., Mellman, I., and Flavell, R.A. (1994). The invariant chain is required for intracellular transport and function of major histocompatibility complex class II molecules. *J. Exp. Med. 179*, 681–694.

Erlich, H.A. and Gyllensten, U.B. (1991). Shared epitopes among HLA class II alleles: gene conversion, common ancestry and balancing selection. *Immunol. Today 12*, 411–414.

Ernst, B., Lee, D.S., Chang, J.M., Sprent, J., and Surh, C.D. (1999). The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity 11*, 173–181.

Fahmy, T.M., Bieler, J.G., Edidin, M., and Schneck, J.P. (2001). Increased TCR avidity after T cell activation: a mechanism for sensing low-density antigen. *Immunity 14*, 135–143.

Fairchild, P.J., Wildgoose, R., Atherton, E., Webb, S., and Wraith, D.C. (1993). An autoantigenic T cell epitope forms unstable complexes with class II MHC: a novel route for escape from tolerance induction. *Int. Immunol. 5*, 1151–1158.

Freitas, A.A. and Rocha, B. (1999). Peripheral T cell survival. *Curr. Opin. Immunol. 11*, 152–156.

Fukui, Y., Ishimoto, T., Utsuyama, M., Gyotoku, T., Koga, T., Nakao, K., Hirokawa, K., Katsuki, M., and Sasazuki, T. (1997). Positive and negative CD4+ thymocyte selection by a single MHC class II/peptide ligand affected by its expression level in the thymus. *Immunity 6*, 401–410.

Fung-Leung, W.-P., Surh, C.D., Liljedahl, M., Pang, J., Leturcq, D., Peterson, P.A., Webb, S.R., and Karlsson, L. (1996). Antigen presentation and T cell development in H2-M-deficient mice. *Science 271*, 1278–1281.

Goldrath, A.W. and Bevan, M.J. (1999). Low-affinity ligands for the TCR drive proliferation of mature CD8+ T cells in lymphopenic hosts. *Immunity 11*, 183–190.

Goverman, J. (1999). Tolerance and autoimmunity in TCR transgenic mice specific for myelin basic protein. *Immunol. Rev. 169*, 147–159.

Groux, H., Bigler, M., De Vries, J.E., and Roncarolo, M.G. (1996). Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. *J. Exp. Med. 184*, 19–29.

Grubin, C.E., Kovats, S., DeRoos, P., and Rudensky, A.Y. (1997). Deficient positive selection of CD4 T cells in mice displaying altered repertoires of MHC class II-bound self-peptides. *Immunity 7*, 197–208.

Hausmann, D.H., Yu, B., Hausmann, S., Wucherpfennig, K.W. (1999). pH-dependent peptide binding properties of the type I diabetes-associated I-Ag7 molecule: rapid release of CLIP at an endosomal pH. *J. Exp. Med. 189*, 1723–1734.

He, X.L., Radu, C., Sidney, J., Sette, A., Ward, E.S., and Garcia, K.C. (2002). Structural snapshot of aberrant antigen presentation linked to autoimmunity: the immunodominant epitope of MBP complexed with I-Au. *Immunity 17*, 83–94.

Hesse, M.D., Karulin, A.Y., Boehm, B.O., Lehmann, P.V., Tary-Lehmann, M. (2001). A T cell clone's avidity is a function of its activation state. *J Immunol. 167*, 1353–1361.

Hill, A.V., Allsopp, C.E., Kwiatkowski, D., Anstey, N.M., Twumasi, P., Rowe, P.A., Bennett, S., Brewster, D., McMichael, A.J., and Greenwood, B.M. (1991). Common west African HLA antigens are associated with protection from severe malaria. *Nature 352*, 595–600.

Holvoet, P., Stassen, J.M., Van Cleemput, J., Collen, D., and Vanhaecke, J. (1998a). Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease. *Arterioscler. Thromb. Vasc. Biol. 18*, 100–107.

Holvoet, P., Vanhaecke, J., Janssens, S., Van de, W.F., and Collen, D. (1998b). Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation 98*, 1487–1494.

Horwitz, D.A., Zheng, S.G., Gray, J.D. (2003). The role of the combination of IL-2 and TGF-beta or IL-10 in the generation and function of CD4+ CD25+ and CD8+ regulatory T cell subsets. *J. Leukoc. Biol. 74*, 471–478.

Huan, J., Subramanian, S., Jones, R., Rich, C., Link, J., Mooney, J., Bourdette, D.N., Vandenbark, A.A., Burrows, G.G., and Offner, H. (2004). Monomeric recombinant TCR ligand reduces relapse rate and severity of experimental autoimmune encephalomyelitis in SJL/J mice through cytokine switch. *J. Immunol. 172*, 4556–4566.

Hughes, A.L. and Nei, M. (1988). Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature 335*, 167–170.

Iezzi, G., Scotet, E., Scheidegger, D., and Lanzavecchia, A. (1999). The interplay between the duration of TCR and cytokine signaling determines T cell polarization. *Eur. J. Immunol. 29*, 4092–4101.

Ignatowicz, L., Winslow, G., Bill, J., Kappler, J., and Marrack, P. (1995). Cell surface expression of class II MHC proteins bound by a single peptide. *J. Immunol. 154*, 3852–3862.

Jameson, S.C., Carbone, F.R., and Bevan, M.J. (1993). Clone-specific T cell receptor antagonist of major histocompatibility complex class I-restricted cytotoxic T cells. *J. Exp. Med. 177*, 1541–1550.

Janeway, C.A., Bottomly, K., Babich, J., Conrad, P., Conzen, S., Jones, B., Kaye, J., Katz, M., McVay, L., Murphy, D.B., and Tite, J. (1984). Quantitative variation in Ia antigen expression plays a central role in immune regulation. *Immunol. Today 5*, 99–105.

Jiang, S., Camara, N., Lombardi, G., and Lechler, R.I. (2003). Induction of allopeptide-specific human CD4+CD25+ regulatory T cells ex vivo. *Blood 102*, 2180–2186.

Jonuleit, H., Schmitt, E., Stassen, M., Tuettenberg, A., Knop, J., and Enk, A.H. (2001). Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J. Exp. Med. 193*, 1285–1294.

Jonuleit, H., Schmitt, E., Kakirman, H., Stassen, M., Knop, J., and Enk, A.H. (2002). Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. *J. Exp. Med. 196*, 255–260.

Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol. 2*, 283–284.

Kappos, L., Comi, G.C., Panitch, H., Oger, J., Antel, J., Conlon, P., Steinman, L., and The Altered Peptide Ligand in Relapsing MS Study Group (2000). Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial" *Nat. Med. 6*, 1176–1182.

Katz-Levy, Y., Neville, K.L., Girvin, A.M., Vanderlugt, C.L., Pope, J.G., Tan, L.J., and Miller, S.D. (1999). Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. *J. Clin. Invest. 104*, 599–610.

Kawahata, K., Misaki, Y., Yamauchi, M., Tsunekawa, S., Setoguchi, K., Miyazaki, J., and Yamamoto, K. (2002). Generation of CD4(+)CD25(+) regulatory T cells from autoreactive T cells simultaneously with their negative selection in the thymus and from nonautoreactive T cells by endogenous TCR expression. *J. Immunol. 168*, 4399–4405.

Kishimoto, H. and Sprent, J. (2001). A defect in central tolerance in NOD mice. *Nat. Immunol. 2*, 1025–1031.

Kohm, A.P., Carpentier, P.A., Anger, H.A., and Miller, S.D. (2002). Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J. Immunol. 169*, 4712–4716.

Kyewski, B., Derbinski, J., Gotter, J., and Klein, L. (2002). Promiscuous gene expression and central T-cell tolerance: more than meets the eye. *Trends Immunol. 23*, 364–371.

Lanzavecchia, A. and Sallusto, F. (2000). Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science 290*, 92–97.

Lanzavecchia, A. and Sallusto, F. (2001). Antigen decoding by T lymphocytes: from synapses to fate determination. *Nat. Immunol. 2*, 487–492.

Lesage, S., Hartley, S.B., Akkaraju, S., Wilson, J., Townsend, M., and Goodnow, C.C. (2002). Failure to censor forbidden clones of CD4 T cells in autoimmune diabetes. *J. Exp. Med. 196*, 1175–1188.

Levings, M.K., Bacchetta, R., Schulz, U., and Roncarolo, M.G. (2002a). The role of IL-10 and TGF-beta in the differentiation and effector function of T regulatory cells. *Int. Arch. Allergy Immunol. 129,* 263–276.

Levings, M.K., Sangregorio, R., Sartirana, C., Moschin, A.L., Battaglia, M., Orban, P.C., and Roncarolo, M.G. (2002b). Human CD25+CD4+ T suppressor cell clones produce transforming growth factor beta, but not interleukin 10, and are distinct from type 1 T regulatory cells. *J. Exp. Med. 196*, 1335–1346.

Liu, G.Y., Fairchild, P.J., Smith, R.M., Prowle, J.R., Kioussis, D., and Wraith, D.C. (1995). Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity 3*, 407–415.

Margulies, D.H. (2001). TCR avidity: it's not how strong you make it, it's how you make it strong. *Nat. Immunol. 2*, 669–670.

Martin, W.D., Hicks, G.G., Mendiratta, S.K., Leva, H.I., Ruley, H.E., and Van Kaer, L. (1996). H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell 84*, 543–550.

Masteller, E.L. and Bluestone, J.A. (2002). Immunotherapy of insulin-dependent diabetes mellitus. *Curr. Opin. Immunol. 14*, 652–659.

Masteller, E.L., Warner, M.R., Ferlin, W., Judkowski, V., Wilson, D., Glaichenhaus, N., and Bluestone, J.A. (2003). Peptide-MHC class II dimers as therapeutics to modulate antigen-specific T cell responses in autoimmune diabetes. *J. Immunol. 171*, 5587–5595.

Miyazaki, T., Wolf, P., Tourne, S., Waltzinger, C., Dierich, A., Barois, N., Ploegh, H., Benoist, C., and Mathis, D. (1996). Mice lacking H2-M complexes, enigmatic elements of the MHC class II peptide-loading pathway. *Cell 84*, 531–541.

Moriyama, H., Abiru, N., Paronen, J., Sikora, K., Liu, E., Miao, D., Devendra, D., Beilke, J., Gianani, R., Gill, R.G., and Eisenbarth, G.S. (2003). Evidence for a primary islet autoantigen (preproinsulin 1) for insulitis and diabetes in the nonobese diabetic mouse. *Proc. Natl. Acad. Sci. USA 100*, 10376–10381.

Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K.J.E., Lalioti, M.D., Mullis, P.E., Antonarakis, S.E., Kawasaki, K., Asakawa, S., Ito, F., and Shimizu, N. (1997). Positional cloning of the APECED gene. *Nat. Genet. 17*, 393–398.

Negishi, I., Motoyama, N., Nakayama, K., Nakayama, K., Senju, S., Hatakeyama, S., Zhang, Q., Chan, A.C., and Loh, D.Y. (1995). Essential role for ZAP-70 in both positive and negative selection of thymocytes. *Nature 376*, 435–438.

Nepom, G.T. (1993). MHC and autoimmune diseases. *Immunol. Ser. 59*, 143–164.

Nepom, G.T. and Erlich, H. (1991). MHC class II molecules and autoimmunity. *Annu. Rev. Immunol. 9*, 493–525.

Nepom, G.T. and Erlich, H. (2002). Immunology and immunogenetics. In *The Genetic Basis of Common Diseases* (pp. 573–586). Oxford: Oxford University Press.

Nepom, G.T. and Nepom, B.S. (1992). Prediction of susceptibility to rheumatoid arthritis by human leukocyte antigen genotyping. In G.T. Nepom (ed.), *Rheumatic Disease Clinics of North America*, vol. 18 (pp. 785–792). Philadelphia: W.B. Saunders.

Nepom, G.T., Hansen, J.A., and Nepom, B.S. (1987). The molecular basis for HLA class II associations with rheumatoid arthritis. *J. Clin. Immunol. 7*, 1–7.

Ollier, W. and Thomson, W. (1992). Population genetics of rheumatoid arthritis. In G.T. Nepom (ed.), *Rheumatic Disease Clinics of North America*, vol. 18 (pp. 741–759). Philadelphia: W.B. Saunders.

Pacini, S., Valensin, S., Telford, J.L., Ladbury, J., and Baldari, C.T. (2000). Temporally regulated assembly of a dynamic signaling complex associated with the activated TCR. *Eur. J. Immunol. 30*, 2620–2631.

Quarsten, H., Molberg, O., Fugger, L., McAdam, S.N., and Sollid, L.M. (1999). HLA binding and T cell recognition of a tissue transglutaminase- modified gliadin epitope. *Eur. J. Immunol. 29*, 2506–2514.

Ramsey, C., Winqvist, O., Puhakka, L., Halonen, M., Moro, A., Kampe, O., Eskelin, P., Pelto-Huikko, M., and Peltonen, L. (2002). Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum. Mol. Genet. 11*, 397–409.

Ridgway, W., Fasso, M., and Fathman, C.G. (1998). Following antigen challenge, T cells up-regulate cell surface expression of CD4 *in vitro* and *in vivo*. *J. Immunol. 161*, 714–720.

Rooke, R., Waltzinger, C., Benoist, C., and Mathis, D. (1997). Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenoviruses. *Immunity 7*, 123–134.

Rosette, C., Werlen, G., Daniels, M.A., Holman, P.O., Alam, S.M., Travers, P.J., Gascoigne, N.R., Palmer, E., and Jameson, S.C. (2001). The impact of duration versus extent of TCR occupancy on T cell activation: a revision of the kinetic proofreading model. *Immunity 15*, 59–70.

Sakaguchi, N., Miyai, K., and Sakaguchi, S. (1994). Ionizing radiation and autoimmunity. Induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells. *J. Immunol. 152*, 2586–2595.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155*, 1151–1164.

Sakaguchi, S., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakiham, T., Matsutani, T., Negishi, I., Nakatsuru, S., and Sakaguchi, S. (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature 426, 454–460.

Scharf, S.J., Friedman, A., Brautbar, C., Szafer, F., Steinman, L., Horn, G., Gyllensten, U., and Erlich, H.A. (1988). HLA class II allelic variation and susceptibility to pemphigus vulgaris. *Proc. Natl. Acad. Sci. USA 85*, 3504–3508.

Slifka, M.K. and Whitton, J.L. (2001). Functional avidity maturation of CD8(+) T cells without selection of higher affinity TCR. *Nat. Immunol. 2*, 711–717.

Sollid, L.M., Markussen, G., Ek, J., Gjerde, H., Vartdal, F., and Thorsby, E. (1989). Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. *J. Exp. Med. 169*, 345–350.

Sorokin, R., Kimura, H., Schroder, K., Wilson, D.H., and Wilson, D.B. (1986). Cyclosporine-induced autoimmunity. Conditions for expressing disease, requirement for intact thymus, and potency estimates of autoimmune lymphocytes in drug-treated rats. *J. Exp. Med. 164*, 1615–1625.

Surh, C.D., Lee, D.S., Fung-Leung, W.-P., Karlsson, L., and Sprent, J. (1997). Thymic selection by a single MHC/peptide ligand produces a semidiverse repertoire of CD4+ T cells. *Immunity 7*, 209–219.

Suto, A., Nakajima, H., Ikeda, K., Kubo, S., Nakayama, T., Taniguchi, M., Saito, Y., and Iwamoto, I. (2002). CD4(+)CD25(+) T-cell development is regulated by at least 2 distinct mechanisms. *Blood 99*, 555–560.

Taams, L.S., Vukmanovic-Stejic, M., Smith, J., Dunne, P.J., Fletcher, J.M., Plunkett, F.J., Ebeling, S.B., Lombardi, G., Rustin, M.H., Bijlsma, J.W., Lafeber, F.P., Salmon, M., and Akbar, A.N. (2002). Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. *Eur. J. Immunol. 32*, 1621–1630.

Taguchi, O. and Nishizuka, Y. (1980). Autoimmune oophoritis in thymectomized mice: T cell requirement in adoptive cell transfer. *Clin. Exp. Immunol. 42*, 324–331.

Taguchi, O., Nishizuka, Y., Sakakura, T., and Kojima, A. (1980). Autoimmune oophoritis in thymectomized mice: detection of circulating antibodies against oocytes. *Clin. Exp. Immunol. 40*, 540–553.

Takeda, S., Rodewald, H.R., Arakawa, H., Bluethmann, H., and Shimizu, T. (1996). MHC class II molecules are not required for survival of newly generated CD4+ T cells, but affect their long-term life span. *Immunity 5*, 217–228.

Taneja, V. and David, C.S. (1998). HLA transgenic mice as humanized mouse models of disease and immunity. *J. Clin. Invest. 101*, 921–926.

Tang, J., Costello, C., Keet, I.P., Rivers, C., Leblanc, S., Karita, E., Allen, S., and Kaslow, R.A. (1999). HLA class I homozygosity accelerates

disease progression in human immunodeficiency virus type 1 infection. *AIDS Res. Hum. Retroviruses 15*, 317–324.

Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med. 199*, 1455–1465.

Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., and Steinman, R.M. (2004). CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med. 199*, 1467–1477.

Thebault-Baumont, K., Dubois-Laforgue, D., Krief, P., Briand, J.P., Halbout, P., Vallon-Geoffroy, K., Morin, J., Laloux, V., Lehuen, A., Carel, J.C., Jami, J., Muller, S., and Boitard, C. (2003). Acceleration of type 1 diabetes mellitus in proinsulin 2-deficient NOD mice. *J. Clin. Invest*. *111*, 851–857.

Thomson, G., Robinson, W.P., Kuhner, M.K., Joe, S., MacDonald, M.J., Gottschall, J.L., Barbosa, J., Rich, S.S., Bertrams, J., Baur, M.P., Partanen, J., Tait, B.D., Schober, E., Mayr, W.R., Ludbigsson, J., Lindblom, B., Farid, N.R., Thompson, C., and Deschamps, I. (1988). Genetic heterogeneity, modes of inheritance, and risk estimates for a joint study of Caucasians with insulin-dependent diabetes mellitus. *Am. J. Hum. Genet*. *43*, 799–816.

Thursz, M.R., Thomas, H.C., Greenwood, B.M., and Hill, A.V. (1997). Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nat. Genet. 17*, 11–12.

Tian, J., Gregori, S., Adorini, L., and Kaufman, D.L. (2001). The frequency of high avidity T cells determines the hierarchy of determinant spreading. *J. Immunol. 166*, 7144–7157.

Tiwari, J. and Terasaki, P. (1985). *HLA and Disease Associations.* New York: Springer-Verlag.

Toda, M., Totsuka, M., Furukawa, S., Yokota, K., Yoshioka, T., Ametani, A., and Kaminogawa, S. (2000). Down-regulation of antigen-specific antibody production by TCR antagonist peptides *in vivo*. *Eur. J. Immunol. 30*, 403–414.

Tourne, S., Miyazaki, T., Oxenius, A., Klein, L., Fehr, T., Kyeski, B., Nenoist, C., and Mathis, D. (1997). Selection of a broad repertoire of CD4+ T cell in H-2Ma$^{0/0}$ mice. *Immunity 7*, 187–196.

van Boekel, M.A., Vossenaar, E.R., van den Hoogen, F.H., and van Venrooij, W.J. (2002). Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res. 4*, 87–93.

van der Merwe, P.A. (2001). The TCR triggering puzzle. *Immunity 14*, 665–668.

Villunger, A., Marsden, V.S., and Strasser, A. (2003). Efficient T cell receptor-mediated apoptosis in nonobese diabetic mouse thymocytes. *Nat. Immunol. 4*, 717.

Viola, A. (2001). The amplification of TCR signaling by dynamic membrane microdomains. *Trends Immunol.* 22, 322–327.

Viville, S., Neefjes, J., Lotteau, V., Dierich, A., Lemeur, M., Ploegh, H., Benoist, C., and Mathis, D. (1993). Mice lacking the MHC class II-associated invariant chain. *Cell 72*, 635–648.

Walker, M.R., Kasprowicz, D.J., Gersuk, V.H., Benard, A., Van Landeghen, M., Buckner, J.H., and Ziegler, S.F. (2003). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+. *J. Clin. Invest*. *112*, 1437–1443.

Walker, M.R., Carson, B.D., Nepom, G.T., Ziegler, S.F., and Buckner, J.H. (2005). De novo generation of antigen-specific CD4+CD25+ regulatory T cells from human CD4+CD25− cells. *Proc. Natl. Acad. Sci. USA 102*, 4103–4108.

Wang, H.Y., Lee, D.A., Peng, G., Guo, Z., Li, Y., Kiniwa, Y., Shevach, E.M., and Wang, R.F. (2004). Tumor-specific human CD4+ regulatory T cells and their ligands: implications for immunotherapy. *Immunity 20*, 107–118.

Wen, L., Wong, F.S., Tang, J., Chen, N.Y., Altieri, M., David, C., Flavell, R., and Sherwin, R. (2000). *In vivo* evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J. Exp. Med. 191*, 97–104.

Winchester, R., Dwyer, E., and Rose, S. (1992). The genetic basis of rheumatoid arthritis: The shared epitope hypothesis. In G.T. Nepom (ed.), *Rheumatic Disease Clinics of North America*, vol. 18 (pp. 761–783). Philadelphia: W.B. Saunders.

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.

Zhao, Z.S., Granucci, F., Yeh, L., Schaffer, P.A., and Cantor, H. (1998). Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science 279*, 1344–1347.

Zheng, S.G., Gray, J.D., Ohtsuka, K., Yamagiwa, S., and Horwitz, D.A. (2002). Generation ex vivo of TGF-beta-producing regulatory T cells from CD4+CD25− precursors. *J. Immunol. 169*, 4183–4189.

Zuo, L., Cullen, C.M., DeLay, M.L., Thornton, S., Myers, L.K., Rosloniec, E.F., Boivin, G.P., and Hirsch, R. (2002). A single-chain class II MHC-IgG3 fusion protein inhibits autoimmune arthritis by induction of antigen-specific hyporesponsiveness. *J. Immunol*. *168*, 2554–2559.

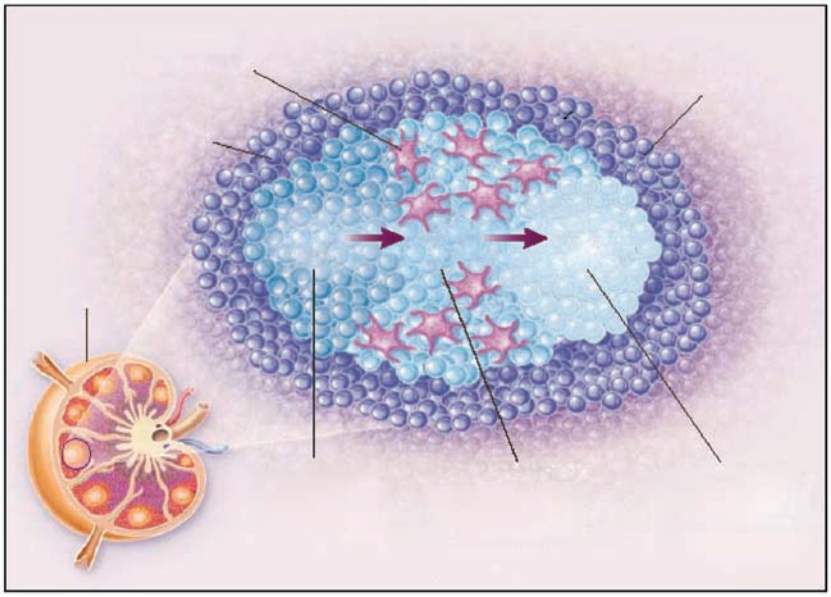


FIGURE 2.7 Germinal center. During the initiation of the acquired immune response, these structures form in the secondary lymphoid tissues in order to generate a microenvironment where all the necessary antigen-specific and innate antigen-presenting cells can interact. Antigen-stimulated B-cell proliferation occurs in the dark zone and is accompanied by affinity maturation due to somatic hypermutation of the immunoglobulin variable region genes. Upon passage into the basal light zone, high-affinity antigen-specific B-cells are positively selected by interaction with antigen, which is present in the form of immune complexes on the surface of follicular dendrite cells. B-cells which fail to be positively selected undergo apoptosis and are phagocytosed by tingible-body macrophages. The positively selected cells migrate to the apical light zone where proliferation continues, class switching occurs, and memory cells and plasma cell precursors are generated.

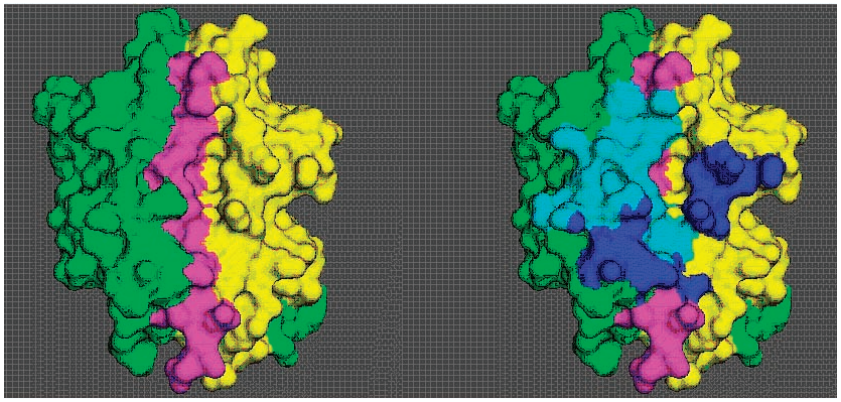


FIGURE 5.2 Trimolecular interaction between MHC, peptide, and T-cell receptor (TCR). The exposed molecular surface of an MHC class II molecule, containing a bound antigen peptide, is shown in the left panel. The MHC α chain is yellow, the β chain is green, and the peptide is shown in pink. Amino acid side chains on the underlying side of the peptide lie buried in the MHC groove, anchoring the binding interaction. This exposed surface forms the contact structure for TCR recognition; the image on the right is colored to show examples of the areas which contact the TCR, with the TCR α chain contacts shown in dark blue and the TCR β chain in light blue. Both peptide and MHC residues are involved in TCR recognition, and both contribute to the overall avidity of the interaction.

Adapted from Koopman, et al., *Arthritis and Allied Conditions*, 15th edition.

CHAPTER

6

T Cells and Autoimmunity

YANG YANG*
**Department of Biochemistry & Molecular Biology*

PERE SANTAMARIA†
*†Department of Microbiology and Infectious Diseases, and *,†Julia McFarlane Diabetes Research Centre, The University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada*

T cells play a fundamental role in most, if not all, adaptive immune responses, including those executed by antibody-producing B lymphocytes. The most fundamental difference between T- and B-cell responses lies in major histocompatibility complex (MHC) restriction. Whereas antigen-specific T cells recognize ligands presented by "self"-MHC molecules on the surface of antigen-presenting cells (APCs), B cells recognize linear or conformational epitopes on antigens in a non-MHC-restricted manner. T cells can be divided into different subpopulations based on surface markers and functional behavior. $CD4^+$ helper T (Th) cells are those capable of promoting B-cell and $CD8^+$ T-cell responses. Cytotoxic T cells, which include cells belonging to both the $CD4^+$ and $CD8^+$ lineages, are those capable of killing target cells. The functions of $CD4^+$ Th cells are predominantly determined by the cytokines they produce, and these T cells are further subclassified into subsets depending on their cytokine profiles (Th0, Th1, Th2 or Th3). Upon antigen stimulation, some $CD4^+$ and $CD8^+$ T cells suppress, rather than drive, immune responses. Evolutionarily speaking, these T cells [also known as regulatory T cells (Tregs)] likely arose out of the immune system's need to control the magnitude of inflammatory reactions against microbial infections, to prevent collateral damage to healthy, noninfected tissue. Disruption of the delicate balance that exists among all these cell types of the adaptive immune system (in health) thus has the potential to cause autoimmunity, which, by definition, results from a loss of tolerance to self. Because of the central role that T cells play in adaptive immune responses, it is not surprising that they, too, play a fundamental role in most, if not all, autoimmune disorders, including those mediated by autoantibodies. This chapter provides a general overview of the roles of MHC, self-antigens and tolerance, T–T interactions, cytokines, T–B collaboration, and T-cell-mediated regulation in autoimmunity.

MAJOR HISTOCOMPATIBILITY COMPLEX POLYMORPHISM AND T-CELL-MEDIATED AUTOIMMUNITY

Imprinting of self-MHC restriction and tolerance to self on the mature T-cell repertoire occurs during thymocyte development. In the thymus, MHC molecules instruct maturing T cells how to discriminate between self- and non-self-antigens. Whereas thymocytes expressing T-cell receptors (TCRs) recognizing self-antigen–MHC with high affinity perish (a process also referred to as negative selec-

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

tion), those expressing low-affinity TCRs (for the same complex) differentiate into the mature T cells that populate the peripheral lymphoid organs (Figure 6.1A). In addition to playing a key role in shaping the peripheral TCR repertoire, MHC molecules present cognate antigenic molecules, including self-antigens, to mature T cells in the periphery.

Human MHC molecules are encoded in several closely linked genes that comprise the HLA system on the short arm of chromosome 6. Murine MHC molecules are encoded in the H-2 complex, located on chromosome 17. HLA and H-2 genes encode MHC class I (A, B, or C in humans, and K, D and L in mice) and class II molecules (DR, DQ or DP in humans, and I-A and I-E in mice). The structure and function of human and murine MHC molecules are very similar. The N-terminal domains of class I heavy chains or class II α and β chains form a molecular pocket with peptide-binding properties. In general, MHC class I and II molecules present antigenic moieties (predominantly peptides) to $CD8^+$ and $CD4^+$ T cells, respectively. HLA and H-2 genes (A, B, C, DRB1, DRB3, DQA1, DQB1, DPA1, and DPB1; and K, D, L, I-Aα, I-Aβ, I-Eα and I-Eβ) are highly polymorphic, such that specific alleles at a given locus may be expressed

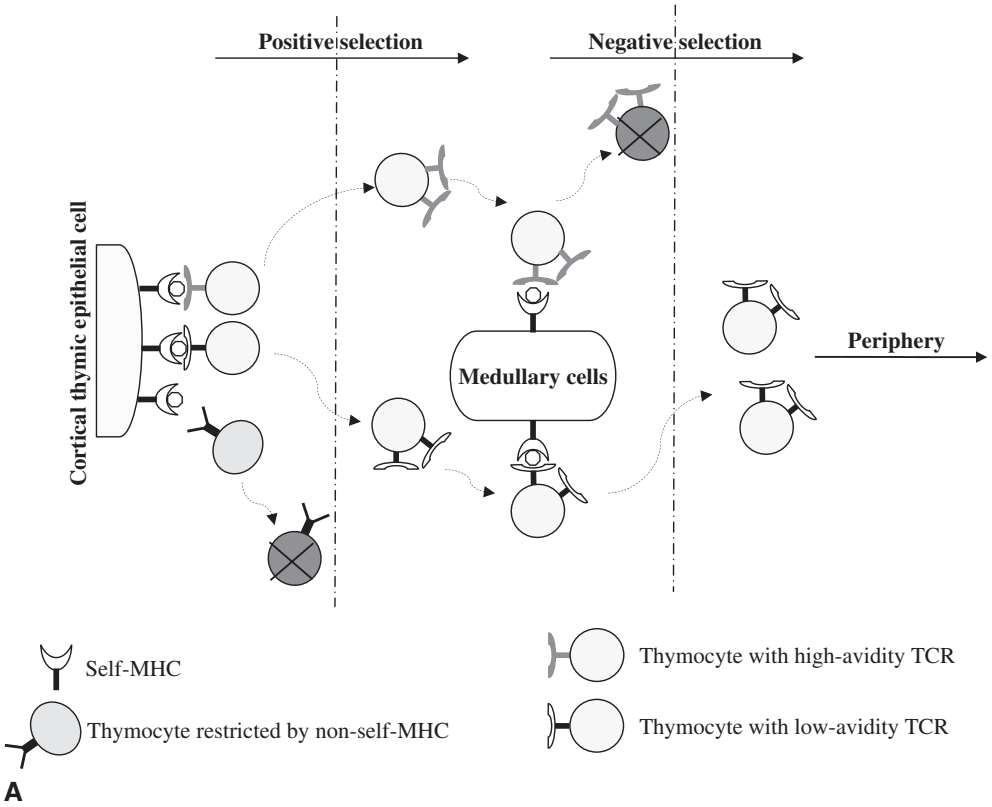
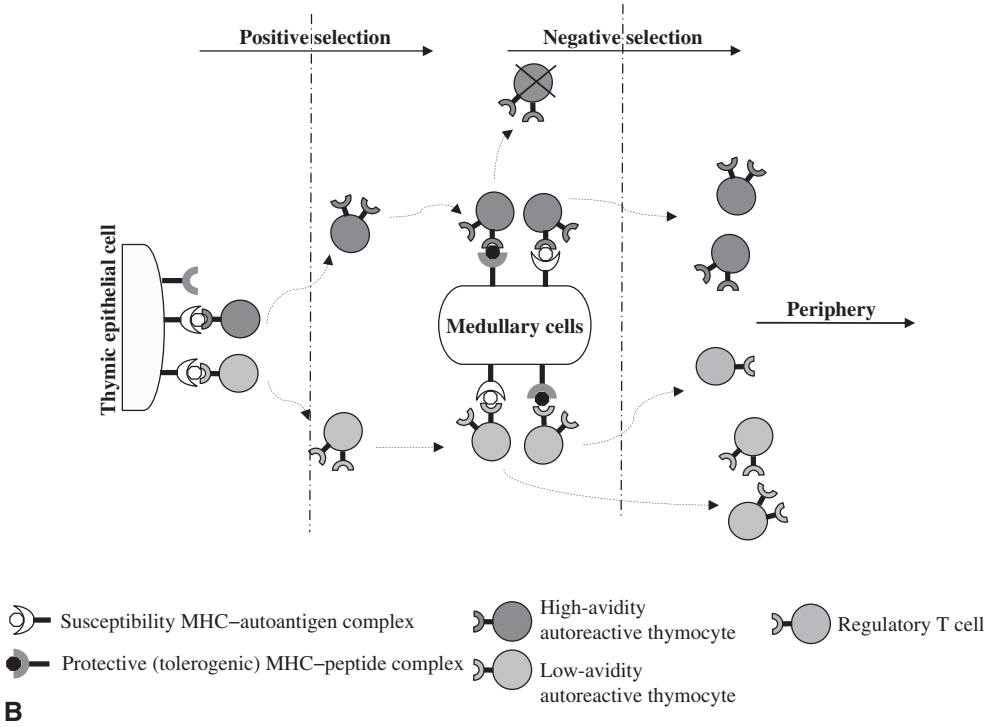
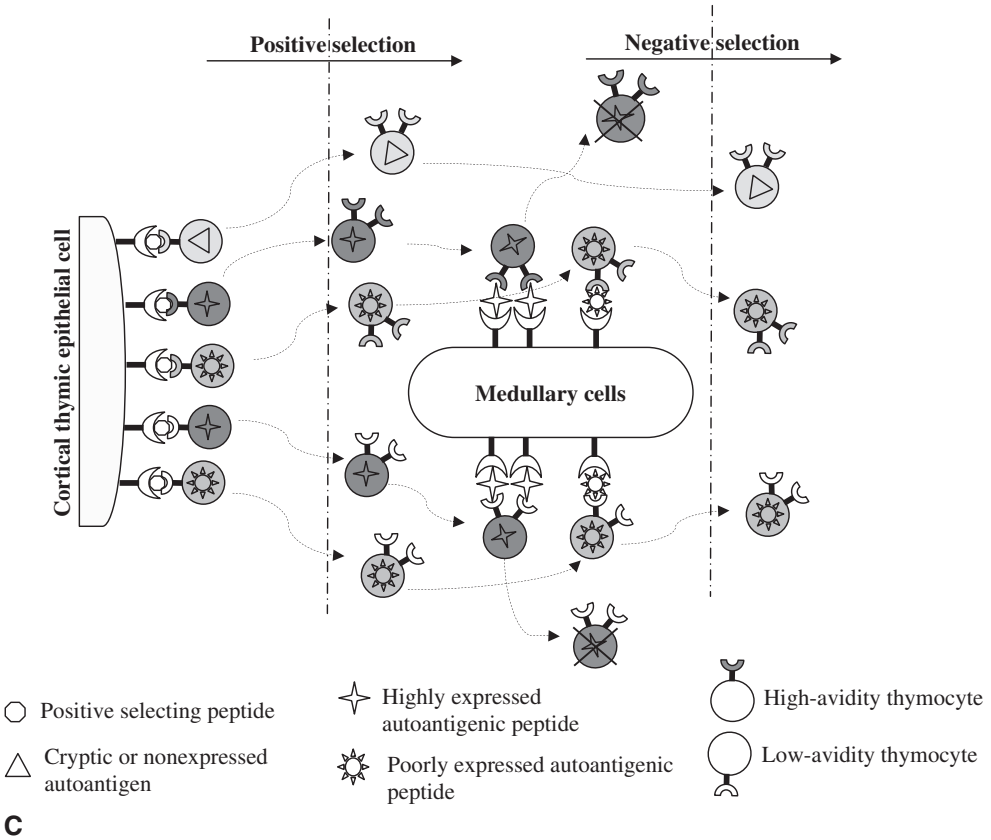


FIGURE 6.1 Hypothetical relationships among proautoimmune and protective MHC molecules, T-cell avidity (for peptide–MHC) and development of diabetogenic or regulatory autoreactive T cells. *A*, Central tolerance versus T-cell receptor (TCR) affinity. *B*, Central tolerance by antidiabetogenic MHC–peptide complexes. *C*, Central tolerance by autoantigen versus avidity. *D*, Developmental fate of autoreactive thymocytes. $CD4^+CD8^+$ thymocytes expressing autoreactive TCRs undergo positive selection by engaging diabetogenic, but not antidiabetogenic, MHC molecules on cortical thymic epithelial cells (*A*, *B*, and *D*). In the thymic medulla, the positively selected $CD4^+CD8^-$ or $CD4^-CD8^+$ thymocytes may or may not recognize proautoimmune or protective MHC molecules. Proautoimmune MHC molecules on medullary thymic epithelial cells or bone marrow-derived antigen-presenting cells (APCs) (i.e., dendritic cells) may or may not present the target autoantigenic peptide that the autoreactive T cells recognize in the periphery. This depends on whether the autoantigen is expressed by the APC or captured by the APC in the periphery and then ferried to the thymus (*B–D*). In some instances, the autoantigen is presented by medullary APCs but the target epitope is a cryptic one (*C* and *D*). High-avidity interactions between thymocytes and APCs will promote deletion, anergy of the thymocytes, and/or their differentiation into regulatory T cells (*B–D*). Low-avidity interactions will tune the activation threshold of the autoreactive thymocyte without inducing tolerance. Absence of autoantigen presentation will allow the migration of high-avidity, and hence potentially pathogenic, mature autoreactive T cells to the periphery (*C* and *D*). We have proposed that these high-avidity autoreactive thymocytes are inherently cross-reactive with protective MHC molecules in a peptide-, but not autoantigen-specific manner (see text and corresponding references). These interactions will selectively promote central tolerance of highly pathogenic autoreactive T cells, sparing low-avidity autoreactive T cells, which may not be pathogenic. See color plate section.



B



C

FIGURE 6.1 (*Continued*)

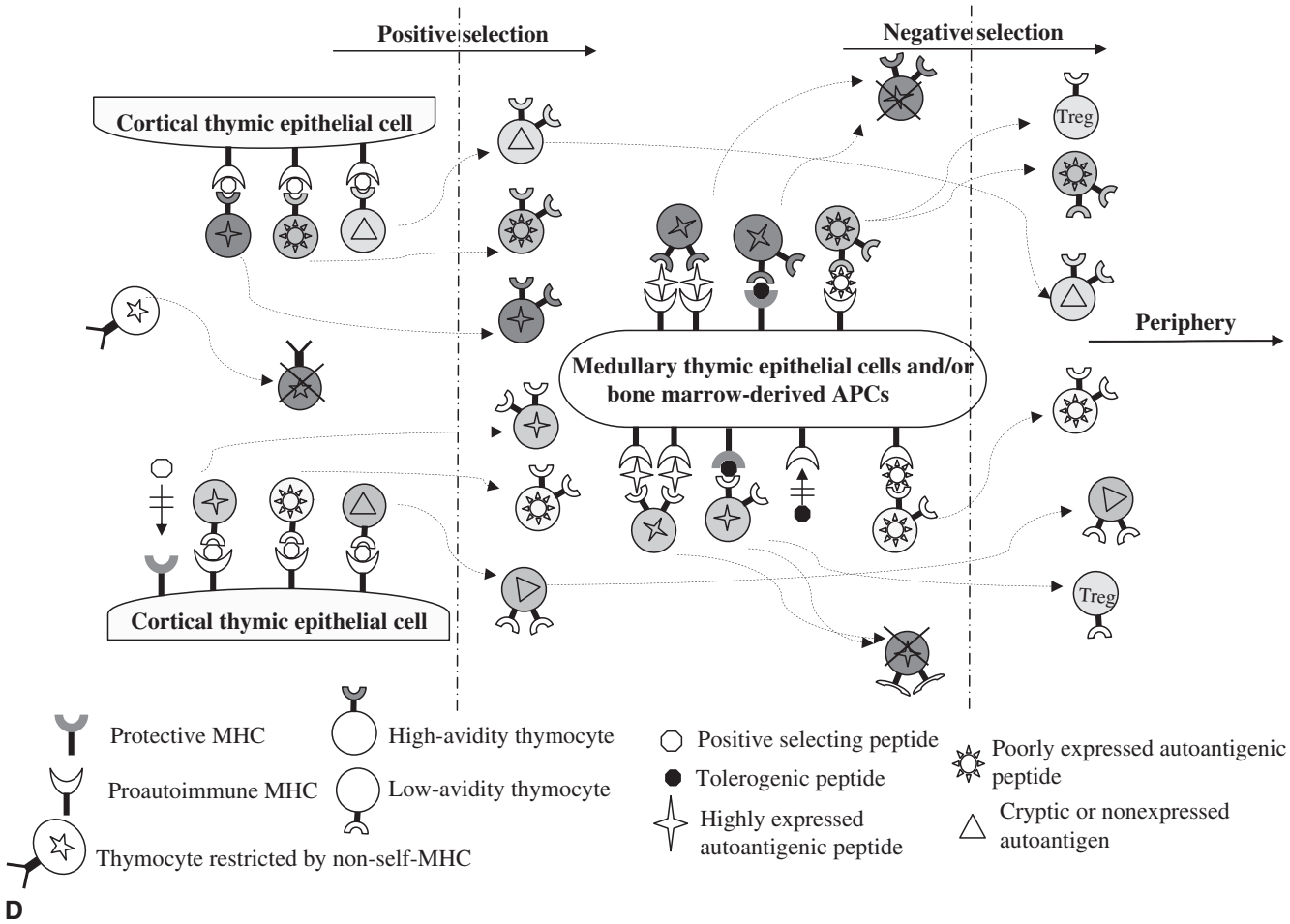


FIGURE 6.1 (*Continued*) (*D*). The balance (in the peripheral T cell repertoire) between high- and low-avidity autoreactive clonotypes and regulatory T cells will determine the overall risk of the individual to a given autoimmune disorder. In diseased individuals, the self-reactive T cells that escape central tolerance undergo activation in the lymph nodes draining the target tissue by recognizing target antigen–MHC complexes on APCs in the presence of proinflammatory signals. See color plate section.

in the context of several different alleles at most other loci. The issues of transcomplementation, whereby α or β chains encoded on alleles of one haplotype can pair with the corresponding partners encoded on the second haplotype, add yet another level of complexity of potential import in genetic susceptibility and/or resistance to autoimmune disorders. Obviously, the extraordinary diversity of the MHC at the population level impacts on an individual's ability to present specific self-antigens, and thus to control the development of autoreactive thymocytes, or the activation of their mature counterparts in the periphery. It is therefore not surprising that susceptibility and/or resistance to specific autoimmune diseases are associated with certain HLA/H-2 alleles and/or haplotypes.

Most autoimmune diseases are primarily associated with MHC class II polymorphisms, but some are almost exclusively associated with polymorphisms at MHC class I loci. To cite a few examples, there are associations between HLA-DR4 and rheumatoid arthritis (RA); between HLA-DR2 and systemic lupus erythematosus (SLE) or multiple sclerosis (MS); between HLA-DR3 and celiac disease, SLE, Graves' disease, myasthenia gravis, and Addison's disease; between HLA-DR8 and juvenile idiopathic arthritis; between HLA-DR3/DR4 and type 1 diabetes (T1D); between HLA-B27 and ankylosing spondylitis, reactive arthritis or anterior uveitis; between HLA-B35 and subacute thyroiditis; and between HLA-Cw6 and pemphigus vulgaris. The MHC class II–disease associations are predominantly accounted for by sequence polymorphisms in the second exon of DQB1, DRB1, and/or DPB1 genes. The MHC-associated susceptibility to T1D, for example, is mostly determined by polymorphisms at the DQB1 locus, particularly at the codon encoding position 57. The presence of alanine, valine or serine at this position affords susceptibility, whereas the presence of aspartic acid affords resistance. RA, on the other hand, is primarily associated with DRB1

alleles encoding DRβ molecules sharing key amino acids between positions 67 and 74. Similar MHC class II–disease associations have been described in the mouse. Susceptibility to T1D in nonobese diabetic (NOD) mice, for example, is associated with a unique I-A allele (I-Aβ^{g7}) in which the histidine and aspartic acid located at positions 56 and 57 in most murine I-Aβ chains (the counterparts of human DQβ chains) are replaced by proline and serine, respectively.

To examine the molecular bases of HLA–disease associations, a number of human MHC genes have been transgenically expressed in rodents lacking endogenous MHC genes (Geluk et al., 1998). When DQ8 molecules (positively associated with T1D) were expressed in mice also expressing a costimulatory CD80 (B7.1) transgene in β cells, the mice became intolerant to islet antigens and developed diabetes (Wen et al., 1998; 2000; 2002). Interestingly, coexpression of DR4 or DQ6 molecules in these animals modulated the diabetes susceptibility provided by the DQ8 transgene (Wen et al., 2001; Kudva et al., 2002). Likewise, whereas expression of DQ8 or DR4 molecules in mice afforded susceptibility to collagen-induced arthritis (CIA), expression of DR2 molecules (negatively associated with RA) afforded disease resistance (Nabozny et al., 1996; Taneja et al., 1998). DQ8 expression also provided susceptibility to experimental autoimmune encephalomyelitis (EAE), an animal model of MS (Das et al., 2000). Expression of DR3 or DR2 molecules in transgenic mice rendered them susceptible or resistant, respectively, to severe experimental autoimmune thyroiditis (EAT), a model of Graves' disease and Hashimoto's thyroiditis in humans (Kong et al., 1996; Flynn et al., 2004). The observations in HLA-B27 transgenic rats and mice are particularly interesting, given the well-established association that exists between HLA-B27 and the spondyloarthropathies (SpA), a group of inflammatory conditions affecting the skeleton, skin, and mucous membranes. These HLA-B27 transgenic animals spontaneously develop autoimmune syndromes that closely mimic SpA, provided that human β2 microglobulin (hβ2M) is coexpressed (in rats) or that the endogenous mβ2M gene is absent (in mice) (Taurog et al., 1999). Interestingly, HLA-B27 molecules can form homodimers in the absence of β2M (Allen et al., 1999) and can support the development of both $CD8^+$ and $CD4^+$ T cells (Roddis et al., 2004). In fact, a role for $CD4^+$ T cells in HLA-B27-associated autoimmunity has been proposed (Breban et al., 1996). Taken together, the above studies strongly support the idea that MHC genes themselves (as opposed to other linked genetic elements) afford autoimmune disease susceptibility and/or resistance.

The roles of murine MHC molecules in autoimmune disease susceptibility and/or resistance have also been explored in MHC-transgenic or -congenic mice. NOD mice have been widely used in these studies because they spontaneously develop a form of autoimmune diabetes that resembles the human condition and is strongly associated with a particular MHC haplotype (H-2^{g7}). Studies of congenic NOD mice expressing non-NOD MHC haplotypes, and of NOD mice expressing I-Eα^d, I-Eα^k, I-Aα^k/I-Aβ^k, I-Aβ^d or modified I-Aβ^{g7} transgenes, provided strong evidence supporting a role for class II molecules in providing susceptibility or resistance to T1D (Tisch and McDevitt, 1996). Although the underlying mechanisms remain unclear, experiments in bone marrow chimeras have shown that the MHC-linked resistance to diabetes is mediated by an hematopoietic cell type, possibly an APC [B lymphocytes, macrophages, and/or dendritic cells (DCs)]. Crystallographic studies have shown that I-A^{g7} molecules have a unique structure that supports the preferential binding of peptides bearing acidic residues at their C-terminal (Chao et al., 1999; Corper et al., 2000). It has been proposed that structural differences resulting from polymorphisms around the I-Aβ chain position 57 account for the ability of I-A^{g7} and protective MHC class II molecules to afford susceptibility or resistance to diabetes, respectively. Evidence for this comes from studies of transgenic mice expressing a diabetogenic TCR. Thymocytes expressing the islet-specific 4.1-TCR, which is highly diabetogenic when expressed in the NOD background (I-A^{g7} homozygous), undergo deletion in NOD mice that coexpress antidiabetogenic MHC class II molecules (Schmidt et al., 1999; Thiessen et al., 2002). Central deletion of 4.1-thymocytes is exclusively mediated by hematopoietic cells and requires the presentation of a specific, but as yet unknown, peptide(s) (Schmidt et al., 1999; Thiessen et al., 2002). Notably, 4.1-thymocyte-deleting class II molecules could restrict neither the positive selection of 4.1-thymocytes on the thymic cortex nor the presentation of their target autoantigen in the periphery, suggesting that the deleting peptide(s) is(are) exclusively expressed by a thymus-resident, tolerogenic APC, possibly a DC. Figure 6.1B represents the interpretation of the data. Namely, the MHC-promiscuous autoreactive T cells undergo central tolerance by engaging protective MHC–peptide complexes on thymic medullary APCs. In the absence of protective MHC molecules, these autoreactive T cells evade central tolerance and migrate to the periphery. Whether these observations account for the MHC-linked resistance of wild-type mice to autoimmune diabetes or are a peculiarity of 4.1-like $CD4^+$ T cells remains to be determined. What is known is that this phenomenon does not target all autoreactive T cells. For example, antidiabetogenic MHC class II molecules, such as I-A^b, I-E^k and I-A^{g7PD} (encoding proline and aspartic acid at positions 56 and 57, respectively), did not trigger deletion of thymocytes expressing another islet antigen-specific, I-A^{g7}-restricted TCR (BDC2.5). Whereas I-A^{g7PD} rendered 4.1-$CD4^+$ thymocytes partially unresponsive to antigen (Thiessen et al., 2002), it fostered the positive selection of BDC2.5 $CD4^+$ thymocytes (Kanagawa et al., 1998). Neither 4.1 nor BDC2.5 $CD4^+$ T cells could recognize islet antigen in the

context of this MHC molecule (Kanagawa et al., 1998). Since the BDC-2.5 TCR is not diabetogenic when expressed as a transgene in immunocompetent NOD mice, it is tempting to speculate that protective MHC class II molecules selectively (or preferentially) target certain highly pathogenic T-cell specificities, rather than all self-reactive T cells regardless of pathogenic potential.

An alternative, albeit certainly not mutually exclusive possibility, is that protective MHC class II molecules act by promoting the selection of non-autoreactive T cells with regulatory properties (Bohme et al., 1990; Parish et al., 1993; Singer et al., 1993) (Figure 6.1B). For example, NOD mice expressing an I-A^k transgene developed a significantly reduced incidence of diabetes, yet T cells from the spleens of these mice could adoptively transfer diabetes into NOD.scid recipients (Slattery et al., 1993). Thus, protective MHC class II molecules need not delete all islet-autoreactive T cells to afford diabetes resistance. Nevertheless, these observations do not prove a contribution of Tregs to disease resistance, or rule out a tolerogenic effect of I-A^k on T-cell specificities that might play key roles in the natural history of the spontaneous disease. Whatever the mechanism, it is clear that MHC class II molecules contribute to diabetes susceptibility and resistance by promoting the development of different TCR repertoires.

TOLERANCE VERSUS BENIGN AUTOREACTIVITY OR PATHOGENIC AUTOIMMUNITY

Not all self-proteins are immunogenic or capable of driving autoimmune responses. Autoreactive T-cell responses preferentially target certain self-proteins, such as thyroid-stimulating hormone receptor in Graves' disease; myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) in EAE/MS; H^+/K^+ ATPase in autoimmune gastritis; or insulin, glutamic acid decarboxylase (GAD), and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) in T1D. Surprisingly, many of these antigenic T-cell specificities are tolerized during T-cell development, at least partially, even in disease-susceptible genetic backgrounds. For example, although NOD mice mount T-cell responses to various GAD65 epitopes, thymocytes expressing a transgenic GAD65-reactive TCR ($GAD65_{286–300}$) underwent negative selection (Tian et al., 1997; Tisch et al., 1999). A similar phenomenon was observed in transgenic mice expressing a TCR recognizing an H^+/K^+ ATPase β chain epitope (Alderuccio et al., 2000). In addition, whereas $MBP_{121–150}$-specific T cells developed normally in MBP gene-targeted mice, they succumbed to central tolerance in MBP-competent animals. Obviously, susceptibility of autoreactive thymocytes to tolerance depends not only on the affinity of the target autoantigenic peptide for self-MHC, but also on the abundance of the corresponding MHC–peptide complex on tolerogenic APCs. For example, $MBP_{1–11}$, which binds self-MHC with significantly lower affinity than $MBP_{121–150}$, did not delete TCR-transgenic thymocytes in MBP-competent mice (Harrington et al., 1998; Huseby et al., 2001b). However, when $MBP_{1–11}$'s affinity (for MHC) or concentration were increased (by introducing single amino acid substitutions in the peptide), deletion was observed (Liu et al., 1995; Pearson et al., 1997). Notwithstanding these observations, not all TCRs capable of recognizing potentially tolerogenic (stable and/or abundant) autoantigen–MHC complexes will necessarily undergo tolerance: the outcome of T-cell development is also a func-tion of the affinity/avidity with which individual TCRs engage specific MHC–peptide complexes. Thus, whereas TCR-transgenic thymocytes recognizing $IGRP_{206–214}/K^d$ with intermediate avidity developed normally, those recognizing the same complex with higher avidity did not (P. Santamaria, unpublished observations). Since high-affinity T cells can remove target MHC–peptide complexes from the surface of APCs (Kedl et al., 2002), high-affinity autoreactive T cells may spare lower-affinity T-cells with identical antigenic specificity from tolerance induction by downregulating the corresponding MHC–peptide complex on APCs (Perchellet et al., 2004). Autoreactive T cells may evade central tolerance if the target autoantigen is only expressed at low levels of tolerogenic APCs (Figure 6.1C).

Another important determinant of tolerance susceptibility is whether or not developing thymocytes encounter MHC–peptide complexes on medullary epithelial cells or bone marrow-derived APCs (Anderson et al., 2002; Ramsdell and Ziegler, 2003). In some cases, autoreactive T cells escape central tolerance because they target epitopes encoded on mRNA splice variants that are not expressed in tolerogenic APCs (Klein et al., 2000; Kuchroo et al., 2002). In other instances, such as appears to be the case for $IGRP_{206–214}/K^d$-reactive thymocytes, tolerance occurs despite the fact that the self-antigen is not expressed by thymic stromal cells (P. Santamaria, unpublished observation). Whether tolerance in this system is induced by delivery of $IGRP_{206–214}/K^d$ complexes to the thymus by autoantigen-loaded DCs, or by a different MHC–peptide complex remains to be determined.

Another important, albeit not always openly recognized, concept is that autoreactivity does not necessarily imply pathogenicity. Transgenic mice expressing TCRs specific for several MBP or MOG epitopes, including $MBP_{1–11}$, $MBP_{84–102}$, $MBP_{121–150}$, and $MOG_{35–55}$, did not spontaneously develop (or only did so with a low incidence) symptoms of EAE when housed in specific pathogen-free environments (Goverman et al., 1993; Lafaille et al., 1994; Madsen et al., 1999; Huseby et al., 2001b). In contrast, mice expressing $PLP_{139–151}$-reactive TCRs developed a high incidence of

disease, regardless of whether the TCRs came from encephalitogenic or non-encephalitogenic T cell clones (Waldner et al., 2000). Likewise, whereas T-cells recognizing an H^+/K^+ ATPase β subunit epitope barely induced autoimmune gastritis in TCR-transgenic BALB/c mice, those recognizing an H^+/K^+ ATPase α subunit epitope were highly gastritogenic (McHugh et al., 2001). Another example of incomplete correlation between autoreactivity and pathogenicity comes from studies of β cell autoreactive TCR-transgenic NOD mice. Whereas expression of the class II-restricted 4.1-TCR led to a dramatic acceleration of onset of diabetes (Schmidt et al., 1997), expression of the BDC2.5 TCR did not. In fact, the incidence of diabetes in BDC2.5 TCR-transgenic NOD mice is significantly lower than that seen in wild-type NOD mice (Kurrer et al., 1997; Luhder et al., 2000). Such effects of antigenic (and epitope) specificity on pathogenicity were also observed when a panel of GAD65-derived peptides were injected into young NOD mice. Certain epitopes protected the animals from T1D by inducing interleukin (IL)-4 producing Th2 cells (Tisch et al., 1999). Interestingly, T cells expressing a $GAD65_{286–300}$-specific TCR not only were nonpathogenic, but actually delayed the onset of diabetes in NOD.scid recipients of diabetogenic T cells (Tarbell et al., 2002).

Another factor that contributes to the expression of clinical autoimmunity is antigen processing. T-cell epitopes can be classified into at least two broad functional groups. The first group includes all naturally processed peptides capable of binding self-MHC molecules. The second includes the so-called cryptic epitopes, i.e., epitopes that are either not presented at all, or presented only inefficiently (See Chapter 14). T cells recognizing cryptic determinants that can be presented by APCs readily escape central tolerance because these determinants are presented at levels that fall below the threshold required for induction of tolerance (Figure 6.1C). Another example of crypticity is related to the ability/inability of autoreactive TCRs to recognize specific core sequences within larger epitopes. For example, MBP-pulsed APCs generate substantial amounts of $MPB_{1–17}$ and $MBP_{1–18}$, and these peptides bind MHC molecules with high affinity (Seamons et al., 2003). However, neither of these two peptides is recognized well by $MBP_{1–11}$-specific T cells. This phenomenon may account for both the lack of tolerance and the low incidence of disease in mice expressing $MBP_{1–11}$-specific TCR transgenes (Goverman et al., 1993; Brabb et al., 1997). Pathogenicity of such cryptic epitope-specific T cells may be enhanced by exposure to certain environmental factors, such as infectious agents capable of eliciting inflammation or molecular mimicry. Lastly, it is important to recognize the possibility that initiation of autoimmunity need not involve T cells recognizing the ultimate cellular target of the autoimmune response (i.e., pancreatic β cells in T1D). Initiation of T1D in both NOD mice and humans, for example, is associated with autoreactive T- and B-cell responses against peri-islet Schwann cells (Winer et al., 2003). Likewise, arthritis in a TCR-transgenic mouse model was triggered by recognition of a ubiquitous autoantigen (see below) (Kouskoff et al., 1996).

In summary, it is clear that pathogenic autoreactive T cells must "jump" through a number of "hoops" before they can establish themselves in the peripheral immune system. Factors such as presence or absence of anti- and pro-autoimmune MHC class II molecules, affinity of autoreactive TCRs for peptide–MHC, and levels of autoantigen expression will ultimately determine whether potentially pathogenic autoreactive T cells will develop. These factors are outlined individually in Figure 6.1A–C. Figure 6.1D attempts to integrate the contribution of each of these variables to the fate of autoreactive T cells.

T–T CELL INTERACTIONS AND SPONTANEOUS AUTOIMMUNITY

Most organ-specific, spontaneous autoimmune diseases require the contribution of $CD4^+$ and $CD8^+$ T cells. Both these T-cell subsets are found in the pancreatic or central nervous system (CNS) lesions of T1D and MS patients, respectively (Hauser et al., 1986; Somoza et al., 1994; Babbe et al., 2000; Skulina et al., 2004; Zang et al., 2004), and both recognize tissue-specific antigens (Tsuchida et al., 1994; Schmidt, 1999; Abiru and Eisenbarth, 2000). The same is true in animal models of these diseases. Unsorted splenic T cells from prediabetic NOD mice can transfer diabetes into NOD.scid or young nondiabetic NOD recipients, but splenic T cells depleted of $CD4^+$ or $CD8^+$ T cells cannot (Bendelac et al., 1987; Miller et al., 1988; Christianson et al., 1993). Since diabetes can be transferred by preactivated islet-specific $CD4^+$ or $CD8^+$ T-cell clones (Bradley et al., 1992; Peterson and Haskins, 1996), it is reasonable to suspect that T–T collaboration is required for activation of the pathogenic activities of autoreactive T cells. Alternatively, transfer of relatively large numbers of clonal T cells overwhelms the naturally-occurring immune-regulatory mechanisms that are responsible for keeping in check the limited numbers of naïve autoreactive T cells that are normally present in the circulation (see below). These considerations may account, at least in part, for the dramatic acceleration of disease that is normally seen in monoclonal TCR-transgenic NOD mice (Verdaguer et al., 1997; Graser et al., 2000; Gonzalez et al., 2001). $CD4^+$ and $CD8^+$ T cells also seem to play an equally important role in the pathogenesis of experimental autoimmune myasthenia gravis (EAMG) (Zhang et al., 1996). Although it has been generally held that EAE is a disease predominantly driven by $CD4^+$ T cells (Pettinelli and McFarlin, 1981; Jiang et al., 1992), recent studies have shown that MBP-specific $CD8^+$ T cells, while not necessary for the initiation of EAE,

contribute to its pathogenesis (Huseby et al., 2001a; Sun et al., 2001; 2003). $CD4^+$ T cells seem to be less critical for development of CIA, because CD4-deficient mice were significantly more susceptible to CIA development than their CD8-deficient counterparts (Tada et al., 1996).

Although there is compelling evidence to suggest that $CD4^+$ T cells are also required (Thivolet et al., 1991; Christianson et al., 1993), studies of $CD8^+$ T-cell-deficient NOD mice have shown that $CD8^+$ T cells are key to the initiation of the diabetogenic process (Wang et al., 1996; Serreze et al., 1997; DiLorenzo et al., 1998). Observations made in MHC class I-restricted, β cell autoreactive TCR-transgenic NOD mice are consistent with the idea that $CD4^+$ T cells contribute to the diabetogenic process by enhancing the recruitment, accumulation, and/or differentiation of Th-dependent autoreactive $CD8^+$ clonotypes. Recruitment of $CD8^+$ T cells expressing the 8.3-TCR, for example, is enhanced by endogenous (non-transgenic) $CD4^+$ T cells or by splenic $CD4^+$ T cells derived from non-transgenic NOD mice (Verdaguer et al., 1997). Not all diabetogenic $CD8^+$ clonotypes, however, require the assistance of $CD4^+$ Th cells to home to and/or accumulate into pancreatic islets. $CD8^+$ T cells expressing the AI4-TCR (recognizing a different antigen from the 8.3-TCR), for example, are as diabetogenic in the presence of $CD4^+$ T cells as they are in their absence (Graser et al., 2000).

Although the mechanisms underlying the helper-dependency or -independency of $CD8^+$ clonotypes remain unclear, there are some informative clues. Progression of diabetes in NOD mice is accompanied by avidity maturation of the $IGRP_{206–214}/K^d$-reactive $CD8^+$ T-cell subpopulation, such that low-avidity clonotypes predominating at the outset of insulitis are progressively replaced by high-avidity ones as the animals age (Amrani et al., 2000). Interestingly, studies employing TCR-transgenic NOD mice have shown that high-avidity $IGRP_{206–214}/K^d$-reactive $CD8^+$ T cells can trigger diabetes in a Th-independent manner (P. Santamaria, unpublished observations), suggesting that the requirement for T-cell help is a function of avidity. In this case scenario, the low-avidity clonotypes that prevail at the outset of the diabetogenic autoimmune response would only be able to differentiate into β cell killers in the presence of $CD4^+$ Th cells. With progressive recruitment of the less prevalent high-avidity clonotypes into islets, this Th-dependent response would evolve into a Th-independent one. This interpretation of the data is consistent with the observation that purified $CD4^+$ and $CD8^+$ T cells from nondiabetic NOD mice cannot transfer disease into NOD.scid mice unless they are transferred together (Bendelac et al., 1987; Miller et al., 1988; Christianson et al., 1993).

Collaboration between $CD4^+$ Th cells and precytotoxic $CD8^+$ T cells is usually bridged by DCs, which can process exogenous antigens through the endogenous pathway of antigen presentation. Engagement of CD40 on DCs by CD154 (CD40 ligand) on antigen-specific $CD4^+$ T cells upregulates the expression of costimulatory molecules on DCs, elicits the production of proinflammatory cytokines, and endows them with the ability to foster the differentiation of naïve $CD8^+$ T cells into cytotoxic effectors (van Essen et al., 1995; Yang and Wilson, 1996). In fact, blockade of the CD40–CD154 interaction prevents the development of several different autoimmune diseases in animal models, including EAE (Grewal et al., 1995; Gerritse et al., 1996; Howard et al., 1999; Girvin et al., 2002), thyroiditis (Carayanniotis et al., 1997), colitis (De Jong et al., 2000), lupus (Kalled et al., 2001), and T1D (Balasa et al., 1997; Green et al., 2000). In addition to triggering the activation of DCs, engagement of CD40 by CD154 is key to $CD4^+$ Th cell activation (Becher et al., 2001; Amrani et al., 2002). Furthermore, since CD40–CD154 interactions are dispensable for the development and function of regulatory $CD4^+CD25^+$ T cells, and since regulatory T cells inhibit DC maturation (Serra et al., 2003), CD40 blockade also prevents autoimmunity by promoting the active suppression of $CD8^+$ T-cell responses. Lastly, it is important to note that autoreactive $CD4^+$ T cells not only contribute to autoimmune responses by providing T-cell help; they, too, can differentiate into effectors capable of killing targets through a number of mechanisms (Bradley et al., 1992; Goverman et al., 1993; Lafaille et al., 1994; Peterson and Haskins, 1996; Verdaguer et al., 1997; Graser et al., 2000; Huseby et al., 2001a; Sun et al., 2001).

Figure 6.2A represents some of the processes underlying T–T collaboration in autoimmunity. Naïve $CD4^+$ T cells undergo activation by recognizing target MHC–peptide complexes on mature autoantigen-loaded DCs. Cytokines and chemokines produced by these activated $CD4^+$ T cells foster the recruitment of additional $CD4^+$ and $CD8^+$ T cells to the site of the autoimmune inflammation. High-avidity autoreactive $CD8^+$ T cells differentiate into effectors in the absence of Th cells. Activation of their low-avidity counterparts, on the other hand, requires the presence of $CD4^+$ Th cells. Such $CD4^+$ Th cells induce costimulatory activity on DCs by ligating CD40. Tissue damage in autoimmunity is ultimately caused by both T-cell types.

CYTOKINES AND T-CELL-MEDIATED AUTOIMMUNITY

Cytokines play pivotal roles in the development and regulation of immune responses in general, and T-cell-mediated autoimmunity in particular. According to their role in immune responses, cytokines can be categorized as being proinflammatory [e.g., interferon (IFN)-γ and TNF-α] or anti-inflammatory (e.g., IL-4 and TGF-β). Depending on the cytokines they produce, most $CD4^+$ T cells can be subclassified into Th1 and Th2 types (See Chapter 7). Th1 cells typ-

ically produce IFN-γ, TNF-α, and IL-2, whereas Th2 cells generally produce IL-4, IL-5, and IL-13. Cytokines secreted by autoreactive lymphocytes, particularly those with proinflammatory activity, can cause tissue damage either directly (i.e. by ligating proapoptotic receptors on target cells) or indirectly [i.e., by inducing the production of secondary mediators, such as nitric oxide (NO)]. Since an indepth discussion of the role of cytokines in autoimmunity is beyond the scope of this chapter, this section focuses on specific examples to illustrate the notion that cytokines often play paradoxical roles in autoimmunity, depending on the location, timing, and magnitude of expression.

Inflammatory lesions in the brains of patients with MS or in the pancreatic islets of patients with T1D contain significant levels of proinflammatory cytokines, such as INF-γ and TNF-α (Brosnan and Raine, 1996; Balashov et al., 1999). Peripheral T cells from both types of patients produce increased levels of IFN-γ, and the magnitude of this phenotype is associated with disease progression (Dettke et al., 1997; Begolka et al., 1998; Petereit et al., 2000; Karni

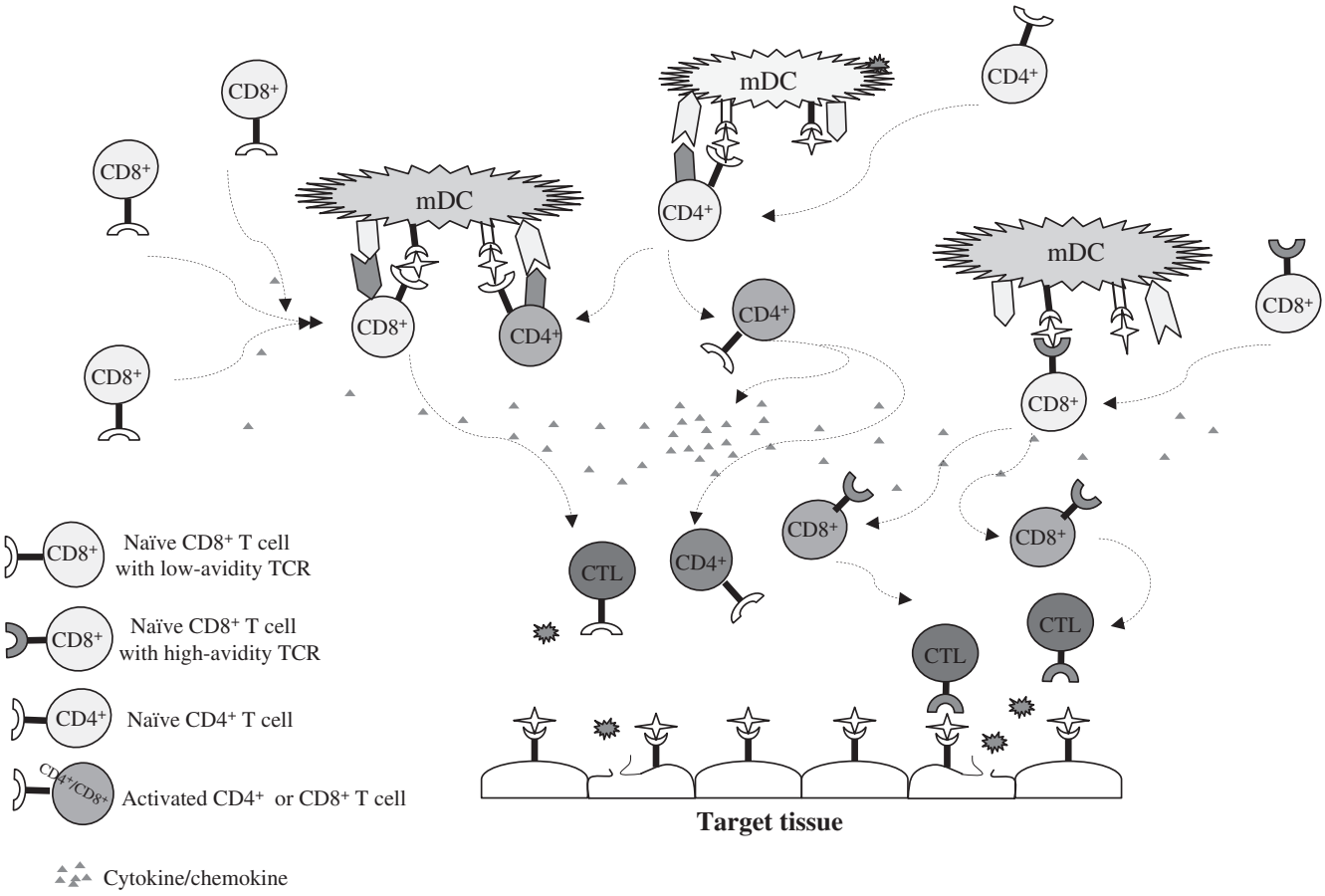
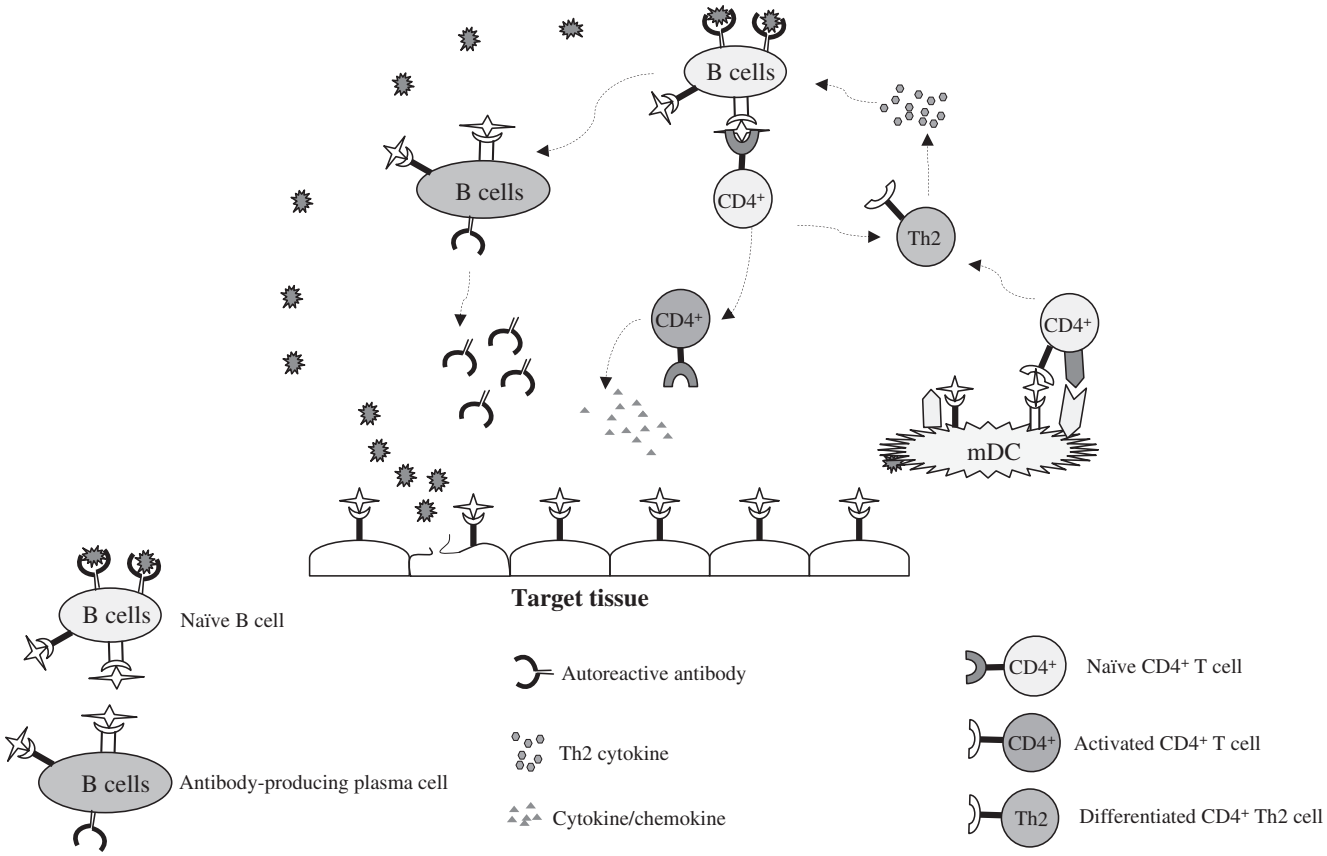
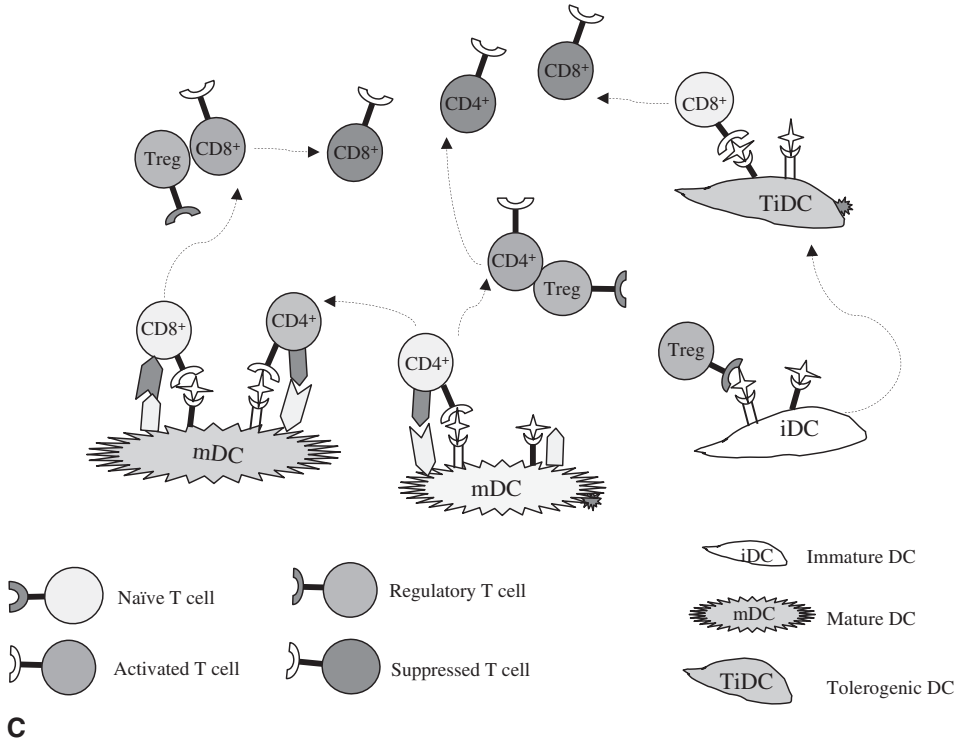


FIGURE 6.2 T–T and T–B interactions in the development of autoimmune responses. *A*, T–T collaboration in autoimmunity. *B*, Autoimmune reactions driven by collaboration between autoreactive T and B cells. *C*, Regulatory T cells suppress the activation of autoreactive T cells. In the steady-state, iDCs are kept in check by regulatory T cells (Treg) and, on capturing autoantigens, may have tolerogenic properties (TiDCs) against naïve autoreactive T cells. In the presence of activated helper T (Th) cells, Toll-like receptor ligands and/or proinflammatory (Th1) cytokines, iDCs are derepressed and differentiate into mature DCs (mDCs). Engagement of DCs by $CD4^+$ Th cells leads to CD154 upregulation and generation of effector $CD4^+$ cytotoxic T lymphocytes (CTLs). Ligation of CD40 on DCs by CD154 endows DCs with the ability to costimulate autoreactive $CD8^+$ T cells, and to foster their differentiation into $CD8^+$ CTLs (*A* and *D*). On capturing autoantigens via cell-surface immunoglobulins (BCR), B cells can activate autoreactive T cells and thus foster their differentiation into Th cells. Th2 cytokines produced by autoreactive $CD4^+$ Th can, in turn, promote the differentiation of naïve autoreactive B cells into autoantibody-secreting plasma cells. These autoantibodies may or may not have pathogenic significance, depending on the autoimmune disease (*B* and *D*). Tregs can suppress T-cell responses by inhibiting DC maturation, or by preventing T-cell activation or CTL differentiation (*C* and *D*). BCR, B-cell receptor; TCR, T-cell receptor. See color plate section.

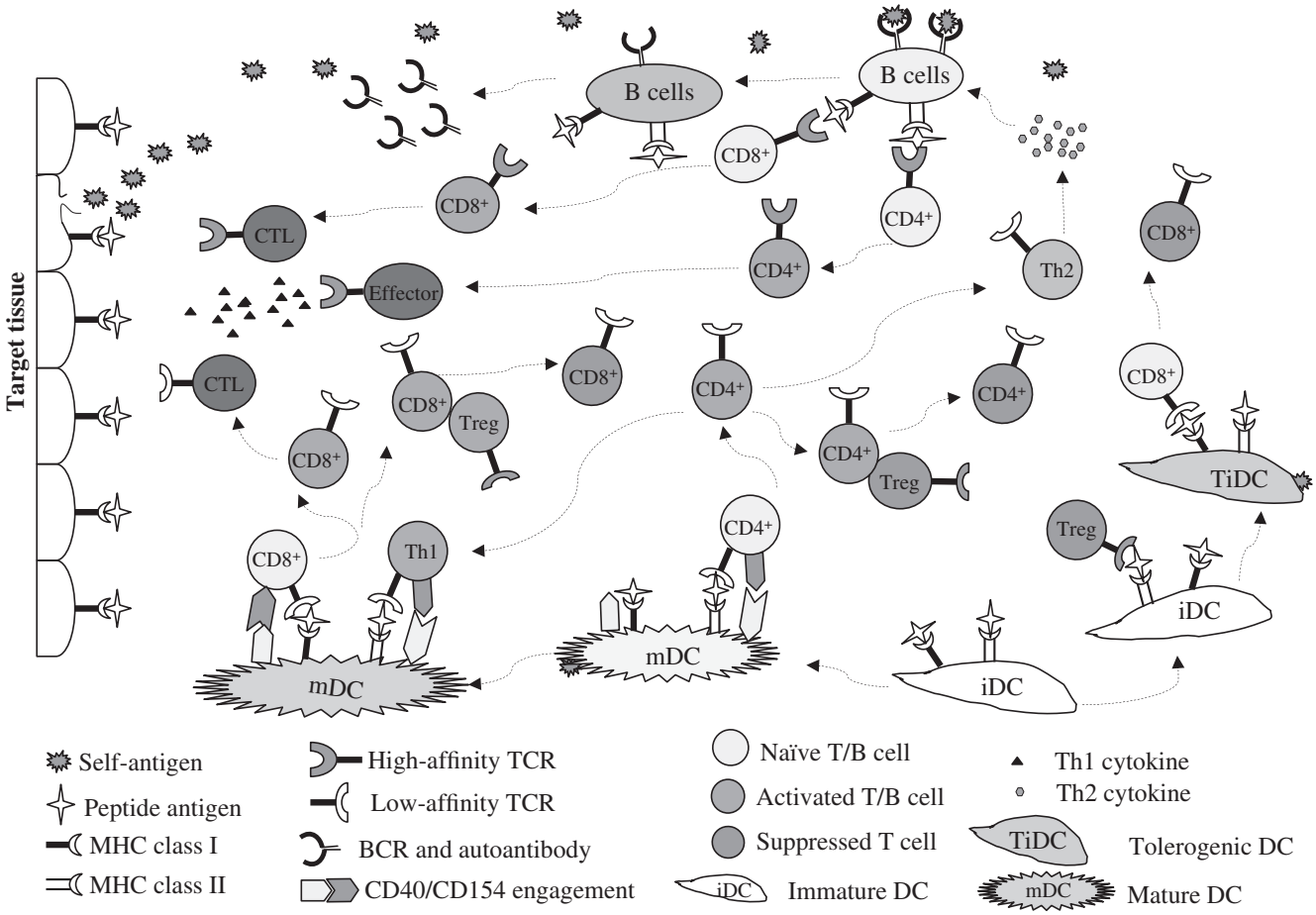


B



C

FIGURE 6.2 (*Continued*)



D

FIGURE 6.2 (*Continued*) *D*, Interactions that fuel or control autoimmunity in the periphery. Immunogenic antigens released from damaged tissues are captured and processed by B cells and immature dendritic cells (iDCs).

et al., 2002; 2004; Arif et al., 2004; Ott et al., 2004). In agreement with this, autoreactive T-cell clones and lines derived from patients and animal models of these two diseases produce large quantities of IFN-γ (Suarez-Pinzon et al., 1996; Bradley et al., 1999; Waldner et al., 2000; Huseby et al., 2001a; Pewe and Perlman, 2002; Bettelli et al., 2003). In addition, decreased production of IFN-γ by such cells correlated with clinical remission or delayed progression of disease (Kuchroo et al., 1995; Nicholson et al., 1995; Faust et al., 1996; Segal and Shevach, 1996; Trembleau et al., 1997). Furthermore, transgenic expression of IFN-γ in pancreatic islets or the CNS enhanced disease progression in animal models of these two diseases (Sarvetnick et al., 1990; Horwitz et al., 1997). Notably, patients with MS treated with IFN-γ displayed an exacerbated clinical course (Panitch et al., 1987). It should be noted, however, that, under certain circumstances, IFN-γ can also play a protective role in autoimmunity. In contrast to the outcome of clinical trials in patients with MS (Panitch et al., 1987), administration of IFN-γ to mouse models of MS and T1D decreased both the severity and incidence of these diseases (Voorthuis et al., 1990; Sobel et al., 2002). IFN-γ blockade with antibodies exacerbated the development of EAE in mice (Lublin et al., 1993; Heremans et al., 1996). Although genetic elimination of IFN-γ or its receptor had no significant effects on disease development (Ferber et al., 1996; Hultgren et al., 1996; Kanagawa et al., 2000; Serreze et al., 2000), animals lacking IFN-γ or IFN-γ receptor genes displayed increased susceptibility to EAE and T1D, depending on the genetic backgrounds that were studied or the disease induction protocols that were employed (Krakowski and Owens, 1996; Willenborg et al., 1996; Serreze et al., 2001; Trembleau et al., 2003). Some evidence suggests that the protective effects of IFN-γ on autoreactive responses are effected mainly in the periphery (Willenborg et al., 1999; Chu et al., 2000), although this remains to be demonstrated. Accordingly, the effects of certain cytokines (in this case, IFN-γ) on autoimmune responses are not always those expected. The timing

of expression is a very important consideration. For example, whereas early expression of TNF-α in pancreatic islets of transgenic mice accelerated diabetes, late expression prevented disease onset (Yang et al., 1994; Grewal et al., 1996; Green et al., 2000).

Th2 cytokines are thought to play a regulatory role in the development of T-cell–mediated autoimmunity. Peripheral autoreactive T cells from both MS and T1D patients expressed lower levels of IL-10 than autoreactive T cells derived from healthy controls (Ferrante et al., 1998; Rapoport et al., 1998; Kallmann et al., 1999). In addition, systemic treatment with, or transgenic expression of, IL-10 (Pennline et al., 1994; Rott et al., 1994) protected animals from EAE, autoimmune colitis, and T1D (Moritani et al., 1996; Bettelli et al., 1998; Nitta et al., 1998; Xiao et al., 1998, Cua et al., 1999; Pauza et al., 1999; Asseman et al., 2003). These observations are in agreement with the idea that many clonal T cells that regulate autoimmune diseases mediate their functions via IL-10 (Burkhart et al., 1999; Maron et al., 1999; Stohlman et al., 1999; Asseman et al., 2003; Bettelli et al., 2003; Frenkel et al., 2003). However, when IL-10 was specifically overexpressed in pancreatic islets, islet cells were destroyed rapidly (Moritani et al., 1994; Wogensen et al., 1994). It is likely that local expression of IL-10 accelerated the pathogenic process by fostering the recruitment of lymphocytes, possibly by upregulating the expression of intercellular adhesion molecule-1 (ICAM-1) (Wogensen et al., 1993; Balasa et al., 2000). In contrast, islet expression of viral–IL-10 mice protected from T1D (Kawamoto et al., 2001). Expression of IL-10 in the CNS also had a protective rather than a pathogenic effect (Cua et al., 2001), possibly because IL-10 expression was not sufficient to promote the recruitment of lymphocytes across the blood–brain barrier. IL-4 is another Th2 cytokine that has anti-inflammatory effects. Whereas increased production of IL-4 had protective effects against a number of autoimmune disorders, targeted disruption of the IL-4 gene had little or no effect on the natural history of these diseases (Rapoport et al., 1993; Mueller et al., 1996; Croxford et al., 1998; Samoilova et al., 1998; Pal et al., 1999; Martino et al., 2000; Serreze et al., 2001). When pathogenic T-cell clones were engineered to produce IL-4, they protected animals from EAE and T1D (Shaw et al., 1997; Maron et al., 1999). However, it was also reported that IL-4 producing autoreactive T cells could trigger EAE and T1D (Lafaille et al., 1997; Pakala et al., 1997; Poulin and Haskins, 2000; Durinovic-Bello et al., 2004). When taken together, these studies have revealed that the effects of cytokines in autoimmunity are more a function of the location and timing of expression than of their pro- or anti-inflammatory nature alone. Another important concept is that the effects of individual cytokines on autoimmune responses need to be studied in the context of all the other cytokines that are produced at the site of inflammation (Santamaria, 2003). Ultimately, it is the combined effects of all these cytokines that determine (or contribute to) the outcome.

B–T CELL COLLABORATION IN AUTOIMMUNITY

B cells often play important roles in the pathogenesis of organ-specific, T-cell-dependent autoimmune diseases. T1D patients and NOD mice produce autoantibodies against numerous islet antigens, including insulin, islet autoantigen-2 (IA-2) and GAD65, presumably as a result of defective B-cell tolerance to soluble autoantigens (Thomas et al., 2002; Silveira et al., 2004). Furthermore, B-cells are prevalent in the insulitis lesions of prediabetic NOD mice and T1D patients. Notwithstanding this, B cells or sera from diabetic NOD mice cannot transfer disease to healthy recipients. Although B cells are not sufficient in diabetogenesis, NOD mice do not spontaneously develop T1D, or do so with significantly reduced incidence and delayed kinetics if they lack B cells, suggesting that B cells play an essential role in diabetogenesis (Serreze et al., 1996; Akashi et al., 1997; Yang et al, 1997; Noorchashm et al., 1997 and 1999). It has been suggested that autoreactive B cells contribute to the disease process by capturing islet antigens via surface immunoglobulins (Falcone et al., 1998; Serreze et al., 1998; Noorchashm et al., 1999). In fact, the role of B cells in murine T1D seems to be restricted to early T-cell priming events, because NOD T cells can readily transfer diabetes into B-cell-deficient recipients if they are allowed to undergo priming in the donors (Charlton et al., 2001). Although monoclonal islet autoreactive TCR-transgenic NOD mice develop diabetes despite lacking mature B cells (Verdaguer et al., 1997; Graser et al., 2000; Gonzalez et al., 2001), the large frequency of circulating autoreactive T cells in these mice may overwhelm (artificially) the need for B cells. Alternatively, B cells are not necessary for the activation of naïve autoreactive T cells, but contribute to the maintenance and/or expansion of the peripheral autoreactive T-cell repertoire (Jaume et al., 2002; Dromey et al., 2004).

The recent description of a B-cell-deficient patient with T1D casts some doubts as to whether B cells are necessary for development of human T1D (Naserke et al., 2001). There is also some controversy as to whether autoantibodies contribute to the pathogenesis of T1D. Although the presence of islet antigen-specific autoantibodies in sera from T1D patients and NOD mice forecasts progression of clinically silent islet autoimmunity to overt T1D, these autoantibodies are generally considered to be nonpathogenic (Devendra et al., 2004). There is some evidence, however, that transplacental transfer of maternal autoantibodies in NOD mice affords risk of diabetes to the offspring (Greeley et al., 2002).

The role of autoreactive B cells in other T-cell-driven autoimmune diseases like MS seems to be different from that in T1D. Autoantibody-producing B cells are present in the CNS lesions of MS patients (Prineas and Wright, 1978; Genc et al., 1997), and in both MS and EAE, autoantibodies are associated with destruction of myelin (Storch et al., 1998; Genain et al., 1999). In EAE, B cells are only required when the disease is induced by immunization with intact protein antigens, but not when it is induced with peptides (Wolf et al., 1996; Lyons et al., 1999; Dittel et al., 2000; Svensson et al., 2002), suggesting that here, too, B cells contribute to the disease process by processing and presenting target autoantigens. Nevertheless, it has been shown that the presence of autoantibodies enhances the severity of EAE (Myers et al., 1992; Genain et al., 1995; Lyons et al., 2002). It has also been reported that autoreactive B cells may contribute to the regulation of EAE, possibly by producing IL-10 (Fillatreau et al., 2002).

T cells play an important role in the development of autoimmune diseases effected by pathogenic autoreactive B cells, such as SLE. Although SLE is characterized by the presence of high titers of pathogenic autoantibodies against several nuclear antigens, including dsDNA, Sm and Ro/SSA (Kotzin, 1996; Morrow et al., 1999), SLE-prone mice do not develop disease in the absence of T cells (Steinberg et al., 1980; Wofsy et al., 1985; Santoro et al., 1988; Jevnikar et al., 1994). Furthermore, disease severity correlates with the expansion of autoreactive T cells (Lang et al., 2003), which recognize the same antigens as antibody-producing B cells (Crow et al., 1994; Kaliyaperumal et al., 1996), and induce the production of autoantibodies by the latter through a CD40–CD154-dependent mechanism (Early et al., 1996; Peng et al., 1997; Voll et al., 1997; Kalled et al., 2001). Although there is evidence for dysregulated B-cell activation in animal models of SLE (Chang et al., 2004), this seems to result, at least in part, from defects in T-cell signaling (Jury et al., 2004, Xu et al., 2004).

Another example of a B-cell-mediated autoimmune disease that is driven by T cells is RA. RA is a chronic inflammatory disease of the joints that is usually associated with presence of rheumatoid factor, and involves cartilage destruction and bone erosion owing to infiltration of synovial tissue by leukocytes, T cells, macrophages, and autoantibody-producing B cells (Mellbye et al., 1990, Kraan et al., 2004). B-cell-deficient mice are refractory to RA-like disease induction (Svensson et al., 1998), and the disease can be transferred by serum or purified immunoglobulins (Stuart and Dixon, 1983; Matsumoto et al., 1999; Maccioni et al., 2002). Although clearly B-cell dependent, susceptibility to RA is associated with specific MHC class II genes (Wordsworth et al., 1989; Devereux et al., 1991), and T-cell depletion impairs disease development in experimental rodent models, demonstrating a key role for T cells in its pathogenesis (Pelegri et al., 1996, Corthay et al., 1999; Ehinger et al., 2001; Pohlers et al., 2004). A number of mice expressing collagen-specific TCR transgenes have been developed (Mori et al., 1992; Osman et al., 1998); these animals develop accelerated RA when immunized with collagen. Another animal model of possible relevance to human RA is based on transgenic expression of a TCR cloned from a disease-unrelated T-cell clone. These mice spontaneously develop RA (Kouskoff et al., 1996) because their transgenic T cells recognize a peptide derived from a ubiquitously expressed self-antigen, glucose-6-phosphate isomerase (G6PI), in the context of the NOD mouse MHC class II molecule I-A^{g7}, and elicit the production of arthritogenic immunoglobulins by B cells (Korganow et al., 1999; Matsumoto et al., 1999). Accumulation of extracellular G6PI on the surface of cartilage attracts autoantibodies from the circulation and triggers pathogenesis (Matsumoto et al., 2002; Wipke et al., 2002). Interestingly, immunization of normal mice with G6PI induced a T-cell-dependent RA-like syndrome (Schubert et al., 2004), and anti-G6IP antibodies were detected in RA patients (Schaller et al., 2001; Matsumoto et al., 2003). Therefore, autoreactivity against a non-tissue-specific autoantigen can result in a tissue-specific autoimmune disorder.

Figure 6.2B represents interactions between autoreactive T and B cells during the development of autoimmunity. On the one hand, B cells "concentrate" autoantigens for effective presentation to autoreactive $CD4^+$ T cells. On the other, activated $CD4^+$ T cells produce cytokines capable of fostering the activation and differentiation of autoreactive B cells into antibody-secreting plasma cells.

REGULATORY T CELLS AND AUTOIMMUNITY

Central (thymic) tolerance is responsible for removing potentially pathogenic autoreactive T cells from the repertoire. Some autoreactive T cells, however, escape central tolerance and harmlessly circulate in the periphery without posing a significant health threat to the individual (Yan et al., 1992; Wekerle et al., 1996). This is so, at least in part, because these cells are normally kept in check by regulatory T cells. The immunosuppressive power of regulatory T cells against autoimmunity is best documented by studies of mice expressing autoreactive TCR transgenes. Despite exporting overwhelmingly high numbers of autoreactive T cells to the periphery, some of these mice either do not develop autoimmunity or do so with very low incidence. However, when development of T cells expressing endogenous TCRs, including regulatory T cells, is abrogated, the mice rapidly develop spontaneous autoimmunity (Lafaille et al., 1994; Olivares-Villagomez et al., 1998; Gonzalez et al., 2001).

Tregs can be categorized into two major functional subgroups: inducible and naturally occurring. Inducible Tregs

arise from peripheral nonregulatory T cells under specific conditions, such as in the presence of cytokines like IL-4, IL-10 or TGF-β (Cobbold et al., 2004; Fantini et al., 2004). A number of experimental therapeutic strategies for autoimmunity exploit the existence of this type of Treg (Harrison et al., 1996, Panoutsakopoulou et al., 2004). In the steady-state, however, autoreactive T cells are predominantly kept in check by thymus-derived, naturally-occurring Treg types like $CD4^+CD25^+$ and natural killer T (NKT) cells. $CD4^+CD25^+$ T cells express heterogeneous TCRs, are thought to arise from autoreactive thymocyte precursors, and are kept (in the periphery) in a state of chronic activation (Bensinger et al., 2001; Jordan et al., 2001; Fisson et al., 2003). Development of these T cells requires IL-2 and CD28, and is controlled by expression of the transcription factor Foxp3 (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). In fact, Tregs arising from conventional T cells in the periphery also express Foxp3 (Hori et al., 2003; Walker et al., 2003; Fantini et al., 2004). The regulatory activity of $CD4^+CD25^+$ T cells seems to be mediated by a poorly understood cell-contact-dependent mechanism that is capable of inhibiting IL-2 production by, and proliferation of, conventional T cells (Sakaguchi, 2004). CTLA-4 is thought to be necessary for this process (Boden et al., 2003; Fallarino et al., 2003; Liu et al., 2003). Recent work has shown that $CD4^+CD25^+$ T cells can inhibit diabetogenic $CD8^+$ T-cell responses indirectly, by suppressing DC maturation *in vivo* (Serra et al., 2003). It has also been shown that $CD4^+CD25^+$ T cells can, under certain circumstances, transform conventional (nonregulatory) $CD4^+$ T cells into inducible Tregs (Stassen et al., 2004, Zheng et al., 2004). Thus, as shown in Figure 6.2C, regulatory T cells may inhibit autoreactive T-cell responses directly by recognizing autoreactive T cells, or indirectly by suppressing DC maturation or by inducing tolerogenic activities in immature DCs.

Unlike $CD4^+CD25^+$ T cells, most NKT cells express an invariant TCR α chain (Benlagha et al., 2002, Kronenberg and Gapin, 2002, Sakaguchi, 2004) and recognize glycolipid antigens in the context of CD1d MHC molecules. Although their natural antigenic targets have not yet been identified, NKT cells can be activated by α-galactosylceramide (α-GalCer), a compound derived from a marine sponge. Like $CD4^+CD25^+$ T cells, NKT cells also arise in the thymus, but under the control of different signaling pathways. Development of NKT cells, for example, requires molecules such as Ets1, Fyn, cathepsin L, and RelB, which are dispensable for development of nonregulatory T cells (Gadue et al., 1999; Walunas et al., 2000; Honey et al., 2002; Elewaut et al., 2003; Sivakumar et al., 2003).

Although both $CD4^+CD25^+$ and NKT cells comprise a relatively small fraction of the peripheral T-cell repertoire, defective development of either subset promotes autoimmunity. For instance, IL-2-, CD80/CD28-, and Foxp3-deficient mice develop systemic or organ-specific autoimmunity, owing to the absence of $CD4^+CD25^+$ T cells (Almeida et al., 2002; Malek et al., 2002; Nelson, 2004). Notably, defective $CD4^+CD25^+$ T cells have been described in patients with specific autoimmune disorders (Kriegel et al., 2004; Ou et al., 2004). Early studies also found defective NKT-cell development in autoimmune-prone mice. In NOD mice, for example, there is impaired NKT-cell development, and NKT-cell transfer can inhibit T1D development (Mieza et al., 1996; Gombert et al., 1996; Hammond et al., 1998). In addition, NKT-cell deficiency enhances the development of T1D and EAE in NOD and C57BL/6 mice, respectively (Shi et al., 2001; Wang et al., 2001; Teige et al., 2004). Although abnormal NKT cell development has also been reported in humans, the contribution of this phenotype to human autoimmunity is less clear (Sumida et al., 1995; 1998; Wilson et al., 1998; Yanagihara et al., 1999; van der Vliet et al., 2001). Because NKT cells produce high levels of cytokines, such as IL-4 and IL-10, shortly upon activation, it is generally thought that these cytokines play a critical role in the suppressive activity of NKT cells (Miyamoto et al., 2001; Laloux et al., 2001; Sharif et al., 2001). These and other less well characterized populations of regulatory cells thus play a fundamental role in ensuring that the individual will respond vigorously to nonself, without mounting responses to self. Disruption of this balance surely has the potential to promote autoimmunity.

Figure 6.2D attempts to integrate the complex cellular interaction summarized in Figures 6.2A–C.

MEMORY T CELLS AND AUTOIMMUNITY

Antigen-primed naïve T cells proliferate and differentiate into effector cells. Upon undergoing clonal expansion, a fraction of these antigen-exposed T cells differentiate into long-lived memory cells. Memory $CD4^+$ and $CD8^+$ T-cells are responsible for the immune system's ability to mount recall responses against antigen re-encounters and thus are responsible for affording long-term protection against pathogens (Dutton et al., 1998). In mice, memory T cells arise from effector cells that exit the cell cycle and down-regulate activation markers, such as CD25 and CD69, while maintaining high levels of CD44 (Opferman et al, 1999; Hu et al., 2001). In humans, memory $CD4^+$ T cells are usually $CD45RA^-CD45RO^+$. Unlike effector cells, which are susceptible to activation-induced cell death, memory T cells can survive in nonlymphoid as well as secondary lymphoid organs without undergoing division (Sabbagh et al., 2004). Although generation and persistence of memory T cells do not always require MHC molecules (Murali-Krishna et al., 1999; Swain et al., 1999), their homeostatic survival is fostered by signals from the TCR and cytokines like IL-7 and

IL-15 (Kaech et al., 2003; Seddon et al., 2003). Importantly, upon antigen re-encounter, memory T cells are recruited rapidly to the site of inflammation, and expand and produce large amounts of cytokines, even in the presence of low levels of antigen and/or costimulatory signals (Pihlgren et al., 1996; Liu et al., 1997; Rogers et al., 2000; Veiga-Fernandes et al., 2000). Because memory T cells have increased functional avidities for ligand as compared to their naïve progenitors, and because the antigen-binding site of the TCR is conformationally flexible and potentially promiscuous, development of a sizeable population of memory T cells in response to an infectious agent bears a theoretical risk of eliciting autoimmunity.

Memory T cells can be categorized into central memory T (T_{CM}) cells, which migrate into secondary lymphoid organs, and effector memory T (T_{EM}) cells, which are recruited to inflamed nonlymphoid tissues (Sallusto et al, 1999; Iezzi et al., 2001; Masopust et al., 2001; Reinhardt et al, 2001). In humans, T_{CM} cells express CCR7 and CD62L, whereas T_{EM} cells are mostly CCR7- and CD62L-negative. T_{EM} cells express chemokine receptors characteristic of Th1 and Th2 cells, including CCR5 and CCR6 for Th1 cells and cytotoxic T lymphocytes, and CCR3 and CCR4 for both Th1 and Th2 cells. The expression pattern of chemokine receptors on T_{EM} cells parallels polarized cytokine profiles retained from effector cells. Indeed, $CD4^+$ T_{EM} cells produce Th1 or Th2 cytokines within hours of antigen stimulation, and $CD8^+$ T_{EM} cells express a large amount of perforin, allowing rapid effector function in response to antigen. T_{CM} cells proliferate vigorously and produce high levels of IL-2 in response to antigen, and differentiate into Th1- or Th2-like effector cells capable of producing abundant IFN-γ or IL-4 on antigen stimulation (Sallusto et al., 2004). In the absence of antigen, both T_{CM} and T_{EM} cells turn over, slowly, mostly in response to homeostatic cytokines. Whereas T_{EM} cells provide immediate protection against infectious agents, T_{CM} cells proliferate and differentiate into T_{EM} cells on secondary antigenic stimulation, ensuring the long-term retention of memory T-cell pools (Seder and Ahmed, 2003; Wherry et al., 2003).

Several lines of evidence suggest a role for memory T cells in autoimmune disease progression or recurrence. Murine T1D or EAE can be transferred to genetically susceptible recipients by splenic lymphocytes from affected individuals, consistent with the presence of autoreactive memory T cells in the spleen (Santamaria, 2001; Rossini et al., 2004). Since splenic memory T cells are probably T_{CM} cells, they likely undergo antigen-independent homeostatic expansion and antigen-dependent proliferation in the recipients before differentiating into effector cells. This may explain why transfer of T1D by splenocytes from diabetic NOD mice usually takes a few weeks. This contrasts with the rapidity with which pancreatic islet-associated T cells from prediabetic NOD mice, which presumably contain both effector and T_{EM} cells, transfer disease into NOD.scid hosts (Rohane et al., 1995). As noted above, homeostatic expansion of memory T cells involves cytokines such as IL-7 and IL-15, whose receptors share the common γ chain. Administration of a blocking anti-γ chain antibody to NOD mice effectively reduced the size of the memory T-cell population, and inhibited disease transfer, suggesting that expansion of memory T cells contributes to the progression of T1D (Demirci et al., 2003). It has also been shown that isolated $CD4^+CCR4^+CD44^{high}$ T cells can transfer insulitis into NOD.scid mice within a week of injection, underscoring their pathogenic potential. Furthermore, blockade of macrophage-derived chemokine (MDC), a ligand for CCR4, inhibited development of T1D in NOD mice (Kim et al., 2002). In addition, when the homeostatic turnover rate of the memory T-cell pool, which controls its size, was slowed by transfer of large numbers of syngenic T cells or by non-antigen-specific stimulation of endogenous T cells, NOD mice were protected from T1D (King et al., 2004). The formidable proclivity of diabetic NOD mice to disease recurrence following syngenic or allogenic islet transplantation provides additional evidence for the contribution of autoreactive memory T cells to the progression of this autoimmune disease. Another observation supporting the idea that memory T cells contribute to the progression of autoimmunity is that autologous hematopoietic stem cell transplantation in patients with severe multiple sclerosis can blunt disease progression in a subset of patients; in these patients, memory T cells are replaced by a diverse repertoire of naïve T cells that arise from new thymic emigrats (Muraro et al., 2005). Taken together, these observations suggest that development of autoreactive memory T cells fuels the progression of autoimmunity.

CONCLUDING REMARKS

In this chapter, we have provided a general overview on the role of T cells in autoimmunity. This is by no means a comprehensive review of the literature but rather a synopsis of recent developments in our understanding of the delicate balance that exists between autoreactive lymphocytes and their regulatory counterparts in both health and disease. Other chapters in this book deal in greater detail with some of the aspects we only touch upon here. Ultimately, we hope that the reader will gain a better understanding of how T cells contribute to autoimmunity, while maintaining an appreciation of the many complexities that remain unresolved.

Acknowledgments

The authors' work described in this review was supported by grants from the Canadian Institutes of Health Research, the Natural Sciences and

Engineering Research Council of Canada (to P. S.), the Juvenile Diabetes Research Foundation and the Canadian Diabetes Association (to Y. Y. and P. S.). P. S. is a Scientist of the Alberta Heritage Foundation for Medical Research. We thank all the members of our laboratories for excellent technical assistance and stimulating discussions. Y. Y. and P. S. are supported by the Julia McFarlane Diabetes Research Centre and the Diabetes Association Foothills. The authors thank Ms V. Crosbie for expert editorial assistance.

References

Abiru, N. and Eisenbarth, G.S. (2000). Multiple genes/multiple autoantigens role in type 1 diabetes. *Clin. Rev. Allergy Immunol. 18*, 27–40.

Akashi, T., Nagafuchi, S., Anzai, K., Kondo, S., Kitamura, D., Wakana, S., Ono, J., Kikuchi, M., Niho, Y., and Watanabe, T. (1997). Direct evidence for the contribution of B cells to the progression of insulitis and the development of diabetes in non-obese diabetic mice. *Int. Immunol. 9*, 1159–1164.

Alderuccio, F., Cataldo, V., van Driel, I.R., Gleeson, P.A., and Toh, B.H. (2000). Tolerance and autoimmunity to a gastritogenic peptide in TCR transgenic mice. *Int. Immunol. 12*, 343–352.

Allen, R.L., O'Callaghan, C.A., McMichael, A.J. and Bowness, P. (1999). Cutting edge: HLA-B27 can form a novel β2-microglobulin-free heavy chain homodimer structure. *J. Immunol. 162*, 5045–5048.

Almeida, A.R., Legrand, N., Papiernik, M., and Freitas, A.A. (2002). Homeostasis of peripheral $CD4^+$ T cells: IL-2Rα and IL-2 shape a population of regulatory cells that controls $CD4^+$ T cell numbers. *J. Immunol. 169*, 4850–4860.

Amrani, A., Verdaguer, J., Serra, P., Tafuro, S., Tan, R., and Santamaria, P. (2000). Progression of autoimmune diabetes driven by avidity maturation of a T-cell population. *Nature 406*, 739–742.

Amrani, A., Serra, P., Yamanouchi, J., Han, B., Thiessen, S., Verdaguer, P., and Santamaria, P. (2002). CD154-dependent activation of diabetogenic $CD4^+$ T cells dissociated from APC activation. *Immunity. 16*, 719–732.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the AIRE protein. *Science 298*, 1395–401.

Arif, S., Tree, T.I., Astill, T.P., Tremble, J.M., Bishop, A.J., Dayan, C.M., Roep, B.O., and Peakman, M. (2004). Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J. Clin. Invest. 113*, 451–463.

Asseman, C., Read, S., and Powrie, F. (2003). Colitogenic Th1 cells are present in the antigen-experienced T cell pool in normal mice: control by $CD4^+$ regulatory T cells and IL-10. *J. Immunol. 171*, 971–978.

Babbe, H., Roers, A., Waisman, A., Lassmann, H., Goebels, N., Hohlfeld, R., Friese, M., Schroder, R., Deckert, M., Schmidt, S., Ravid, R., and Rajewsky, K. (2000). Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J. Exp. Med. 192*, 393–404.

Balasa, B., Krahl, T., Pastone, G., Lee, J., Tisch, R., McDevitt, H., and Sarvetnick, N. (1997). CD40 ligand–CD40 interactions are necessary for the initiation of insulitis and diabetes in nonobese diabetic mice. *J. Immunol. 159*, 4620–4627.

Balasa, B., La Cava, A., Van Gunst, K., Mocnik, L., Balakrishna, D., Nguyen, N., Tucker, L., and Sarvetnick, N. (2000). A mechanism for IL-10-mediated diabetes in the nonobese diabetic (NOD) mouse: ICAM-1 deficiency blocks accelerated diabetes. *J. Immunol. 165*, 7330–7337.

Balashov, K.E., Rottman, J.B., Weiner, H.L., and Hancock, W.W. (1999). CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1α and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. USA 96*, 6873–6878.

Becher, B., Durell, B.G., Miga, A.V., Hickey, W.F., and Noelle, R.J. (2001). The clinical course of experimental autoimmune encephalomyelitis and inflammation is controlled by the expression of CD40 within the central nervous system. *J. Exp. Med. 193*, 967–974.

Begolka, W.S., Vanderlugt, C.L., Rahbe, S.M., and Miller, S.D. (1998). Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. *J. Immunol. 161*, 4437–4446.

Bendelac, A., Carnaud, C., Boitard, C., and Bach, J.F. (1987). Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both L3T4+ and Lyt-2+ T cells. *J. Exp. Med. 166*, 823–832.

Benlagha, K., Kyin, T., Beavis, A., Teyton, L., and Bendelac, A. (2002). A thymic precursor to the NK T cell lineage. *Science 296*, 553–555.

Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J., and Laufer, T.M. (2001). Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4(+)25(+) immunoregulatory T cells. *J. Exp. Med.* 194, 427–438.

Bettelli, E., Das, M.P., Howard, E.D., Weiner, H.L., Sobel, R.A., and Kuchroo, V.K. (1998). IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J. Immunol. 161*, 3299–3306.

Bettelli, E., Pagany, M., Weiner, H.L., Linington, C., Sobel, R.A., and Kuchroo, V.K. (2003). Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. *J. Exp. Med. 197*, 1073–1081.

Boden, E., Tang, Q., Bour-Jordan, H., and Bluestone, J.A. (2003). The role of CD28 and CTLA4 in the function and homeostasis of $CD4^+CD25^+$ regulatory T cells. *Novartis Found. Symp. 252*, 55–63, discussion 63–66, 106–114.

Bohme, J., Schuhbaur, B., Kanagawa, O., Benoist, C., and Mathis, D. (1990). MHC-linked protection from diabetes dissociated from clonal deletion of T cells. *Science* 249, 293–295.

Brabb, T., Goldrath, A.W., von Dassow, P., Paez, A., Liggitt, H.D., and Goverman, J. (1997). Triggers of autoimmune disease in a murine TCR-transgenic model for multiple sclerosis. *J. Immunol. 159*, 497–507.

Bradley, B., Haskins, K., Rosa, F.L., and Lafferty, K. (1992). CD8 T cells are not required for islet destruction induced by a $CD4^+$ islet-specific T cell clone. *Diabetes 41*, 1603–1608.

Bradley, L.M., Asensio, V.C., Schioetz, L.K., Harbertson, J., Krahl, T., Patstone, G., Woolf, N., Campbell, I.L., and Sarvetnick, N. (1999). Islet-specific Th1, but not Th2, cells secrete multiple chemokines and promote rapid induction of autoimmune diabetes. *J. Immunol. 162*, 2511–2520.

Breban, M., Fernandez-Sueiro, J.L., Richardson, J.A., Hadavand, R.R., Maika, S.D., Hammer, R.E., and Taurog, J.D. (1996). T cells, but not thymic exposure to HLA-B27, are required for the inflammatory disease of HLA-B27 transgenic rats. *J. Immunol. 156*, 794–803.

Brosnan, C.F. and Raine, C.S. (1996). Mechanisms of immune injury in multiple sclerosis. *Brain Pathol. 6*, 243–257.

Burkhart, C., Liu, G.Y., Anderton, S.M., Metzler, B., and Wraith, D.C. (1999). Peptide-induced T cell regulation of experimental autoimmune encephalomyelitis: a role for IL-10. *Int. Immunol. 11*, 1625–1634.

Carayanniotis, G., Masters, S.R., and Noelle, R.J. (1997). Suppression of murine thyroiditis via blockade of the CD40–CD40L interaction. *Immunology 90*, 421–426.

Chang, N.H., MacLeod, R., and Wither, J.E. (2004). Autoreactive B cells in lupus-prone New Zealand black mice exhibit aberrant survival and proliferation in the presence of self-antigen *in vivo*. *J. Immunol. 172*, 1553–1560.

Chao, C.C., Sytwu, H.K., Chen, E.L., Toma, J., and McDevitt, H.O. (1999). The role of MHC class II molecules in susceptibility to type I diabetes:

identification of peptide epitopes and characterization of the T cell repertoire. *Proc. Natl. Acad. Sci. USA 96*, 9299–9304.

Charlton, B., Zhang, M.D., and Slattery, R.M. (2001). B lymphocytes not required for progression from insulitis to diabetes in non-obese diabetic mice. *Immunol. Cell. Biol. 79*, 597–601.

Christianson, S., Shultz, L., and Leiter, E. (1993). Adoptive transfer of diabetes into immunodeficient NOD-SCID/SCID mice. Relative contributions of $CD4^+$ and $CD8^+$ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes 42*, 44–55.

Chu, C., Wittmer, S., and Dalton, D.K. (2000). Failure to suppress the expansion of the activated CD4 T cell population in interferon γ-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. *J. Exp. Med. 192*, 123–128.

Cobbold, S.P., Castejon, R., Adams, E., Zelenika, D., Graca, L., Humm, S., and Waldmann, H. (2004). Induction of foxP3(+) regulatory T cells in the periphery of T cell receptor transgenic mice tolerized to transplants. *J. Immunol. 172*, 6003–6010.

Corper, A.L., Stratmann, T., Apostolopoulos, V., Scott, C.A., Garcia, K.C., Kang, A.S., Wilson, I.A., and Teyton, L. (2000). A structural framework for deciphering the link between I-Ag7 and autoimmune diabetes. *Science 288*, 505–511.

Corthay, A., Johansson, A., Vestberg, M., and Holmdahl, R. (1999). Collagen-induced arthritis development requires αβ T cells but not γδ T cells: studies with T cell-deficient (TCR mutant) mice. *Int. Immunol. 11*, 1065–1073.

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.

Croxford, J.L., Triantaphyllopoulos, K., Podhajcer, O.L., Feldmann, M., Baker, D., and Chernajovsky, Y. (1998). Cytokine gene therapy in experimental allergic encephalomyelitis by injection of plasmid DNA-cationic liposome complex into the central nervous system. *J. Immunol. 160*, 5181–5187.

Cua, D.J., Groux, H., Hinton, D.R., Stohlman, S.A., and Coffman, R.L. (1999). Transgenic interleukin 10 prevents induction of experimental autoimmune encephalomyelitis. *J. Exp. Med. 189*, 1005–1010.

Cua, D.J., Hutchins, B., LaFace, D.M., Stohlman, S.A., and Coffman, R.L. (2001). Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. *J. Immunol. 166*, 602–608.

Das, P., Drescher, K.M., Geluk, A., Bradley, D.S., Rodriguez, M., and David, C.S. (2000). Complementation between specific HLA-DR and HLA-DQ genes in transgenic mice determines susceptibility to experimental autoimmune encephalomyelitis. *Hum. Immunol. 61*, 279–289.

De Jong, Y.P., Comiskey, M., Kalled, S.L., Mizoguchi, E., Flavell, R.A., Bhan, A.K., and Terhorst, C. (2000). Chronic murine colitis is dependent on the CD154/CD40 pathway and can be attenuated by anti-CD154 administration. *Gastroenterology 119*, 715–723.

Demirci, G., Strom, T.B., and Li, X.C. (2003). Islet allograft rejection in nonobese diabetic mice involves the common γ-chain and CD28/CD154-dependent and -independent mechanisms. *J. Immunol. 171*, 3878–3885.

Dettke, M., Scheidt, P., Prange, H., and Kirchner, H. (1997). Correlation between interferon production and clinical disease activity in patients with multiple sclerosis. *J. Clin. Immunol*. *17*, 293–300.

Devendra, D., Liu, E., and Eisenbarth, G.S. (2004). Type 1 diabetes: recent developments. *BMJ 328*, 750–754.

Devereux, D., O'Hehir, R.E., McGuire, J., Schooten, W.C.A.V., and Lamb, J.R. (1991). HLA-DR4Dw4-restricted T cell recognition of self antigen(s) in the rheumatoid synovial compartment. *Int. Immunol.* 3, 635–640.

DiLorenzo, T., Graser, T., Ono, T., Christianson, G., Chapman, H., Roopenian, D., Nathenson, S., and Serreze, D. (1998). MHC class I-restricted T-cells are required for all but end stages of diabetes development and utilize a prevalent T cell receptor α chain gene rearrangement. *Proc. Natl. Acad. Sci. USA 95*, 12538–12542.

Dittel, B.N., Urbania, T.H., and Janeway, C.A., Jr. (2000). Relapsing and remitting experimental autoimmune encephalomyelitis in B cell deficient mice. *J. Autoimmun. 14*, 311–318.

Dromey, J.A., Weenink, S.M., Peters, G.H., Endl, J., Tighe, P.J., Todd, I., and Christie, M.R. (2004). Mapping of epitopes for autoantibodies to the type 1 diabetes autoantigen IA-2 by peptide phage display and molecular modeling: overlap of antibody and T cell determinants. *J. Immunol. 172*, 4084–4090.

Durinovic-Bello, I., Schlosser, M., Riedl, M., Maisel, N., Rosinger, S., Kalbacher, H., Deeg, M., Ziegler, M., Elliott, J., Roep, B.O., Karges, W., and Boehm, B.O. (2004). Pro- and anti-inflammatory cytokine production by autoimmune T cells against preproinsulin in HLA-DRB1*04, DQ8 Type 1 diabetes. *Diabetologia 47*, 439–450.

Dutton, R.W., Bradley, L.M. and Swain, S.L. (1998). T cell memory. *Annu. Rev. Immunol. 16*, 201–223.

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.

Ehinger, M., Vestberg, M., Johansson, A.C., Johannesson, M., Svensson, A., and Holmdahl, R. (2001). Influence of CD4 or CD8 deficiency on collagen-induced arthritis. *Immunology 103*, 291–300.

Elewaut, D., Shaikh, R.B., Hammond, K.J., De Winter, H., Leishman, A.J., Sidobre, S., Turovskaya, O., Prigozy, T.I., Ma, L., Banks, T.A., Lo, D., Ware, C.F., Cheroutre, H., and Kronenberg, M. (2003). NIK-dependent RelB activation defines a unique signaling pathway for the development of Vα14i NKT cells. *J. Exp. Med. 197*, 1623–1633.

Falcone, M., Lee, J., Patstone, G., Yeung, B., and Sarvetnick, N. (1998). B lymphocytes are crucial antigen-presenting cells in the pathogenic autoimmune response to GAD65 antigen in nonobese diabetic mice. *J. Immunol. 161*, 1163–1168.

Fallarino, F., Grohmann, U., Hwang, K.W., Orabona, C., Vacca, C., Bianchi, R., Belladonna, M.L., Fioretti, M.C., Alegre, M.L. and Puccetti, P. (2003). Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol. 4*, 1206–1212.

Fantini, M.C., Becker, C., Monteleone, G., Pallone, F., Galle, P.R. and Neurath, M.F. (2004). Cutting edge: TGF-β induces a regulatory phenotype in $CD4^+CD25^-$ T cells through Foxp3 induction and down-regulation of Smad7. *J. Immunol. 172*, 5149–5153.

Faust, A., Rothe, H., Schade, U., Lampeter, E. and Kolb, H. (1996). Primary nonfunction of islet grafts in autoimmune diabetic nonobese diabetic mice is prevented by treatment with interleukin-4 and interleukin-10. *Transplantation 62*, 648–652.

Ferber, I.A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., Dalton, D. and Fathman, C.G. (1996). Mice with a disrupted IFN-γ gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J. Immunol. 156*, 5–7.

Ferrante, P., Fusi, M.L., Saresella, M., Caputo, D., Biasin, M., Trabattoni, D., Salvaggio, A., Clerici, E., de Vries, J.E., Aversa, G., Cazzullo, C.L., and Clerici, M. (1998). Cytokine production and surface marker expression in acute and stable multiple sclerosis: altered IL-12 production and augmented signaling lymphocytic activation molecule (SLAM)-expressing lymphocytes in acute multiple sclerosis. *J. Immunol. 160*, 1514–1521.

Fillatreau, S., Sweenie, C.H., McGeachy, M.J., Gray, D., and Anderton, S.M. (2002). B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol. 3*, 944–950.

Fisson, S., Darrasse-Jeze, G., Litvinova, E., Septier, F., Klatzmann, D., Liblau, R., and Salomon, B.L. (2003). Continuous activation of autoreactive $CD4^+$ $CD25^+$ regulatory T cells in the steady state. *J. Exp. Med. 198*, 737–746.

Flynn, J.C., Rao, P.V., Gora, M., Alsharabi, G., Wei, W., Giraldo, A.A., David, C.S., Banga, J.P., and Kong, Y.M. (2004). Graves' hyperthyroidism and thyroiditis in HLA-DRB1*0301 (DR3) transgenic mice after immunization with thyrotropin receptor DNA. *Clin. Exp. Immunol. 135*, 35–40.

Fontenot, J.D., Gavin, M.A. and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD^+ $CD25^+$ regulatory T cells. *Nat. Immunol. 4*, 330–336.

Frenkel, D., Huang, Z., Maron, R., Koldzic, D.N., Hancock, W.W., Moskowitz, M.A., and Weiner, H.L. (2003). Nasal vaccination with myelin oligodendrocyte glycoprotein reduces stroke size by inducing IL-10-producing $CD4^+$ T cells. *J. Immunol. 171*, 6549–6555.

Gadue, P., Morton, N., and Stein, P.L. (1999). The Src family tyrosine kinase Fyn regulates natural killer T cell development. *J. Exp. Med. 190*, 1189–1196.

Geluk, A., Taneja, V., van Meijgaarden, K.E., Zanelli, E., Abou-Zeid, C., Thole, J.E., de Vries, R.R., David, C.S., and Ottenhoff, T.H. (1998). Identification of HLA class II-restricted determinants of *Mycobacterium tuberculosis*-derived proteins by using HLA-transgenic, class II-deficient mice. *Proc. Natl. Acad. Sci. USA 95*, 10797–10802.

Genain, C.P., Nguyen, M.H., Letvin, N.L., Pearl, R., Davis, R.L., Adelman, M., Lees, M.B., Linington, C., and Hauser, S.L. (1995). Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. *J. Clin. Invest. 96*, 2966–2974.

Genain, C.P., Cannella, B., Hauser, S.L., and Raine, C.S. (1999). Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat. Med. 5*, 170–175.

Genc, K., Dona, D.L., and Reder, A.T. (1997). Increased CD80(+) B cells in active multiple sclerosis and reversal by interferon β-1b therapy. *J. Clin. Invest. 99*, 2664–2671.

Gerritse, K., Laman, J.D., Noelle, R.J., Aruffo, A., Ledbetter, J.A., Boersma, W.J., and Claassen, E. (1996). CD40–CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci USA 93*, 2499–2504.

Girvin, A.M., Dal Canto, M.C. and Miller, S.D. (2002). CD40/CD40L interaction is essential for the induction of EAE in the absence of CD28-mediated co-stimulation. *J. Autoimmun. 18*, 83–94.

Gombert, J.M., Herbelin, A., Tancrede-Bohin, E., Dy, M., Carnaud, C., and Bach, J.F. (1996). Early quantitative and functional deficiency of NK1+-like thymocytes in the NOD mouse. *Eur. J. Immunol. 26*, 2989–2998.

Gonzalez, A., Andre-Schmutz, I., Carnaud, C., Mathis, D., and Benoist, C. (2001). Damage control, rather than unresponsiveness, effected by protective DX5+ T cells in autoimmune diabetes. *Nat. Immunol. 2*, 1117–1125.

Goverman, J., Woods, A., Larson, L., Weiner, L., Hood, L., and Zaller, D. (1993). Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell 72*, 551–560.

Graser, R.T., DiLorenzo, T.P., Wang, F., Christianson, G.J., Chapman, H.D., Roopenian, D.C., Nathenson, S.G. and Serreze, D.V. (2000). Identification of a CD8 T cell that can independently mediate autoimmune diabetes development in the complete absence of CD4 T cell helper functions. *J. Immunol. 164*, 3913–3918.

Greeley, S.A., Katsumata, M., Yu, L., Eisenbarth, G.S., Moore, D.J., Goodarzi, H., Barker, C.F., Naji, A. and Noorchashm, H. (2002). Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. *Nat. Med. 8*, 399–402.

Green, E.A., Wong, F.S., Eshima, K., Mora, C., and Flavell, R.A. (2000). Neonatal tumor necrosis factor α promotes diabetes in nonobese diabetic mice by CD154-independent antigen presentation to CD8(+) T cells. *J. Exp. Med. 191*, 225–238.

Grewal, I., Xu, J., and Flavell, R. (1995). Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. *Nature 378*, 617–620.

Grewal, I.S., Grewal, K.D., Wong, F.S., Picarella, D.E., Janeway, C.A., Jr., and Flavell, R.A. (1996). Local expression of transgene encoded TNF α in islets prevents autoimmune diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J. Exp. Med. 184*, 1963–1974.

Hammond, K.J., Poulton, L.D., Palmisano, L.J., Silveira, P.A., Godfrey, D.I., and Baxter, A.G. (1998). α/β-T cell receptor (TCR)+$CD4^-CD8^-$ (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J. Exp. Med. 187*, 1047–1056.

Harrington, C., Paez, A., Hunkapiller, T., Mannikko, V., Brabb, T., Ahearn, M., Beeson, C., and Governman, J. (1998). Differential tolerance is induced in T cells recognizing distinct epitopes of myelin basic protein. *Immunity 8*, 571–580.

Harrison, L.C., Dempsey-Collier, M., Kramer, D.R., and Takahashi, K. (1996). Aerosol insulin induces regulatory CD8 γδ T cells that prevent murine insulin-dependent diabetes. *J. Exp. Med. 184*, 2167–2174.

Hauser, S.L., Bhan, A.K., Gilles, F., Kemp, M., Kerr, C., and Weiner, H.L. (1986). Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol. 19*, 578–587.

Heremans, H., Dillen, C., Groenen, M., Martens, E., and Billiau, A. (1996). Chronic relapsing experimental autoimmune encephalomyelitis (CREAE) in mice: enhancement by monoclonal antibodies against interferon-γ. *Eur. J. Immunol. 26*, 2393–2398.

Honey, K., Benlagha, K., Beers, C., Forbush, K., Teyton, L., Kleijmeer, M.J., Rudensky, A.Y., and Bendelac, A. (2002). Thymocyte expression of cathepsin L is essential for NKT cell development. *Nat. Immunol. 3*, 1069–1074.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science 299*, 1057–1061.

Horwitz, M.S., Evans, C.F., McGavern, D.B., Rodriguez, M., and Oldstone, M.B. (1997). Primary demyelination in transgenic mice expressing interferon-γ. *Nat. Med. 3*, 1037–1041.

Howard, L.M., Miga, A.J., Vanderlugt, C.L., Dal Canto, M.C., Laman, J.D., Noelle, R.J., and Miller, S.D. (1999). Mechanisms of immunotherapeutic intervention by anti-CD40L (CD154) antibody in an animal model of multiple sclerosis. *J. Clin. Invest. 103*, 281–290.

Hu, H., Huston, G., Duso, D., Lepak, N., Roman, E., and Swain, S.L. (2001). CD4(+) T cell effectors can become memory cells with high efficiency and without further division. *Nat. Immunol. 2*, 705–710.

Hultgren, B., Huang, X., Dybal, N., and Stewart, T. (1996). Genetic absence of γ-interferon delays but does not prevent diabetes in NOD mice. *Diabetes 45*, 812–817.

Huseby, E.S., Liggitt, D., Brabb, T., Schnabel, B., Ohlen, C., and Goverman, J. (2001a). A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *J. Exp. Med. 194*, 669–676.

Huseby, E.S., Sather, B., Huseby, P.G., and Goverman, J. (2001b). Age-dependent T cell tolerance and autoimmunity to myelin basic protein. *Immunity 14*, 471–481.

Iezzi, G., Scheidegger, D., and Lanzavecchia, A. (2001). Migration and function of antigen-primed nonpolarized T lymphocytes *in vivo*. *J. Exp. Med. 193*, 987–993.

Jaume, J.C., Parry, S.L., Madec, A.M., Sonderstrup, G., and Baekkeskov, S. (2002). Suppressive effect of glutamic acid decarboxylase 65-specific autoimmune B lymphocytes on processing of T cell determinants located within the antibody epitope. *J. Immunol.* 169, 665–672.

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, 1137–1143.

Jiang, H., Zhang, S.I., and Pernis, B. (1992). Role of CD8+ T cells in murine experimental allergic encephalomyelitis. *Science 256*, 1213–1215.

Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of $CD4^+CD25^+$ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol. 2*, 301–306.

Jury, E.C., Kabouridis, P.S., Flores-Borja, F., Mageed, R.A., and Isenberg, D.A. (2004). Altered lipid raft-associated signaling and ganglioside expression in T lymphocytes from patients with systemic lupus erythematosus. *J. Clin. Invest. 113*, 1176–1187.

Kaech, S.M., Tan, J.T., Wherry, E.J., Konieczny, B.T., Surh, C.D., and Ahmed, R. (2003). Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat. Immunol. 4*, 1191–1198.

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.

Kalled, S.L., Cutler, A.H., and Burkly, L.C. (2001). Apoptosis and altered dendritic cell homeostasis in lupus nephritis are limited by anti-CD154 treatment. *J. Immunol. 167*, 1740–1747.

Kallmann, B.A., Lampeter, E.F., Hanifi-Moghaddam, P., Hawa, M., Leslie, R.D., and Kolb, H. (1999). Cytokine secretion patterns in twins discordant for Type I diabetes. *Diabetologia 42*, 1080–1085.

Kanagawa, O., Vaupel, B.A., Xu, G., Unanue, E.R., and Katz, J.D. (1998). Thymic positive selection and peripheral activation of islet antigen-specific T cells: separation of two diabetogenic steps by an I-A(g7) class II MHC β-chain mutant. *J. Immunol. 161*, 4489–4492.

Kanagawa, O., Xu, G., Tevaarwerk, A., and Vaupel, B.A. (2000). Protection of nonobese diabetic mice from diabetes by gene(s) closely linked to IFN-γ receptor loci. *J. Immunol. 164*, 3919–3923.

Karni, A., Koldzic, D.N., Bharanidharan, P., Khoury, S.J., and Weiner, H.L. (2002). IL-18 is linked to raised IFN-γ in multiple sclerosis and is induced by activated CD4(+) T cells via CD40–CD40 ligand interactions. *J. Neuroimmunol. 125*, 134–140.

Karni, A., Balashov, K., Hancock, W.W., Bharanidharan, P., Abraham, M., Khoury, S.J., and Weiner, H.L. (2004). Cyclophosphamide modulates $CD4^+$ T cells into a T helper type 2 phenotype and reverses increased IFN-γ production of $CD8^+$ T cells in secondary progressive multiple sclerosis. *J. Neuroimmunol. 146*, 189–198.

Kawamoto, S., Nitta, Y., Tashiro, F., Nakano, A., Yamato, E., Tahara, H., Tabayashi, K., and Miyazaki, J. (2001). Suppression of T(h)1 cell activation and prevention of autoimmune diabetes in NOD mice by local expression of viral IL-10. *Int. Immunol. 13*, 685–694.

Kedl, R.M., Schaefer, B.C., Kappler, J.W., and Marrack, P. (2002). T cells down-modulate peptide-MHC complexes on APCs *in vivo*. *Nat. Immunol. 3*, 27–32.

Khattri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003). An essential role for Scurfin in $CD4^+$ $CD25^+$ T regulatory cells. *Nat. Immunol. 4*, 337–342.

Kim, S.H., Cleary, M.M., Fox, H.S., Chantry, D., and Sarvetnick, N. (2002). CCR4-bearing T cells participate in autoimmune diabetes. *J. Clin. Invest. 110*, 1675–1686.

King, C., Ilic, A., Koelsch, K., and Sarvetnick, N. (2004). Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell 117*, 265–277.

Klein, L., Klugmann, M., Nave, K.A., and Kyewski, B. (2000). Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. *Nat. Med. 6*, 56–61.

Kong, Y.C., Lomo, L.C., Motte, R.W., Giraldo, A.A., Baisch, J., Strauss, G., Hammerling, G.J., and David, C.S. (1996). HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis in transgenic mice: definitive association with HLA-DRB1*0301 (DR3) gene. *J. Exp. Med. 184*, 1167–1172.

Korganow, A.S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J.L., Benoist, C., and Mathis, D. (1999). From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity 10*, 451–461.

Kotzin, B.L. (1996). Systemic lupus erythematosus. *Cell 85*, 303–306.

Kouskoff, V., Korganow, A.S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (1996). Organ-specific disease provoked by systemic autoimmunity. *Cell 87*, 811–822.

Kraan, M.C., Haringman, J.J., Weedon, H., Barg, E.C., Smith, M.D., Ahern, M.J., Smeets, T.J., Breedveld, F.C., and Tak, P.P. (2004). T cells, fibroblast-like synoviocytes, and granzyme B+ cytotoxic cells are associated with joint damage in patients with recent onset rheumatoid arthritis. *Ann. Rheum. Dis. 63*, 483–488.

Krakowski, M. and Owens, T. (1996). Interferon-γ confers resistance to experimental allergic encephalomyelitis. *Eur. J. Immunol. 26*, 1641–1646.

Kriegel, M.A., Lohmann, T., Gabler, C., Blank, N., Kalden, J.R. and Lorenz, H.M. (2004). Defective suppressor function of human $CD4^+$ $CD25^+$ regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med. 199*, 1285–1291.

Kronenberg, M. and Gapin, L. (2002). The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol. 2*, 557–568.

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.

Kuchroo, V.K., Anderson, A.C., Waldner, H., Munder, M., Bettelli, E., and Nicholson, L.B. (2002). T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annu. Rev. Immunol. 20*, 101–123.

Kudva, Y.C., Rajagopalan, G., Raju, R., Abraham, R.S., Smart, M., Hanson, J., and David, C.S. (2002). Modulation of insulitis and type 1 diabetes by transgenic HLA-DR3 and DQ8 in NOD mice lacking endogenous MHC class II. *Hum. Immunol. 63*, 987–999.

Kurrer, M., Pakala, S., Hanson, H., and Katz, J. (1997). β-cell apoptosis in T cell-mediated autoimmune diabetes. *Proc. Natl. Acad. Sci. USA 94*, 213–218.

Lafaille, J., Nagashima, K., Katsuki, M., and Tonegawa, S. (1994). High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptors transgenic mice. *Cell 78*, 399–408.

Lafaille, J.J., Keere, F.V., Hsu, A.L., Baron, J.L., Haas, W., Raine, C.S., and Tonegawa, S. (1997). Myelin basic protein-specific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. *J. Exp. Med. 186*, 307–312.

Laloux, V., Beaudoin, L., Jeske, D., Carnaud, C., and Lehuen, A. (2001). NK T cell-induced protection against diabetes in Vα14-Jα281 transgenic nonobese diabetic mice is associated with a Th2 shift circumscribed regionally to the islets and functionally to islet autoantigen. *J. Immunol. 166*, 3749–3756.

Lang, T.J., Nguyen, P., Papadimitriou, J.C., and Via, C.S. (2003). Increased severity of murine lupus in female mice is due to enhanced expansion of pathogenic T cells. *J. Immunol. 171*, 5795–5801.

Liu, G.Y., Fairchild, P.J., Smith, R.M., Prowle, J.R., Kioussis, D., and Wraith, D.C. (1995). Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity 3*, 407–415.

Liu, H., Hu, B., Xu, D., and Liew, F.Y. (2003). $CD4^+CD25^+$ regulatory T cells cure murine colitis: the role of IL-10, TGF-β, and CTLA4. *J. Immunol. 171*, 5012–5017.

Liu, Y., Wenger, R.H., Zhao, M., and Nielsen, P.J. (1997). Distinct costimulatory molecules are required for the induction of effector and memory cytotoxic T lymphocytes. *J. Exp. Med. 185*, 251–262.

Lublin, F.D., Knobler, R.L., Kalman, B., Goldhaber, M., Marini, J., Perrault, M., D'Imperio, C., Joseph, J., Alkan, S.S., and Korngold, R. (1993). Monoclonal anti-γ interferon antibodies enhance experimental allergic encephalomyelitis. *Autoimmunity 16*, 267–274.

Luhder, F., Chambers, C., Allison, J.P., Benoist, C., and Mathis, D. (2000). Pinpointing when T cell costimulatory receptor CTLA-4 must be engaged to dampen diabetogenic T cells. *Proc. Natl. Acad. Sci. USA 97*, 12204–12209.

Lyons, J.A., San, M., Happ, M.P., and Cross, A.H. (1999). B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. *Eur. J. Immunol. 29*, 3432–3439.

Lyons, J.A., Ramsbottom, M.J., and Cross, A.H. (2002). Critical role of antigen-specific antibody in experimental autoimmune encephalomyelitis induced by recombinant myelin oligodendrocyte glycoprotein. *Eur. J. Immunol. 32*, 1905–1913.

Maccioni, M., Zeder-Lutz, G., Huang, H., Ebel, C., Gerber, P., Hergueux, J., Marchal, P., Duchatelle, V., Degott, C., van Regenmortel, M., Benoist, C., and Mathis, D. (2002). Arthritogenic monoclonal antibodies from K/BxN mice. *J. Exp. Med. 195*, 1071–1077.

Madsen, L.S., Andersson, E.C., Jansson, L., Krogsgaard, M., Andersen, C.B., Engberg, J., Strominger, J.L., Svejgaard, A., Hjorth, J.P., Holmdahl, R., Wucherpfennig, K.W., and Fugger, L. (1999). A humanized model for multiple sclerosis using HLA-DR2 and a human T-cell receptor. *Nat. Genet. 23*, 343–347.

Malek, T.R., Yu, A., Vincek, V., Scibelli, P., and Kong, L. (2002). CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice. Implications for the nonredundant function of IL–2. *Immunity 17*, 167–178.

Maron, R., Melican, N.S., and Weiner, H.L. (1999). Regulatory Th2-type T cell lines against insulin and GAD peptides derived from orally- and nasally-treated NOD mice suppress diabetes. *J. Autoimmun.* 12, 251–258.

Martino, G., Poliani, P.L., Furlan, R., Marconi, P., Glorioso, J.C., Adorini, L., and Comi, G. (2000). Cytokine therapy in immune-mediated demyelinating diseases of the central nervous system: a novel gene therapy approach. *J. Neuroimmunol. 107*, 184–190.

Masopust, D., Vezys, V., Marzo, A.L., and Lefrancois, L. (2001). Preferential localization of effector memory cells in nonlymphoid tissue. *Science 291*, 2413–2417.

Matsumoto, I., Staub, A., Benoist, C., and Mathis, D. (1999). Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science 286*, 1732–1735.

Matsumoto, I., Maccioni, M., Lee, D.M., Maurice, M., Simmons, B., Brenner, M., Mathis, D., and Benoist, C. (2002). How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat. Immunol. 3*, 360–365.

Matsumoto, I., Lee, D.M., Goldbach-Mansky, R., Sumida, T., Hitchon, C.A., Schur, P.H., Anderson, R.J., Coblyn, J.S., Weinblatt, M.E., Brenner, M., Duclos, B., Pasquali, J.L., El-Gabalawy, H., Mathis, D., and Benoist, C. (2003). Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. *Arthritis Rheum. 48*, 944–954.

McHugh, R.S., Shevach, E.M., Margulies, D.H., and Natarajan, K. (2001). A T cell receptor transgenic model of severe, spontaneous organ-specific autoimmunity. *Eur. J. Immunol. 31*, 2094–2103.

Mellbye, O.J., Vartdal, F., Pahle, J., and Mollnes, T.E. (1990). IgG and IgA subclass distribution of total immunoglobulin and rheumatoid factors in rheumatoid tissue plasma cells. *Scand. J. Rheumatol. 19*, 333–340.

Mieza, M.A., Itoh, T., Cui, J.Q., Makino, Y., Kawano, T., Tsuchida, K., Koike, T., Shirai, T., Yagita, H., Matsuzawa, A., Koseki, H., and Taniguchi, M. (1996). Selective reduction of Vα14+ NK T cells associated with disease development in autoimmune-prone mice. *J. Immunol. 156*, 4035–4040.

Miller, B.J., Appel, M.C., O'Neil, J.J., and Wicker, L. S. (1988). Both the Lyt–2+ and L3T4+ T cell subsets are required for the transfer of diabetes in non-obese diabetic mice. *J. Immunol. 140*, 52–58.

Miyamoto, K., Miyake, S., and Yamamura, T. (2001). A synthetic glycolipid prevents autoimmune encephalomyelitis by inducting Th2 bias of natural killer T cells. *Nature 413*, 531–534.

Mori, L., Loetscher, H., Kakimoto, K., Bluethmann, H., and Steinmetz, M. (1992). Expression of a transgenic T cell receptor β chain enhances collagen-induced arthritis. *J. Exp. Med. 176*, 381–8.

Moritani, M., Yoshimoto, K., Tashiro, F., Hashimoto, C., Miyazaki, J., Ii, S., Kudo, E., Iwahana, H., Hayashi, Y., Sano, T. (1994). Transgenic expression of IL-10 in pancreatic islet A cells accelerates autoimmune insulitis and diabetes in non-obese diabetic mice. *Int. Immunol. 6*, 1927–1936.

Moritani, M., Yoshimoto, K., Li, S., Kondo, M., Iwahana, H., Yamaoka, T., Sano, T., Nakano, N., Kikutani, H., and Itakura, M. (1996). Prevention of adoptively transferred diabetes in nonobese diabetic mice with IL-10-transduced islet-specific Th1 lymphocytes. A gene therapy model for autoimmune diabetes. *J. Clin. Invest. 98*, 1851–1859.

Morrow, J., Nelson, L., Watts, R., and Isenberg, D. (1999). Systemic lupus erythematosus. In *Autoimmune Rheumatic Disease* (pp. 56–103). Oxford: Oxford University Press.

Mueller, R., Krahl, T., and Sarvetnick, N. (1996). Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J. Exp. Med. 184*, 1093–1099.

Murali-Krishna, K., Lau, L.L., Sambhara, S., Lemonnier, F., Altman, J., and Ahmed, R. (1999). Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science 286*, 1377–1381.

Muraro, P.A., Douek, D.C., Packer, A., Chung, K., Guenaga, F.J., Cassiani-Ingoni, R., Campbell, C., Memon, S., Nagle, J.W., Hakim, F.T., Gress, R.E., McFarland, H.F., Burt, R.K., and Martin, R. (2005). Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *J. Exp. Med. 201*, 805–816.

Myers, K.J., Sprent, J., Dougherty, J.P., and Ron, Y. (1992). Synergy between encephalitogenic T cells and myelin basic protein-specific antibodies in the induction of experimental autoimmune encephalomyelitis. *J. Neuroimmunol. 41*, 1–8.

Nabozny, G.H., Baisch, J.M., Cheng, S., Cosgrove, D., Griffiths, M.M., Luthra, H.S., and David, C.S. (1996). HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. *J. Exp. Med. 183*, 27–37.

Naserke, H.E., Bonifacio, E., and Ziegler, A.G. (2001). Prevalence, characteristics and diabetes risk associated with transient maternally acquired islet antibodies and persistent islet antibodies in offspring of parents with type 1 diabetes. *J. Clin. Endocrinol. Metab. 86*, 4826–4833.

Nelson, B.H. (2004). IL-2, regulatory T cells, and tolerance. *J. Immunol. 172*, 3983–3988.

Nicholson, L.B., Greer, J.M., Sobel, R.A., Lees, M.B., and Kuchroo, V.K. (1995). An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis. *Immunity 3*, 397–405.

Nitta, Y., Tashiro, F., Tokui, M., Shimada, A., Takei, I., Tabayashi, K., and Miyazaki, J. (1998). Systemic delivery of interleukin 10 by intramuscular injection of expression plasmid DNA prevents autoimmune diabetes in nonobese diabetic mice. *Hum. Gene Ther. 9*, 1701–1707.

Noorchashm, H., Noorchashm, N., Kern, J., Rostami, S.Y., Barker, C.F., and Naji, A. (1997). B-cells are required for the initiation of insulitis and sialitis in nonobese diabetic mice. *Diabetes 46*, 941–946.

Noorchashm, H., Lieu, Y.K., Noorchashm, N., Rostami, S.Y., Greeley, S.A., Schlachterman, A., Song, H.K., Noto, L.E., Jevnikar, A.M., Barker, C.F., and Naji, A. (1999). I-Ag7-mediated antigen presentation by B

lymphocytes is critical in overcoming a checkpoint in T cell tolerance to islet β cells of nonobese diabetic mice. *J. Immunol. 163*, 743–750.

Olivares-Villagomez, D., Wang, Y. and Lafaille, J. (1998). Regulatory $CD4^+$ T cells expressing endogenous T cell receptor chains protect myelin basic protein-specific transgenic mice from spontaneous autoimmune encephalomyelitis. *J. Exp. Med. 188*, 1883–1894.

Opferman, J., Ober, B., and Ashton-Rickardt, P. (1999). Linear differentiation of cytotoxic effectors into memory T lymphocytes. *Science 283*, 1745–1752.

Osman, G.E., Cheunsuk, S., Allen, S.E., Chi, E., Liggitt, H.D., Hood, L.E., and Ladiges, W.C. (1998). Expression of a type II collagen-specific TCR transgene accelerates the onset of arthritis in mice. *Int. Immunol. 10*, 1613–1622.

Ott, P.A., Berner, B.R., Herzog, B.A., Guerkov, R., Yonkers, N.L., Durinovic-Bello, I., Tary-Lehmann, M., Lehmann, P.V., and Anthony, D.D. (2004). CD28 costimulation enhances the sensitivity of the ELISPOT assay for detection of antigen-specific memory effector CD4 and CD8 cell populations in human diseases. *J. Immunol. Methods 285*, 223–235.

Ou, L.S., Goleva, E., Hall, C., and Leung, D.Y. (2004). T regulatory cells in atopic dermatitis and subversion of their activity by superantigens. *J. Allergy Clin. Immunol. 113*, 756–763.

Pakala, S.V., Kurrer, M.O., and Katz, J.D. (1997). T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. *J. Exp. Med. 186*, 299–306.

Pal, E., Yamamura, T., and Tabira, T. (1999). Autonomic regulation of experimental autoimmune encephalomyelitis in IL-4 knockout mice. *J. Neuroimmunol. 100*, 149–155.

Panitch, H.S., Hirsch, R.L., Schindler, J., and Johnson, K.P. (1987). Treatment of multiple sclerosis with γ interferon: exacerbations associated with activation of the immune system. *Neurology 37*, 1097–1102.

Panoutsakopoulou, V., Huster, K.M., McCarty, N., Feinberg, E., Wang, R., Wucherpfennig, K.W., and Cantor, H. (2004). Suppression of autoimmune disease after vaccination with autoreactive T cells that express Qa-1 peptide complexes. *J. Clin. Invest. 113*, 1218–1224.

Parish, N.M., Chandler, P., Quartey-Papafio, R., Simpson, E., and Cooke, A. (1993). The effect of bone marrow and thymus chimerism between non-obese diabetic (NOD) and NOD-E transgenic mice, on the expression and prevention of diabetes. *Eur. J. Immunol. 23*, 2667–2675.

Pauza, M.E., Neal, H., Hagenbaugh, A., Cheroutre, H., and Lo, D. (1999). T-cell production of an inducible interleukin-10 transgene provides limited protection from autoimmune diabetes. *Diabetes 48*, 1948–1953.

Pearson, C.I., van Ewijk, W., and McDevitt, H.O. (1997). Induction of apoptosis and T helper 2 (Th2) responses correlates with peptide affinity for the major histocompatibility complex in self-reactive T cell receptor transgenic mice. *J. Exp. Med. 185*, 583–599.

Pelegri, C., Morante, M.P., Castellote, C., Franch, A., and Castell, M. (1996). Treatment with an anti-CD4 monoclonal antibody strongly ameliorates established rat adjuvant arthritis. *Clin. Exp. Immunol. 103*, 273–278.

Peng, S.L., McNiff, J.M., Madaio, M.P., Ma, J., Owen, M.J., Flavell, R.A., Hayday, A.C., and Craft, J. (1997). αβ T cell regulation and CD40 ligand dependence in murine systemic autoimmunity. *J. Immunol. 158*, 2464–2470.

Pennline, K.J., Roque-Gaffney, E., and Monahan, M. (1994). Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin. Immunol. Immunopathol. 71*, 169–175.

Perchellet, A., Stromnes, I., Pang, J.M., and Goverman, J. (2004). $CD8^+$ T cells maintain tolerance to myelin basic protein by 'epitope theft'. *Nat. Immunol. 5*, 606–614.

Petereit, H.F., Richter, N., Pukrop, R., and Bamborschke, S. (2000). Interferon γ production in blood lymphocytes correlates with disability score in multiple sclerosis patients. *Mult. Scler. 6*, 19–23.

Peterson, J. and Haskins, K. (1996). Transfer of diabetes in the NOD-scid mouse by CD4 T cell clones. Differential requirement for CD8 T cells. *Diabetes 45*, 328–336.

Pettinelli, C.B. and McFarlin, D.E. (1981). Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after *in vitro* activation of lymph node cells by myelin basic protein: requirement for Lyt 1+ 2- T lymphocytes. *J. Immunol. 127*, 1420–1423.

Pewe, L. and Perlman, S. (2002). Cutting edge: CD8 T cell-mediated demyelination is IFN-γ dependent in mice infected with a neurotropic coronavirus. *J. Immunol. 168*, 1547–1551.

Pihlgren, M., Dubois, P.M., Tomkowiak, M., Sjögren, T., and Marvel, J. (1996). Resting memory $CD8^+$ T cells are hyperreactive to antigenic challenge *in vitro*. *J. Exp. Med. 184*, 2141–2151.

Pohlers, D., Nissler, K., Frey, O., Simon, J., Petrow, P.K., Kinne, R.W., and Brauer, R. (2004). Anti-CD4 monoclonal antibody treatment in acute and early chronic antigen-induced arthritis: influence on T helper cell activation. *Clin. Exp. Immunol. 135*, 409–415.

Poulin, M. and Haskins, K. (2000). Induction of diabetes in nonobese diabetic mice by Th2 T cell clones from a TCR transgenic mouse. *J. Immunol. 164*, 3072–3078.

Prineas, J.W. and Wright, R.G. (1978). Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab. Invest. 38*, 409–421.

Ramsdell, F. and Ziegler, S.F. (2003). Transcription factors in autoimmunity. *Curr. Opin. Immunol. 15*, 718–724.

Rapoport, M., Jaramillo, A., Zipris, D., Lazarus, A., Serreze, D., Leiter, E., Cyopick, P., Danska, J., and Delovitch, T. (1993). Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in Nonobese diabetic mice. *J. Exp. Med. 178*, 87–99.

Rapoport, M.J., Mor, A., Vardi, P., Ramot, Y., Winker, R., Hindi, A., and Bistritzer, T. (1998). Decreased secretion of Th2 cytokines precedes upregulated and delayed secretion of Th1 cytokines in activated peripheral blood mononuclear cells from patients with insulin-dependent diabetes mellitus. *J. Autoimmun.* 11, 635–642.

Reinhardt, R.L., Khoruts, A., Merica, R., Zell, T., and Jenkins, M.K. (2001). Visualizing the generation of memory CD4 T cells in the whole body. *Nature 410*, 101–105.

Roddis, M., Carter, R.W., Sun, M.Y., Weissensteiner, T., McMichael, A.J., Bowness, P., and Bodmer, H.C. (2004). Fully functional HLA B27-restricted $CD4^+$ as well as $CD8^+$ T cell responses in TCR transgenic mice. *J. Immunol. 172*, 155–161.

Rogers, P.R., Dubey, C., and Swain, S.L. (2000). Qualitative changes accompany memory T cell generation: faster, more effective responses at lower doses of antigen. *J. Immunol. 164*, 2338–2346.

Rohane, P.W., Shimada, A., Kim, D.T., Edwards, C.T., Charlton, B., Shultz, L.D., and Fathman, C.G. (1995). Islet-infiltrating lymphocytes from prediabetic NOD mice rapidly transfer diabetes to NOD-scid/scid mice. *Diabetes 44*, 550–554.

Rossini, A.A., Mordes, J.P., Greiner, D.L., and Stoff, J.S. (2004). Islet cell transplantation tolerance. *Transplantation* 72(8 Suppl), S43–46.

Rott, O., Fleischer, B. and Cash, E. (1994). Interleukin-10 prevents experimental allergic encephalomyelitis in rats. *Eur. J. Immunol. 24*, 1434–1440.

Sabbagh, L., Kaech, S.M., Bourbonniere, M., Woo, M., Cohen, L.Y., Haddad, E.K., Labrecque, N., Ahmed, R., and Sekaly, R.P. (2004). The selective increase in caspase-3 expression in effector but not memory T cells allows susceptibility to apoptosis. *J. Immunol. 173*, 5425–5433.

Sakaguchi, S. (2004). Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature 401*, 708–712.

Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu. Rev. Immunol. 22*, 745–763.

Samoilova, E.B., Horton, J.L., and Chen, Y. (1998). Acceleration of experimental autoimmune encephalomyelitis in interleukin-10-deficient mice: roles of interleukin-10 in disease progression and recovery. *Cell. Immunol. 188*, 118–124.

Santamaria, P. (2001). Effector lymphocytes in autoimmunity. *Curr. Opin. Immunol. 13*, 663–669.

Santamaria, P. (2003). Cytokines and chemokines in autoimmune disease: an overview. *Adv. Exp. Med. Biol. 520*, 1–7.

Santoro, T.J., Portanova, J.P., and Kotzin, B.L. (1988). The contribution of L3T4+ T cells to lymphoproliferation and autoantibody production in MRL-lpr/lpr mice. *J. Exp. Med. 167*, 1713–1718.

Sarvetnick, N., Shizuru, J., Liggit, D., Martin, L., McIntyre, B., Gregory, A., Parslow, T., and Stewart, T. (1990). Loss of pancreatic islet tolerance induced by β cell-expression of interferon-γ. *Nature* 346, 844–847.

Schaller, M., Burton, D.R., and Ditzel, H.J. (2001). Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. *Nat. Immunol. 2*, 746–753.

Schmidt, D., Verdaguer, J., Averill, N., and Santamaria, P. (1997). A mechanism for the major histocompatibility complex-linked resistance to autoimmunity. *J. Exp. Med. 186*, 1059–1075.

Schmidt, D., Amrani, A., Verdaguer, J., Bou, S., and Santamaria, P. (1999). Autoantigen-independent deletion of diabetogenic $CD4^+$ thymocytes by protective MHC class II molecules. *J. Immunol. 162*, 4627–4636.

Schmidt, S. (1999). Candidate autoantigens in multiple sclerosis. *Mult. Scler. 5*, 147–160.

Schubert, D., Maier, B., Morawietz, L., Krenn, V., and Kamradt, T. (2004). Immunization with glucose-6-phosphate isomerase induces T cell-dependent peripheral polyarthritis in genetically unaltered mice. *J. Immunol. 172*, 4503–4509.

Seamons, A., Sutton, J., Bai, D., Baird, E., Bonn, N., Kafsack, B.F., Shabanowitz, J., Hunt, D.F., Beeson, C., and Goverman, J. (2003). Competition between two MHC binding registers in a single peptide processed from myelin basic protein influences tolerance and susceptibility to autoimmunity. *J. Exp. Med. 197*, 1391–1397.

Seddon, B., Tomlinson, P., and Zamoyska, R. (2003). Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nat. Immunol. 4*, 680–686.

Seder, R.A. and Ahmed, R. (2003). Similarities and differences in $CD4^+$ and $CD8^+$ effector and memory T cell generation. *Nat. Immunol. 4*, 835–842.

Segal, B.M. and Shevach, E.M. (1996). IL-12 unmasks latent autoimmune disease in resistant mice. *J. Exp. Med. 184*, 771–775.

Serra, P., Amrani, A., Yamanouchi, J., Han, B., Thiessen, S., Utsugi, T., Verdaguer, J., and Santamaria, P. (2003). CD40 ligation releases immature dendritic cells from the control of regulatory $CD4^+CD25^+$ T cells. *Immunity 19*, 877–889.

Serreze, D., Chapman, H., Varnum, D., Hanson, M., Reifsnyder, P., Richard, S., Fleming, S., Leiter, E., and Shultz, L. (1996). B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.$Ig\mu^{null}$ mice. *J. Exp. Med. 184*, 2049–2053.

Serreze, D., Chapman, H., Varnum, D., Gerling, I., Leiter, E., and Shultz, L. (1997). Initiation of autoimmune diabetes in NOD/Lt mice is MHC class I-dependent. *J. Immunol. 157*, 3978–3986.

Serreze, D., Fleming, S., Chapman, H., Richard, S., Leiter, E., and Tisch, R. (1998). B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J. Immunol. 161*, 3912–3918.

Serreze, D.V., Post, C.M., Chapman, H.D., Johnson, E.A., Lu, B., and Rothman, P.B. (2000). Interferon-γ receptor signaling is dispensable in the development of autoimmune type 1 diabetes in NOD mice. *Diabetes 49*, 2007–2011.

Serreze, D.V., Chapman, H.D., Post, C.M., Johnson, E.A., Suarez-Pinzon, W.L., and Rabinovitch, A. (2001). Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. *J. Immunol. 166*, 1352–1359.

Sharif, S., Arreaza, G.A., Zucker, P., Mi, Q.S., Sondhi, J., Naidenko, O.V., Kronenberg, M., Koezuka, Y., Delovitch, T.L., Gombert, J.M., Leite-De-Moraes, M., Gouarin, C., Zhu, R., Hameg, A., Nakayama, T., Taniguchi, M., Lepault, F., Lehuen, A., Bach, J.F., and Herbelin, A. (2001). Activation of natural killer T cells by α-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat. Med. 7*, 1057–1062.

Shaw, M.K., Lorens, J.B., Dhawan, A., DalCanto, R., Tse, H.Y., Tran, A.B., Bonpane, C., Eswaran, S.L., Brocke, S., Sarvetnick, N., Steinman, L., Nolan, G.P., and Fathman, C.G. (1997). Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. *J. Exp. Med. 185*, 1711–1714.

Shi, F.D., Flodstrom, M., Balasa, B., Kim, S.H., Van Gunst, K., Strominger, J.L., Wilson, S.B., and Sarvetnick, N. (2001). Germ line deletion of the CD1 locus exacerbates diabetes in the NOD mouse. *Proc. Natl. Acad. Sci. USA 98*, 6777–6782.

Silveira, P.A., Dombrowsky, J., Johnson, E., Chapman, H.D., Nemazee, D., and Serreze, D.V. (2004). B cell selection defects underlie the development of diabetogenic APCs in nonobese diabetic mice. *J. Immunol. 172*, 5086–5094.

Singer, S.M., Tisch, R., Yang, X.D., and McDevitt, H.O. (1993). An Abd transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. *Proc. Natl. Acad. Sci. USA 90*, 9566–9570.

Sivakumar, V., Hammond, K.J., Howells, N., Pfeffer, K., and Weih, F. (2003). Differential requirement for Rel/nuclear factor κ B family members in natural killer T cell development. *J. Exp. Med. 197*, 1613–1621.

Skulina, C., Schmidt, S., Dornmair, K., Babbe, H., Roers, A., Rajewsky, K., Wekerle, H., Hohlfeld, R., and Goebels, N. (2004). Multiple sclerosis: brain-infiltrating $CD8^+$ T cells persist as clonal expansions in the cerebrospinal fluid and blood. *Proc. Natl. Acad. Sci. USA 101*, 2428–2433.

Slattery, R.M., Miller, J.F., Heath, W.R., and Charlton, B. (1993). Failure of a protective major histocompatibility complex class II molecule to delete autoreactive T cells in autoimmune diabetes. *Proc. Natl. Acad. Sci. USA 90*, 10808–10810.

Sobel, D.O., Han, J., Williams, J., Yoon, J.W., Jun, H.S., and Ahvazi, B. (2002). γ Interferon paradoxically inhibits the development of diabetes in the NOD mouse. *J. Autoimmun. 19*, 129–137.

Somoza, N., Vargas, F., Roura-Mir, C., Vives-Pi, M., Fernandez-Figueras, M.T., Ariza, A., Gomis, R., Bragado, R., Marti, M., and Jaraquemada, D. (1994). Pancreas in recent onset insulin-dependent diabetes mellitus. Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor V β usage, and cytokine profile. *J. Immunol. 153*, 1360–1377.

Stassen, M., Fondel, S., Bopp, T., Richter, C., Muller, C., Kubach, J., Becker, C., Knop, J., Enk, A.H., Schmitt, S., Schmitt, E., and Jonuleit, H. (2004). Human $CD25^+$ regulatory T cells: two subsets defined by the integrins α4β7 or α4β1 confer distinct suppressive properties upon $CD4^+$ T helper cells. *Eur. J. Immunol. 34*, 1303–1311.

Steinberg, A.D., Roths, J.B., Murphy, E.D., Steinberg, R.T., and Raveche, E.S. (1980). Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. *J. Immunol. 125*, 871–873.

Stohlman, S.A., Pei, L., Cua, D.J., Li, Z., and Hinton, D.R. (1999). Activation of regulatory cells suppresses experimental allergic encephalomyelitis via secretion of IL-10. *J. Immunol. 163*, 6338–6344.

Storch, M.K., Stefferl, A., Brehm, U., Weissert, R., Wallstrom, E., Kerschensteiner, M., Olsson, T., Linington, C., and Lassmann, H. (1998). Autoimmunity to myelin oligodendrocyte glycoprotein in rats mimics the spectrum of multiple sclerosis pathology. *Brain Pathol. 8*, 681–694.

Stuart, J.M. and Dixon, F.J. (1983). Serum transfer of collagen-induced arthritis in mice. *J. Exp. Med. 158*, 378–392.

Suarez-Pinzon, W., Rajotte, R.V., Mosmann, T.R., and Rabinovitch, A. (1996). Both CD4+ and $CD8^+$ T-cells in syngeneic islet grafts in NOD mice produce interferon-γ during β-cell destruction. *Diabetes 45*, 1350–1357.

Sumida, T., Sakamoto, A., Murata, H., Makino, Y., Takahashi, H., Yoshida, S., Nishioka, K., Iwamoto, I., and Taniguchi, M. (1995). Selective reduction of T cells bearing invariant Vα24JαQ antigen receptor in patients with systemic sclerosis. *J. Exp. Med. 182*, 1163–1168.

Sumida, T., Maeda, T., Taniguchi, M., Nishioka, K., and Stohl, W. (1998). TCR AV24 gene expression in double negative T cells in systemic lupus erythematosus. *Lupus 7*, 565–568.

Sun, D., Whitaker, J.N., Huang, Z., Liu, D., Coleclough, C., Wekerle, H., and Raine, C.S. (2001). Myelin antigen-specific $CD8^+$ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J. Immunol. 166*, 7579–7587.

Sun, D., Zhang, Y., Wei, B., Peiper, S.C., Shao, H., and Kaplan, H.J. (2003). Encephalitogenic activity of truncated myelin oligodendrocyte glycoprotein (MOG) peptides and their recognition by $CD8^+$ MOG-specific T cells on oligomeric MHC class I molecules. *Int. Immunol. 15*, 261–268.

Svensson, L., Jirholt, J., Holmdahl, R., and Jansson, L. (1998). B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). *Clin. Exp. Immunol. 111*, 521–526.

Svensson, L., Abdul-Majid, K.B., Bauer, J., Lassmann, H., Harris, R.A., and Holmdahl, R. (2002). A comparative analysis of B cell-mediated myelin oligodendrocyte glycoprotein-experimental autoimmune encephalomyelitis pathogenesis in B cell-deficient mice reveals an effect on demyelination. *Eur. J. Immunol. 32*, 1939–1946.

Swain, S.L., Hu, H., and Huston, G. (1999). Class II-independent generation of CD4 memory T cells from effectors. *Science 286*, 1381–1383.

Tada, Y., Ho, A., Koh, D.R., and Mak, T.W. (1996). Collagen-induced arthritis in $CD4^-$ or $CD8^-$ deficient mice: $CD8^+$ T cells play a role in initiation and regulate recovery phase of collagen-induced arthritis. *J. Immunol. 156*, 4520–4526.

Taneja, V., Griffiths, M.M., Luthra, H., and David, C.S. (1998). Modulation of HLA-DQ-restricted collagen-induced arthritis by HLA-DRB1 polymorphism. *Int. Immunol. 10*, 1449–1457.

Tarbell, K.V., Lee, M., Ranheim, E., Chao, C.C., Sanna, M., Kim, S.K., Dickie, P., Teyton, L., Davis, M., and McDevitt, H. (2002). $CD4^+$ T cells from glutamic acid decarboxylase (GAD)65-specific T cell receptor transgenic mice are not diabetogenic and can delay diabetes transfer. *J. Exp. Med. 196*, 481–492.

Taurog, J.D., Maika, S.D., Satumtira, N., Dorris, M.L., McLean, I.L., Yanagisawa, H., Sayad, A., Stagg, A.J., Fox, G.M., Le O'Brien, A., Rehman, M., Zhou, M., Weiner, A.L., Splawski, J.B., Richardson, J.A., and Hammer, R.E. (1999). Inflammatory disease in HLA-B27 transgenic rats. *Immunol. Rev. 169*, 209–223.

Teige, A., Teige, I., Lavasani, S., Bockermann, R., Mondoc, E., Holmdahl, R., and Issazadeh-Navikas, S. (2004). CD1-dependent regulation of chronic central nervous system inflammation in experimental autoimmune encephalomyelitis. *J. Immunol. 172*, 186–194.

Thiessen, S., Serra, P., Amrani, A., Verdaguer, J., and Santamaria, P. (2002). T-cell tolerance by dendritic cells and macrophages as a mechanism for the major histocompatibility complex-linked resistance to autoimmune diabetes. *Diabetes 51*, 325–338.

Thivolet, C., Bendelac, A., Bedossa, P., Bach, J.F., and Carnaud, C. (1991). $CD8^+$ T cell homing to the pancreas in the nonobese diabetic mouse is $CD4^+$ T cell-dependent. *J. Immunol. 146*, 85–88.

Thomas, J.W., Kendall, P.L., and Mitchell, H.G. (2002). The natural autoantibody repertoire of nonobese diabetic mice is highly active. *J. Immunol. 169*, 6617–6624.

Tian, J., Lehmann, P.V., and Kaufman, D.L. (1997). Determinant spreading of T helper cell 2 (Th2) responses to pancreatic islet autoantigens. *J. Exp. Med. 186*, 2039–2043.

Tisch, R. and McDevitt, H. (1996). Insulin-dependent diabetes mellitus. *Cell 85*, 291–297.

Tisch, R., Wang, B., and Serreze, D.V. (1999). Induction of glutamic acid decarboxylase 65-specific Th2 cells and suppression of autoimmune diabetes at late stages of disease is epitope dependent. *J. Immunol. 163*, 1178–1187.

Trembleau, S., Penna, G., Gregori, S., Gately, M.K., and Adorini, L. (1997). Deviation of pancreas-infiltrating cells to Th2 by interleukin-12 antagonist administration inhibits autoimmune diabetes. *Eur. J. Immunol. 27*, 2330–2339.

Trembleau, S., Penna, G., Gregori, S., Giarratana, N., and Adorini, L. (2003). IL-12 administration accelerates autoimmune diabetes in both wild-type and IFN-γ-deficient nonobese diabetic mice, revealing pathogenic and protective effects of IL-12-induced IFN-γ. *J. Immunol. 170*, 5491–5501.

Tsuchida, T., Parker, K.C., Turner, R.V., McFarland, H.F., Coligan, J.E., and Biddison, W.E. (1994). Autoreactive $CD8^+$ T-cell responses to human myelin protein-derived peptides. *Proc. Natl. Acad. Sci. USA 91*, 10859–10863.

van der Vliet, H.J., von Blomberg, B.M., Nishi, N., Reijm, M., Voskuyl, A.E., van Bodegraven, A.A., Polman, C.H., Rustemeyer, T., Lips, P., van den Eertwegh, A.J., Giaccone, G., Scheper, R.J., and Pinedo, H.M. (2001). Circulating V(α24+) Vβ11+ NKT cell numbers are decreased in a wide variety of diseases that are characterized by autoreactive tissue damage. *Clin. Immunol. 100*, 144–148.

van Essen, D., Kikutani, H., and Gray, D. (1995). CD40 ligand-transduced co-stimulation of T cells in the development of helper function. *Nature 378*, 620–623.

Veiga-Fernandes, H., Walter, U., Bourgeois, C., McLean, A., and Rocha, B. (2000). Response of naive and memory $CD8^+$ T cells to antigen stimulation *in vivo*. *Nat. Immunol. 1*, 47–53.

Verdaguer, J., Schmidt, D., Amrani, A., Anderson, B., Averill, N., and Santamaria, P. (1997). Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. *J. Exp. Med. 186*, 1663–1676.

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.

Voorthuis, J.A., Uitdehaag, B.M., De Groot, C.J., Goede, P.H., van der Meide, P.H., and Dijkstra, C.D. (1990). Suppression of experimental allergic encephalomyelitis by intraventricular administration of interferon-γ in Lewis rats. *Clin. Exp. Immunol. 81*, 183–188.

Waldner, H., Whitters, M.J., Sobel, R.A., Collins, M., and Kuchroo, V.K. (2000). Fulminant spontaneous autoimmunity of the central nervous system in mice transgenic for the myelin proteolipid protein-specific T cell receptor. *Proc. Natl. Acad. Sci. USA 97*, 3412–3417.

Walker, M.R., Kasprowicz, D.J., Gersuk, V.H., Benard, A., Van Landeghen, M., Buckner, J.H., and Ziegler, S.F. (2003). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human $CD4^+CD25^-$ T cells. *J. Clin. Invest. 112*, 1437–1443.

Walunas, T.L., Wang, B., Wang, C.R., and Leiden, J.M. (2000). Cutting edge: the Ets1 transcription factor is required for the development of NK T cells in mice. *J. Immunol. 164*, 2857–2860.

Wang, B., Gonzalez, A., Benoist, C., and Mathis, D. (1996). The role of $CD8^+$ T-cells in initiation of insulin-dependent diabetes mellitus. *Eur. J. Immunol. 26*, 1762–1769.

Wang, B., Geng, Y.B., and Wang, C.R. (2001). CD1-restricted NK T cells protect nonobese diabetic mice from developing diabetes. *J. Exp. Med. 194*, 313–320.

Wekerle, H., Bradl, M., Linington, C., Kaab, G., and Kojima, K. (1996). The shaping of the brain-specific T lymphocyte repertoire in the thymus. *Immunol. Rev. 149*, 231–243.

Wen, L., Wong, F., Burkly, L., Altieri, M., Mamalaki, C., Kioussis, D., Flavell, R., and Sherwin, R. (1998). Induction of insulitis by glutamic acid decarboxylase peptide-specific and HLA-DQ8-restricted $CD4^+$ T cells from human DQ transgenic mice. *J. Clin. Invest. 102*, 947–957.

Wen, L., Wong, F.S., Tang, J., Chen, N.Y., Altieri, M., David, C., Flavell, R., and Sherwin, R. (2000). *In vivo* evidence for the contribution of human histocompatibility leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J. Exp. Med. 191*, 97–104.

Wen, L., Chen, N.Y., Tang, J., Sherwin, R., and Wong, F.S. (2001). The regulatory role of DR4 in a spontaneous diabetes DQ8 transgenic model. *J. Clin. Invest. 107*, 871–880.

Wen, L., Wong, F.S., Sherwin, R., and Mora, C. (2002). Human DQ8 can substitute for murine I-Ag7 in the selection of diabetogenic T cells restricted to I-Ag7. *J. Immunol. 168*, 3635–3640.

Wherry, E.J., Teichgraber, V., Becker, T.C., Masopust, D., Kaech, S.M., Antia, R., Von Andrian, U.H., and Ahmed, R. (2003). Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat. Immunol. 4*, 225–234.

Willenborg, D.O., Fordham, S., Bernard, C.C., Cowden, W.B., and Ramshaw, I.A. (1996). IFN-γ plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J. Immunol. 157*, 3223–3227.

Willenborg, D.O., Fordham, S.A., Staykova, M.A., Ramshaw, I.A., and Cowden, W.B. (1999). IFN-γ is critical to the control of murine autoimmune encephalomyelitis and regulates both in the periphery and in the target tissue: a possible role for nitric oxide. *J. Immunol. 163*, 5278–5286.

Wilson, S., Kent, S., Patton, K., Orban, T., Jackson, R., Exley, M., Porcelli, S., Schatz, D., Atkinson, M., Balk, S., Strominger, J., and Hafler, D. (1998). Extreme Th1 bias of invariant Vα24JαQT cells in type 1 diabetes. *Nature 391*, 177–181.

Winer, S., Tsui, H., Lau, A., Song, A., Li, X., Cheung, R.K., Sampson, A., Afifiyan, F., Elford, A., Jackowski, G., Becker, D.J., Santamaria, P., Ohashi, P., and Dosch, H.M. (2003). Autoimmune islet destruction in spontaneous type 1 diabetes is not β-cell exclusive. *Nat. Med. 9*, 198–205.

Wipke, B.T., Wang, Z., Kim, J., McCarthy, T.J., and Allen, P.M. (2002). Dynamic visualization of a joint-specific autoimmune response through positron emission tomography. *Nat. Immunol. 3*, 366–372.

Wofsy, D., Ledbetter, J.A., Hendler, P.L., and Seaman, W.E. (1985). Treatment of murine lupus with monoclonal anti-T cell antibody. *J. Immunol. 134*, 852–857.

Wogensen, L., Huang, X., and Sarvetnick, N. (1993). Leukocyte extravasation into the pancreatic tissue in transgenic mice expressing interleukin 10 in the islets of Langerhans. *J. Exp. Med. 178*, 175.

Wogensen, L., Lee, M.S., and Sarvetnick, N. (1994). Production of interleukin 10 by islet cells accelerates immune-mediated destruction of β cells in nonobese diabetic mice. *J. Exp. Med. 179*, 1379–1384.

Wolf, S.D., Dittel, B.N., Hardardottir, F., and Janeway, C.A., Jr. (1996). Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J. Exp. Med. 184*, 2271–2278.

Wordsworth, B.P., Lanchbury, J.S., Sakkas, L.I., Welsh, K.I., Panayi, G.S., and Bell, J.I. (1989). HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DRB1 is the major susceptibility locus within the HLA class II region. *Proc. Natl. Acad. Sci. USA 86*, 10049–10053.

Xiao, B.G., Bai, X.F., Zhang, G.X., and Link, H. (1998). Suppression of acute and protracted-relapsing experimental allergic encephalomyelitis by nasal administration of low-dose IL-10 in rats. *J. Neuroimmunol. 84*, 230–237.

Xu, L., Zhang, L., Yi, Y., Kang, H.K., and Datta, S.K. (2004). Human lupus T cells resist inactivation and escape death by upregulating COX-2. *Nat. Med. 10*, 411–415.

Yan, T., Burkhardt, H., Ritter, T., Broker, B., Mann, K.H., Bertling, W.M., von der Mark, K., and Emmrich, F. (1992). Specificity and T cell receptor β chain usage of a human collagen type II-reactive T cell clone derived from a healthy individual. *Eur. J. Immunol. 22*, 51–56.

Yanagihara, Y., Shiozawa, K., Takai, M., Kyogoku, M., and Shiozawa, S. (1999). Natural killer (NK) T cells are significantly decreased in the peripheral blood of patients with rheumatoid arthritis (RA). *Clin. Exp. Immunol. 118*, 131–6.

Yang, X.D., Tisch, R., Singer, S.M., Cao, Z.A., Liblau, R.S., Schreiber, R.D., and McDevitt, H.O. (1994). Effect of tumor necrosis factor α on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J. Exp. Med. 180*, 995–1004.

Yang, Y. and Wilson, J. (1996). CD40 ligand-dependent T-cell activation: requirement of B7-CD28 signalling through CD40. *Science 273*, 1862–1865.

Yang M., Charlton B., and Gautam A.M. (1997). Development of insulitis and diabetes in B cell-deficient NOD mice. *J. Autoimmun. 10*, 257–260

Zang, Y.C., Li, S., Rivera, V.M., Hong, J., Robinson, R.R., Breitbach, W.T., Killian, J., and Zhang, J.Z. (2004). Increased $CD8^+$ cytotoxic T cell responses to myelin basic protein in multiple sclerosis. *J. Immunol. 172*, 5120–5127.

Zhang, G.X., Xiao, B.G., Bakhiet, M., van der Meide, P., Wigzell, H., Link, H., and Olsson, T. (1996). Both $CD4^+$ and $CD8^+$ T cells are essential to induce experimental autoimmune myasthenia gravis. *J. Exp. Med. 184*, 349–356.

Zheng, S.G., Wang, J.H., Gray, J.D., Soucier, H. and Horwitz, D.A. (2004). Natural and induced $CD4^+CD25^+$ cells educate $CD4^+CD25^-$ cells to develop suppressive activity: the role of IL-2, TGF-β, and IL-10. *J. Immunol. 172*, 5213–5221.

CHAPTER

7

Role of Th1 and Th2 Cells in Autoimmunity

LORENZO EMMI AND SERGIO ROMAGNAMI

Center for Research, Transfer and High Education, MCIDNENT, University of Florence, Italy

DEFINITION AND FUNCTIONAL PROPERTIES OF HUMAN Th1 AND Th2 CELLS

Type 1 and type 2 T-helper (Th1 and Th2) cells do not represent distinct subsets, but rather are polarized forms of the $CD4^+$ Th cell-mediated immune response, which occurs under particular experimental or pathophysiologic conditions. In mice, Th1 responses are characterized by the prevalent production of interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β, without production of IL-4, IL-5, IL-9, IL-10, and IL-13. By contrast, Th2 responses are characterized by the prevalent production of IL-4, IL-5, IL-9, IL-10, and IL-13, in the absence of production of IFN-γ and TNF-β. Th cell responses characterized by the conjunct production of Th1 and Th2 cytokines are commonly defined as type 0 Th (Th0) responses (Mosmann and Coffman, 1989). The Th1/Th2 polarization is clear-cut in murine models based on artificial immunization, whereas it is usually less restricted among human Th cell-mediated responses (Romagnani, 1995). Moreover, in humans, IL-10 is produced by both Th1 and Th2 cells (Romagnani, 1995). In general, Th1-polarized responses are highly protective against infections by the majority of microbes, especially the intracellular parasites, because of the ability of Th1 cytokines to activate phagocytes and to promote the production by B-lymphocytes of opsonizing and complement-fixing antibodies (phagocyte-dependent host defense) (Romagnani, 1995). However, when the microbe is not rapidly removed from the body, the Th1 response may become dangerous for the host, because of the strong and chronic inflammatory reaction evoked. By contrast, cytokines produced by Th2 cells induce the differentiation, activation, and *in situ* survival of eosinophils (through IL-5), promote the production by B-lymphocytes of high amounts of antibodies, including IgE (through IL-4 and IL-13), as well as the growth and degranulation of mast cells and basophils (through IL-4 and IL-9). Moreover, IL-4 and IL-13 inhibit several macrophage functions and IL-4 can suppress the development of Th1 cells (Romagnani, 1997). Thus, the phagocyte-independent Th2 response is usually less protective than the Th1 response against the majority of infectious agents, with the exception of some gastrointestinal nematodes (Finkelman and Urban, 2001). In addition to their protective activity against some nematodes, Th2 cells probably also play a regulatory role in the immune system, because a switch from Th1 to Th2 may provide a protective effect when the Th1 response threatens to become a dangerous event for the host (Romagnani, 1997) (Figure 7.1).

Besides the selective production of IL-4, IL-5, IL-6, IL-9, and IL-13, human Th2 cells also exhibit the preferential expression of some surface molecules, such as CD30, CCR4, CCR8, and the chemoattractant receptor of Th2 cells

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

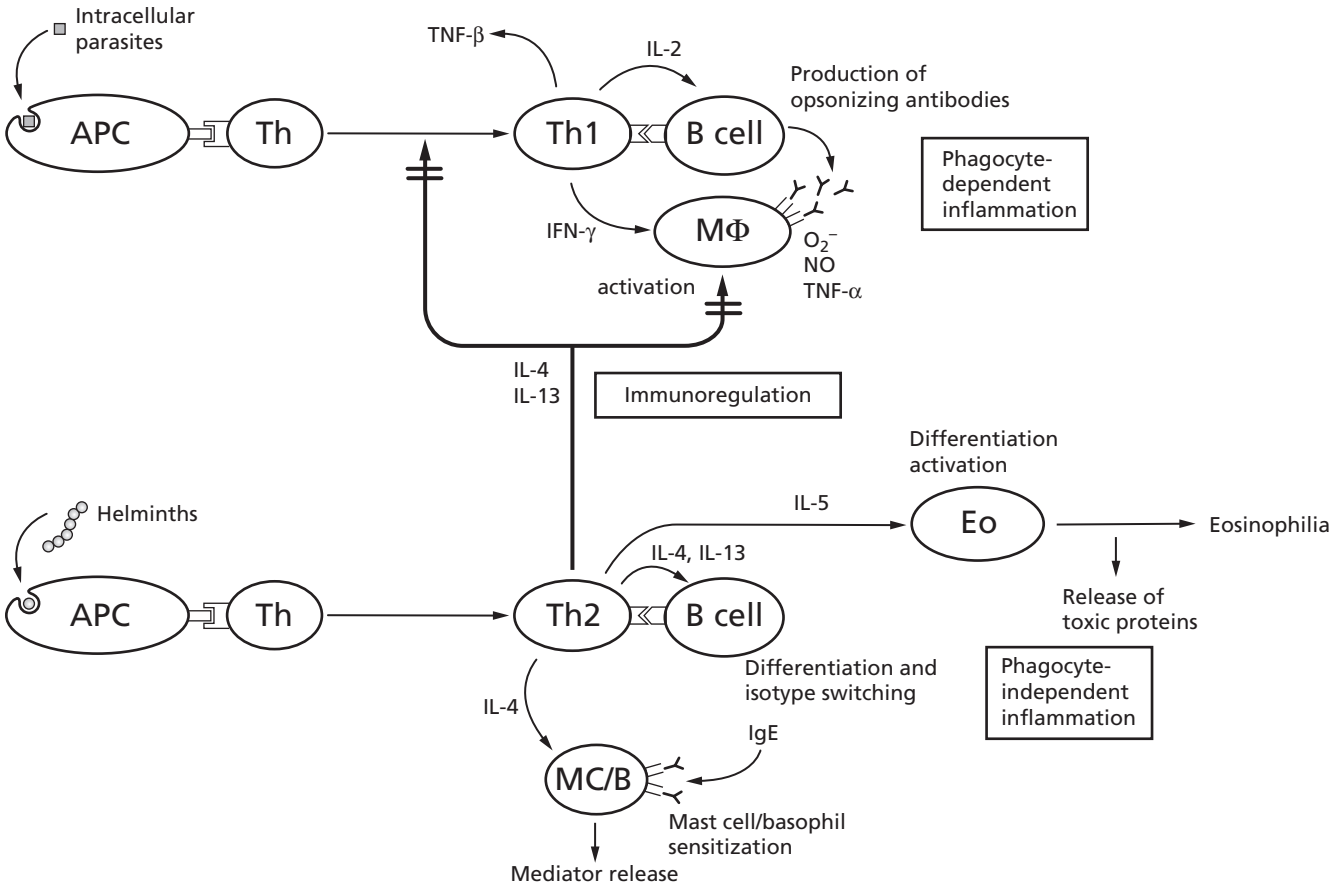


FIGURE 7.1 Th1/Th2 paradigm. Different infectious agents can polarize the helper T (Th)-cell-mediated immune response into different patterns of cytokine production. Intracellular microbes usually induce Th1 responses which are characterized by the production of interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β. IFN-γ is the main activator of phagocytic cells, whereas IL-2 favors the production of opsonizing antibodies by B-cells, which together promote the removal of the infectious agent (phagocyte-dependent inflammation). Helminths usually induce Th2 responses, producing IL-4 and IL-13, which promote the production of high concentrations of antibodies, including IgE and IL-5, which activate eosinophil granulocytes. Moreover, IL-4 and IL-13 inhibit different macrophage activities (phagocyte-independent inflammation). APC, antigen-presenting cell; B, basophil; Eo, eosinophil; Mø, macrophage; MC, mast cell.

(CRTH2) (Romagnani, 1997), whereas human Th1 cells preferentially express the lymphocyte activation gene-3 (LAG-3), and the chemokine receptors CXCR3 and CCR5 (Romagnani, 1997). The chemoattractant receptors prevalently expressed on the surface of Th1 or Th2 cells are important for recruitment and homing in target tissues of effector Th cells.

Th1/Th2 POLARIZATION

Nature of Th1 and Th2 Polarizing Signals

Clear evidence suggests that Th1 and Th2 cells develop from the same Th cell precursor under the influence of both environmental and genetic factors acting at the level of antigen presentation. Suggested environmental factors include the route of antigen entry, physical form of the immunogen, type of adjuvant, and dose of antigen (Abbas et al., 1996; Romagnani, 1997). The genetic mechanisms involved in controlling the type of Th cell differentiation remain elusive. Genetic and environmental factors can influence, independently or in association, a series of modulatory factors, including: 1) ligation of the T-cell receptor (TCR); 2) activation of costimulatory molecules, such as B7/CD28, OX40/OX40L, and LAF-3/ICAM-1; 3) predominance of a given cytokine in the microenvironment of the responding Th cell, such as IL-4, IL-12, IL-18, IFN-γ, and IFN-α; and 4) the type of Notch ligands activated by the exogenous stimulus on the antigen-presenting cell (APC).

It has been known for many years that the production of IL-12, IL-18, and IFNs (γ and α) by cells of the natural immunity, such as dendritic cells (DCs) and natural killer (NK) cells, favors the development of Th1 cells. More recent data have allowed an understanding of why many microbial agents usually induce the production of IL-12 and IFNs (Maggi et al., 1992; Manetti et al., 1993). Both DCs and NK

cells possess receptors on their surface, named Toll-like receptors (TLRs) because of their homology with the archetypal *Drosophila* Toll protein. So far, 10 distinct TLRs have been identified, which are able to recognize constitutive and highly conserved pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), double-stranded RNA, and flagellin, thus resulting in strong activation of, and cytokine production by, DCs and NK cells (Sabroe et al., 2003) (Figure 7.2).

IL-12 produced by DCs is the most powerful Th1-inducing agent and its production is upregulated by both CD40L–CD40 interaction and the production of IFN-γ by NK and Th1-polarized cells. Of interest, IFN-γ, but not IFN-α, promotes Th1 differentiation in mice, whereas both IFN-γ and IFN-α (which is also produced by a subset of DCs) play an important role in humans; IFN-α upregulates the expression of the IL-12 receptor (IL-12R) β chain. In contrast to the production of IL-12 and IFNs, early IL-4 expression during an immune response is critical for the development of Th2 cells. It has been suggested that naïve Th cells are able to produce small amounts of IL-4 from their initial activation, and the concentration of IL-4 that accumulates at the level of the Th cell response increases with increasing lymphocyte activation. The inducing effect of IL-4 dominates over other cytokines, so that if IL-4 levels reach a necessary threshold, differentiation of the Th cell into the Th2 phenotype occurs. Other possible sources of early IL-4 production may be NK T-cells, basophils, mast cells, and eosinophils, but IL-4 produced by these cells is certainly more important in the amplification rather than in the initiation of a Th2 response (Seder and Paul, 1994).

Signals through cytokine receptors elicit a complex series of molecular interactions in the naïve Th cell that culminate in the binding of cell-type-specific transcription factors to multiple regulatory elements in the promotors of cytokine genes, and their subsequent activation (Figure 7.3). At least five groups of transcription factor play an important role in Th2 differentiation. The interaction of IL-4 with its receptor on the surface of the naïve Th cell results in the activation of STAT6 (signal transducer and activator of transcription). Proteins of the NFAT (nuclear factor of activated T cells) family are also involved, since they bind specifically to the promotor region of the IL-4 gene and cooperate with activator protein (AP-1) factors, like Fra and Jun, to induce IL-4 transcription. However, NFAT and AP-1 are expressed by both Th1 and Th2 cells and their role in the selective Th cell differentiation appears to be very complex. By contrast, the proto-oncogene c-Maf is specifically expressed by Th2, but not Th1, cells and binds to a Maf response element (MARE) within the IL-4 proximal promotor. However, c-Maf is specific for IL-4 and is critical for high levels of IL-4 production, but is not sufficient for the initiation of IL-4 transcription. An even more important transcription factor for Th2 differentiation is GATA3, which is undetectable in Th1 cells. GATA3 inhibits the production of IFN-γ, increases the transactivation of the IL-4 promotor, and also directly regulates IL-5 and IL-13 expression (Rengarajan et al., 2000).

While the binding of IL-4 to its receptor activates STAT6, the IL-12–IL-12R interaction results in the activation of STAT4. This transcription factor, as well as IFN regulatory factor (IRF)-1, have been implicated in Th1 differentiation, because mice deficient for each of these factors lack Th1 cells. A substantial advance in elucidating Th1 lineage commitment and IFN-γ gene expression came with the isolation of the protein T-box expressed in T cells (T-bet).

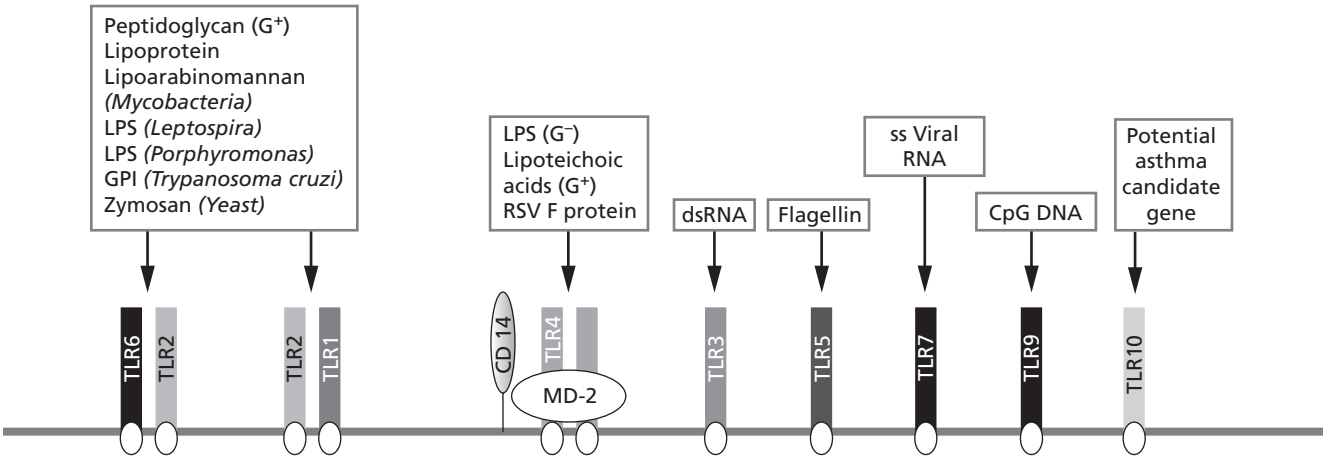


FIGURE 7.2 Schematic illustration of Toll-like receptors (TLRs) present on the surface of dendritic cells (DCs) and other cell types. Each of the 10 known TLRs can recognize constitutive and conserved microbial products. Interaction of TLRs with their respective ligands results in the activation of cells of innate immunity, such as DCs and natural killer (NK) cells, and the production of cytokines, including IL-12 and IFNs, which can influence the subsequent profile of the adaptive immune response. GPI, glycosylphosphatidylinositol; G^-/G^+, LPS, lipopolysaccharide; MD-2, an adaptor molecule required for TLR4 signaling; RSV, respiratory syncytial virus.

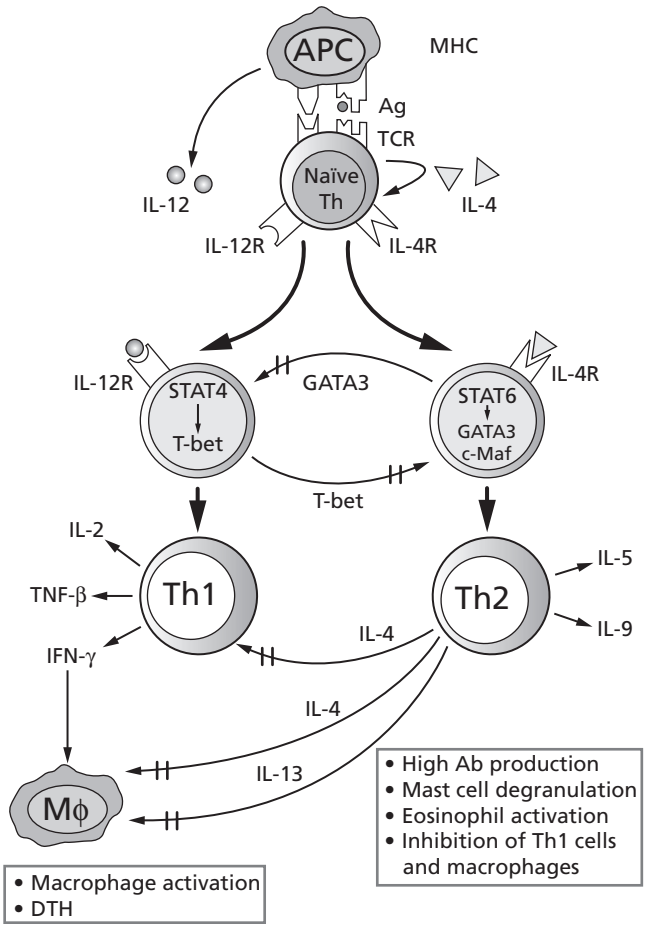


FIGURE 7.3 Main mechanisms involved in Th1 or Th2 polarization. The interaction of interleukin (IL)-12, produced by dendritic cells (DCs) in response to the stimulation of some Toll-like receptors, with its receptor (IL-12R) on the surface of the naïve Th cell, results in the activation of the transcription factors STAT4 and T-bet, which promote Th1 polarization. The early production of IL-4 and its interaction with its receptor (IL-4R) on the surface of the naïve Th cell results in the activation of the transcription factors STAT6, c-Maf, and GATA3, which promote Th2 polarization. Th2 polarization may also occur through the interaction of Jagged ligands expressed on DCs and the Notch receptors present on the naïve Th cell, resulting in the direct activation of the IL-4 promotor (see text). Of note, T-bet not only promotes Th1 polarization but also antagonizes Th2 polarization, whereas GATA3 promotes Th2 polarization and antagonizes Th1 polarization. APC, antigen-presenting cell.

T-bet expression strongly correlates with IFN-γ expression; it is specifically upregulated in primary Th cells differentiated along the Th1, but not the Th2, pathway, and it directly binds to the IFN-γ promotor. Thus, a model for Th1/Th2 polarization that involves a balance between the Th1-specific T-bet and the Th2-specific GATA3 transcription factors may presently be envisaged (Szabo et al., 2000) (see Figure 7.3).

A novel model of Th1/Th2 polarization recently has been described (Amsen et al., 2004). According to this model, APCs also use the Notch pathway to instruct T-cell differentiation. Notch is an evolutionarily conserved receptor involved in cell fate decisions. On binding ligand, its intracellular domain (ICD) is released from the membrane through proteolytic cleavages, enabling cytoplasmic and nuclear functions. A target of the Notch ICD is RBPJk, which is converted from a transcriptional repressor to an activator. Mammals express four Notch genes and, in addition, five genes encode ligands for Notch from two conserved families, Jagged and Delta. Strikingly, the Delta family of Notch ligands present on APCs induces Th1, while the Jagged family induces the alternate Th2 fate. Expression of these different Notch ligands on APCs is induced by Th1- or Th2-promoting stimuli. Interestingly, the Jagged-mediated signaling for Th2 differentiation appears to be independent of the IL-4/STAT6 pathway described above. Notch was indeed found to direct Th2 differentiation by inducing GATA3 upregulation and nuclear translocation, but also by directly regulating IL-4 gene transcription through RPBJk sites in a 3′ enhancer. These findings suggest that Th2 differentiation is not the result of a default pathway but, like Th1 polarization, is strictly controlled by cells of the natural immunity. Indeed, when DCs expressing the Delta family of ligands are stimulated, Notch receptors present on the naïve Th cell induce STAT6-independent upregulation of GATA3 and direct activation of the IL-4 gene. This early production of IL-4 by the naïve Th cell probably originates as an autocrine/paracrine circuit that then amplifies Th2 development via the previously described IL-4/IL-4R/STAT6 pathway (Amsen et al., 2004). By contrast, the role of Delta-mediated Th1 polarization is still unclear, although the Delta ligand on APCs does appear to stimulate Th1 responses.

Finally, it should be noted that the Th cell fate is regulated not only by differentiative signals delivered by early produced cytokines, contact-dependent factors, and the antagonism of transcription factors, but is also dependent on cell cycle expression, i.e., the number of postactivation cell divisions. Several studies suggest that there is an opportunity for a Th cell to initiate cytokine gene expression at each cell division and that the probability of this event varies between cytokines. It is of interest that these division relationships and probabilities of expression appear not to be heavily dependent on the genetic background of the T cells, but rather on epigenetic events that may control cytokine gene accessibility (Bird et al., 1998). Thus, in addition to a deterministic process related to the above-mentioned factors, a probabilistic process may influence the final cytokine pattern produced during the specific Th cell response.

Regulatory Mechanisms

Under some conditions, the T-cell effector response (either Th1- or Th2-polarized) may become dangerous for

the host and therefore, needs to be controlled. Under these conditions, the Th cell immune response can shift from a prevalent Th1 to a prevalent Th2 profile or vice versa. This switching process has been defined as "immune deviation." However, besides the mutual antagonisms at the level of cytokines and transcription factors mentioned above, Th1 and Th2 functions can also be regulated by other T-cell types, called regulatory T-cells (Tregs). Tregs are a highly heterogeneous family, which includes type 3 Th (Th3) cells, T regulatory 1 (Tr1) cells, and $CD4^+CD25^+$ T cells. Th3 cells mainly produce transforming growth factor (TGF)-β and their regulatory function is due to a TGF-β-dependent mechanism, whereas Tr1 cells mainly produce IL-10, with or without TGF-β. By contrast, $CD4^+CD25^+$ T cells do not produce cytokines and act via a contact-dependent mechanism, which probably involves the activity of both membrane CTLA (cytotoxic T-lymphocyte-associated antigen)-4 and TGF-β (Bluestone and Abbas, 2003). Another feature of $CD4^+CD25^+$ T cells is the expression of the products of FoxP3 and glucocorticoid-induced TNF receptor (TNFR)-related (GITR) genes (Fontenot et al., 2003).

Due to the heterogeneity of the Treg family, a distinction between "natural" and "adaptive" Tregs has recently been suggested (Bluestone and Abbas, 2003) (Figure 7.4). Natural Tregs are generated in the thymus and normally function to prevent the activation of other self-reactive T cells that have the potential to develop into effector cells. Indeed, both thymectomy and depletion of $CD4^+CD25^+$ T cells result in mice developing spontaneous autoimmune disorders, suggesting the important protective role of natural Tregs against autoimmunization. These natural Tregs act mainly by T–T-cell–APC contact in a cytokine-independent fashion. Similar to natural Tregs, adaptive Tregs also originate from the thymus, but they then further differentiate and acquire their suppressive activity in the periphery under certain conditions of antigenic stimulation. Their expression of CD25 is variable and their mechanism of suppression is mediated by the production of inhibitory cytokines, such as IL-10 and TGF-β. Natural and adaptive Tregs might function in different immunologic settings, depending on the context of antigen exposure, the nature of the inflammatory response, and the TCR repertoires of the individual cells. The relationship between microbial stimulation of the TLR pathway and Tregs is unclear. However, it has been shown that microbial induction of the TLR pathway can also block the suppressive effect of $CD4^+CD25^+$ Tregs, allowing activation of pathogen-specific adaptive immune responses (Netea et al., 2004). Thus, a complex and still partially unknown network of cells of innate immunity, Th1 or Th2 effector cells, and Tregs, seems to be operating (Figure 7.5).

Pathophysiologic Manifestations

Th1 and Th2 cells play a protective role against different infectious agents, but sometimes their wrong or exaggerated

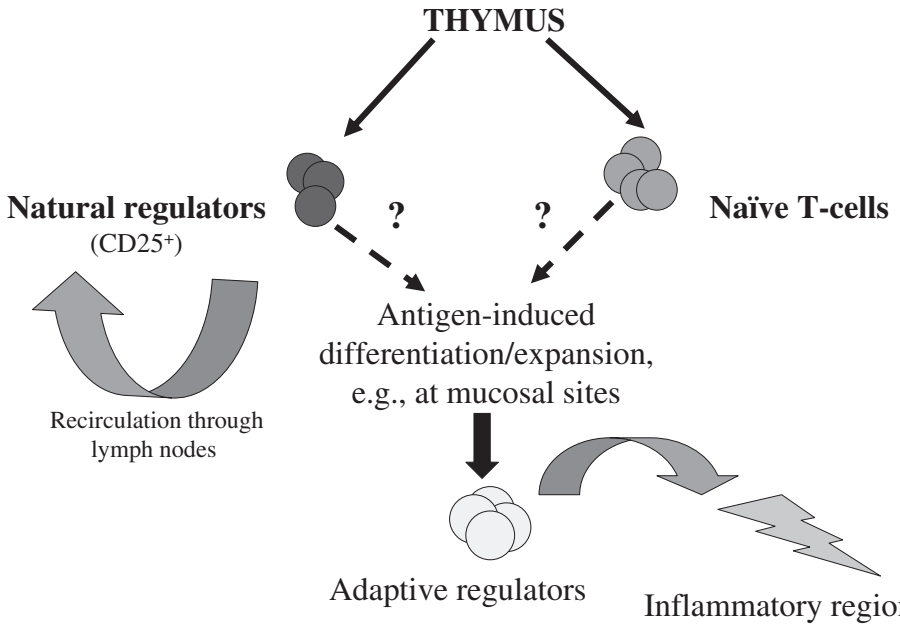


FIGURE 7.4 Regulatory T cells (Tregs). Natural Tregs originate from the thymus as $CD4^+CD25^+$ T cells and suppress the function of autoantigen-specific T cells not only in the thymus but also in the periphery. A proportion of the naïve T cell ($CD25^-$) population that originates from the thymus may encounter exogenous antigens in the periphery, which, under particular conditions, confer the ability to suppress the immune response with acquisition of CD25 positivity (adaptive Tregs) in an inflammatory site.

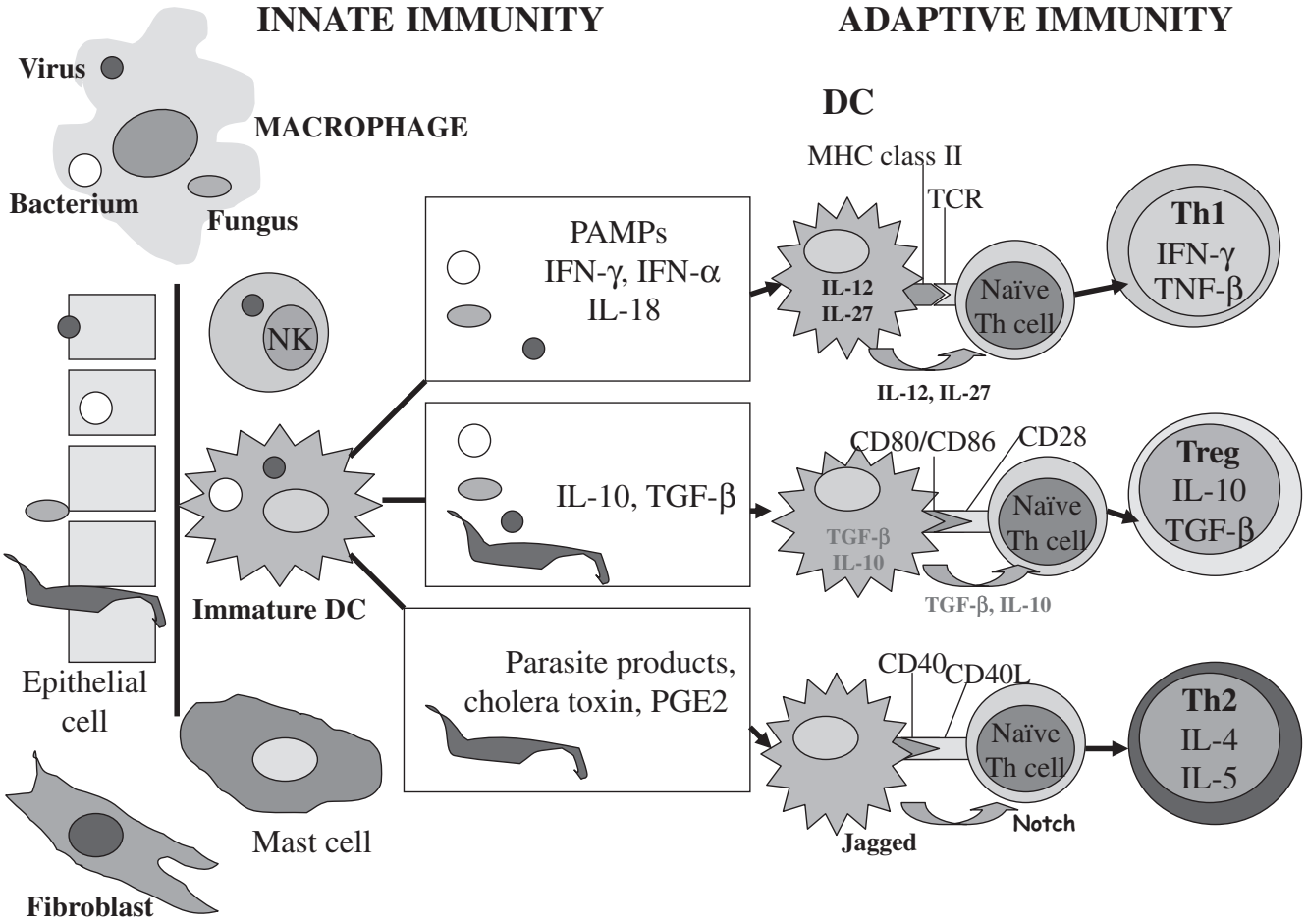


FIGURE 7.5 Complex bridge linking innate immunity, adaptive immunity, and immune regulation. Different pathogens can stimulate different types of dendritic cells (DCs) through interaction with the Toll-like receptors (TLRs) present on these cells, which may result in Th1 or Th2 polarization, as well as the development of regulatory T cells (Tregs), which are then able to suppress the Th cell effector responses. The production by DCs of interleukin (IL)-12 and interferon (IFN)-α, together with the production of IFN-γ by natural killer (NK) cells, promotes Th1 polarization. The production by DCs of IL-10 and tumor growth factor (TGF)-β promotes the development of Tregs. The production of IL-4 by mast cells, but mainly the interaction of Jagged ligands from DCs and Notch receptors present on the naïve Th cell, may promote Th2 differentiation. PAMPs, pathogen-associated molecular patterns; PGE2, prostaglandin E2; TCR, T-cell receptor.

response may cause an abnormal or chronic pathologic condition. Allergic disorders represent the prototypical immune-mediated conditions related to a polarized Th2 response against common environmental allergens. More often, however, Th1 cells are involved in the induction and maintenance of chronic inflammatory processes. These processes are usually initiated by an antimicrobial Th1 response that is unable to remove the invading agent, thus resulting in the perpetuation of an inflammatory response which is mainly sustained by the activation of phagocytic cells and the release of high concentrations of inflammatory cytokines. Under some conditions, and based on a particular genetic background, this may result in the accessibility of sequestered autoantigens and the aberrant expression of costimulatory molecules by APCs. This series of events is commonly known as "bystander activation." Infectious agents may also express antigens cross-reactive with self-determinants and this may trigger a Th1-mediated response against some peptide(s) shared by one or more autoantigen (epitope mimicry). These two possible events are now considered to be the most important mechanisms of autoimmunization that may follow a Th1 response triggered by a microbial agent, and are probably the main reason why the great majority of organ-specific autoimmune diseases have been found to be characterized by the prevalence of Th1 cells, their cytokines, and other Th1-related molecules.

Th1/Th2 BALANCE IN AUTOIMMUNE DISORDERS

Several clinical and experimental findings suggest that autoimmune diseases develop as a result of abnormalities in the immune response mediated by T cells and T-cell-derived lymphokines. Evidence is now accumulating in both experimental animal models and human pathologic conditions to suggest that the relative contribution of either Th1- or Th2-dominated reactions can determine not only the development of a particular autoimmune response, but also influence whether or not this response leads to clinical symptoms. Moreover, an important interplay with the activity of Tregs is also emerging.

Multiple Sclerosis

Multiple sclerosis (MS) is presumed to be an autoimmune disease of the central nervous system (CNS). Active lesions in the brains of patients with MS are characterized by lymphocyte (mainly $CD4^+$ T cells) and macrophage infiltration, but the relationship between these cells and the demyelinating process is still partially unclear. Although the etiology of the disease is unknown, it is thought to involve an autoimmune reaction against myelin antigens, such as myelin basic protein (MBP), proteolipid protein (PLP), and possibly other proteins, as shown in its classical animal disease counterpart, experimental autoimmune encephalomyelitis (EAE) (Windhagen et al., 1995).

Several features of MS lesions suggest that in the human disease the inflammatory process is mainly driven by a Th1-mediated autoimmune response. Several studies have suggested a primary pathogenic role for TNF-α and IFN-γ in MS. First, high levels of TNF-α were found in the cerebrospinal fluid (CSF) of patients with chronic progressive MS (Sharief and Hentges, 1991; Tsukada et al., 1991; Rieckmann et al., 1994; 1995). Most clones derived from both the peripheral blood and CSF of patients with MS showed a Th1 profile (Brod et al., 1991; Benvenuto et al., 1991; Correale et al., 1995). More recently, several Th1-type cytokine receptors (Fas, TNFR-1, and TNFR-2) have been described on oligodendrocytes (with ligands occurring on microglia) and show enhanced expression around MS lesions. Other studies have detected the presence of TRAIL-R2 and TRAIL-R4 on the same cells (Weber et al., 2004). Cytokine receptors on oligodendrocytes may play a role in both cell fate decisions and broader immunologic responses, a feature of particular relevance in a disease such as MS, where the oligodendrocyte is the major target. TNF-β expression was associated with T lymphocytes, whereas TNF-α was associated with astrocytes in all areas of the lesion (Selmaj et al., 1991). By using both semiquantitative reverse transcriptase-polymerase chain reactions (RT-PCRs) and immunohistochemistry, the cytokine IL-12p40 appeared to be predominantly upregulated in acute MS plaques in early disease, suggesting that an early event in the initiation of MS is the upregulation of costimulatory molecules and IL-12 (Windhagen et al., 1995). IL-18, another Th1-polarizing cytokine produced by DCs, has also been shown to be produced by activated microglia and may contribute to inflammation in the brain through synergism in a cascade of cytokines associated with the innate response, including IL-12 and IL-15. These probably represent the conditions that maximally stimulate T-cell activation and induce Th1-type immune responses. Finally, a high number of chemokines, such as CCL2, CCL3, CCL4, CCL5, CCL7, CXCL9, and CXCL10, produced by myelin-reactive T cells, macrophages, and glial cells have also been found to be expressed at the CNS level in MS. These factors can initiate a pathogenic cascade in the CNS, leading to inflammation, demyelination, and axonal damage, which contribute to the functional deficiencies. More importantly, in patients with MS, CXCL10 levels in the CSF are significantly higher than in controls (Franciotta et al., 2001; Sørensen et al., 2001), and Th1 cells expressing CXCR3 have been detected in the brain and CSF (Balashov et al., 1999; Sørenson et al., 1999; Misu et al., 2001).

However, there is evidence that cells other than classical Th1 cells contribute to the inflammatory response in MS lesions. Numerically, $CD8^+$ major histocompatibility complex (MHC) class I-restricted T cells outnumber $CD4^+$ T cells. Furthermore, class I-restricted $CD8^+$ T cells predominate at the site of tissue destruction in actively demyelinatimg lesions, whereas $CD4^+$ T cells are retained mainly in perivascular inflammatory infiltrates. Recent studies based on PCR analysis at the single-cell level show that clonal expansion is much more prominent in the $CD8^+$ than in the $CD4^+$ T-cell population. Finally, recent data based on the use of microarray analysis showed upregulation of the tryptase gene in MS plaques, suggesting a role for mast cell-derived products in the genesis of MS lesions (Pedotti et al., 2003). Thus, although the possible inflammatory role of Th2-related effector phenomena in MS is certainly less important than Th1 cell-triggered inflammation, the emerging picture is more complex than a pure Th1-mediated disorder (Lassmann and Ransohoff, 2004). It probably also includes a loss of functional suppression by $CD4^+CD25^+$ Tregs (Viglietta et al., 2004).

A hypothetical scheme illustrating the main immunologic events involved in the pathogenesis of MS is shown in Figure 7.6.

Autoimmune Thyroid Diseases

Autoimmune thyroid diseases include Hashimoto' thyroiditis (HT) and Graves' disease (GD). HT is an organ-specific autoimmune disease characterized by massive infiltration of lymphoid cells in the thyroid and parenchymal

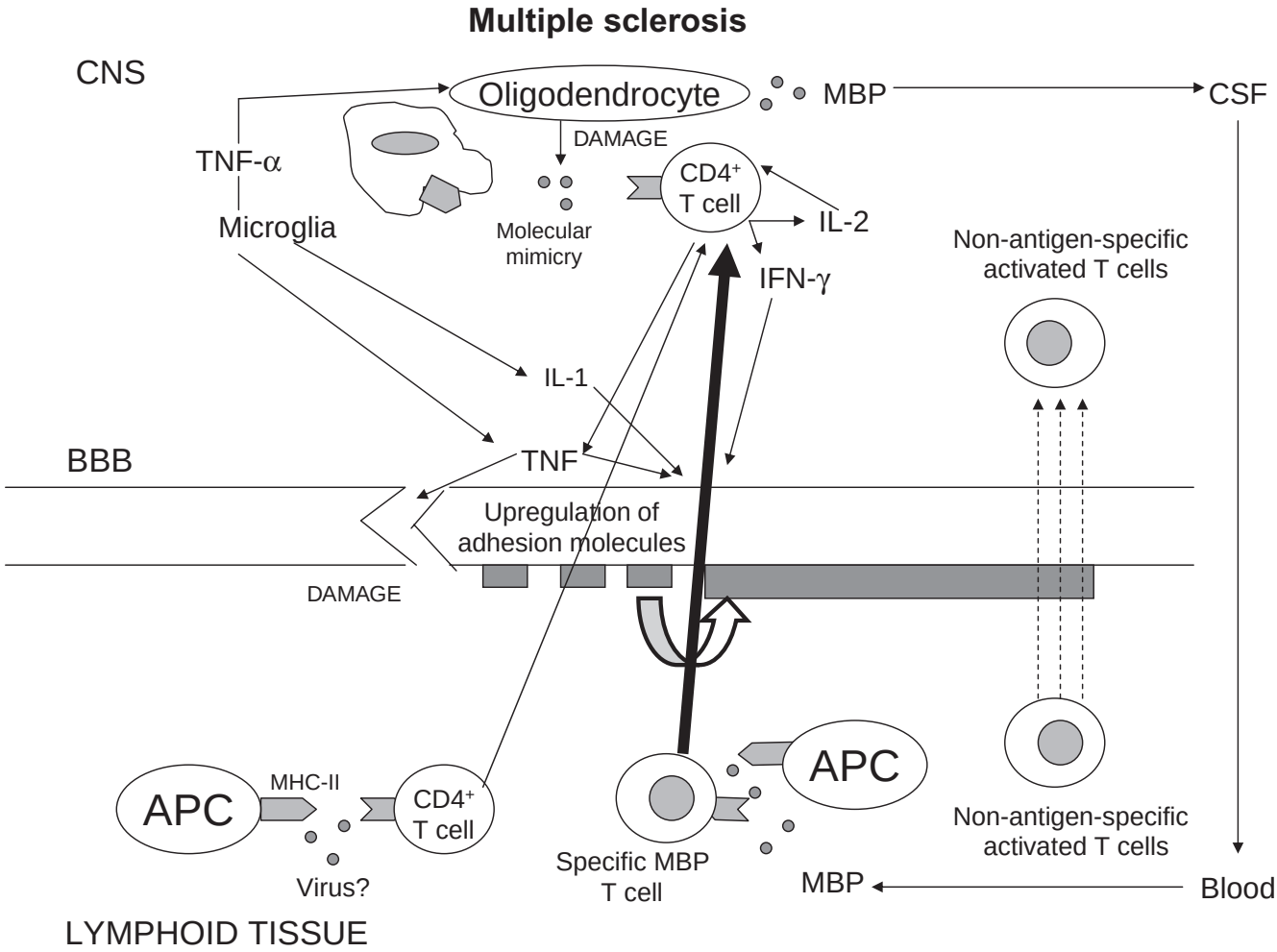


FIGURE 7.6 Possible events contributing to the development of multiple sclerosis. T-lymphocytes gain access to the central nervous system (CNS) by diapedesis through the blood–brain barrier. T-cell recruitment is a complex multistep process that can be influenced by several mediators, including those derived from macrophages, microglia, mast cells, and T cells themselves. Once within the CNS, T cells can release inflammatory cytokines in response to appropriate stimulation. Macrophages recruited by T cells and resident microglia cells also contribute to the tissue damage. In addition to promoting myelin sheath destruction through the direct effects of certain secreted mediators, T cells, macrophages, microglia, and mast cells can also contribute to the development of a local inflammatory response, in which additional recruited cells and mediators might also contribute to the destruction of the myelin sheath. Myelin basic protein (MBP) is shown as an illustrative autoantigen since it is operative in the experimental autoimmune encephalomyelitis (EAE) model. APC, antigen-presenting cell; CSF, cerebrospinal fluid; BBB, blood–brain barrier; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

destruction leading to hypothyroidism. GD shows a histologic picture often indistinguishable from HT, but is characterized by both the production of thyroid-stimulating antibodies leading to hyperthyroidism and an associated ophthalmopathy.

Although the precise etiology of HT remains largely unknown, the role of infiltrating T-lymphocytes in the destruction of the target organ is generally accepted, and has been definitively proved in a "humanized" model of transgenic mice expressing a human TCR derived from the thyroid-infiltrating T cell of a patient with thyroiditis and specific for a cryptic thyroid-peroxidase epitope. These mice spontaneously developed destructive thryoiditis with histologic, clinical, and hormonal signs comparable with human autoimmune hypothyroidism (Quaratino et al., 2004). Accordingly, studies performed in humans by different laboratories showed that T cells from lymphocytic thyroid infiltrates of patients with HT or GD had a clear-cut Th1 lymphokine profile, with production of high TNF-α and IFN-γ concentrations, and exhibited strong cytolytic

potential (Del Prete et al., 1987; 1989). Moreover, quite a homogenous Th1 profile was also observed in $CD4^+$ T-cell clones derived from retroorbital infiltrates of patients with Graves' ophthalmopathy (De Carli et al., 1993). In contrast, either a Th1-like (Watson et al., 1994) or a more heterogenous cytokine profile (Grubeck-Loebenstein et al., 1994; McLachlan et al., 1994; Paschke et al., 1994; Roura-Mir et al., 1996) was found using PCR in both the thyroid gland and the retroorbital infiltrates of patients with GD (see Chapter 35). Th2 responses have been implicated in GD, which is caused by autoantibodies to the thyrotropin receptor (TSHR), which stimulates the thyroid [reviewed in Rapoport and McLachlan (2001)]. Indeed, a number of studies indicate that Th2 cytokines, or responses reflecting Th2 cytokines, are elevated in patients with GD. In addition, TSHR-specific clones derived from patients with GD secrete IL-4 and relatively little IFN-γ. Furthermore, elevated serum concentrations of eosinophil-derived neurotoxin (EDN) have been detected in GD, but not in HT. Moreover, the levels of EDN correlated with TSHR antibody activity measured by inhibition of thyrotropin (TSH) binding, suggesting that Th2-type responses are crucial for GD (Hidaka et al., 2003).

One explanation for the discrepancy between findings suggesting a Th1 predominance and those indicating a Th2 predominance in GD may emerge from recent experiments assessing the levels of the IFN-γ-induced chemokine CXCL10, as well as the presence of its receptor, CXCR3, in the thyroid gland and/or serum of patients examined at different times from the onset of GD. Both CXCR3 and CXCL10 were found to be expressed in the gland of patients in the early phases of GD, the former at the level of infiltrating cells and the latter at the level of follicular epithelial cells. Moreover, high CXCL10 levels were found in the sera of these patients. However, CXCL10 values declined in later phases and the CXCL10/CXCL22 (a Th2-related chemokine) ratio progressively decreased with time (Romagnani et al., 2002). These findings provide strong, although indirect, evidence that GD is initiated by a Th1-dominated inflammatory process, due to the recruitment of CXCR3-expressing Th1 cells by CXCL10 produced by follicular epithelial cells in response to some still unknown triggering agent (Romagnani et al., 2002). Then, Th1 cells produce IFN-γ, which further stimulates the production of CXCL10, thus contributing to the amplification of the inflammatory process. In subsequent phases, an immune deviation from Th1 to Th2 can occur to dampen the chronic Th1 inflammation, and this may account for the documented presence at glandular level of Th2-type cytokines. It is also of note that patients with MS treated with the Campath-1H antibody show a strong depletion of T cells, including those biased towards the Th1 profile, and the appearance of Graves' disease in a third of them (Coles et al., 1999). This finding suggests a possible major role for Tregs in controlling the breakdown in self-tolerance mechanisms that initiate thyroid autoimmunity.

Type 1 (Insulin-Dependent) Diabetes Mellitus

A cellular autoimmune process that selectively destroys the pancreatic islet β cells is thought to be responsible for the development of type 1 diabetes (T1D) in humans (Eisenbarth, 1986) and the spontaneous animal models, the Bio Breeding (BB) rat (Marliss et al., 1983) and the nonobese diabetic (NOD) mouse (Leiter et al., 1987) (see Chapter 36). A common histopathologic feature associated with the development of T1D is insulitis, characterized by the presence within and around the islets of mononuclear cells consisting predominantly of T lymphocytes and macrophages (Bottazzo et al., 1985; Foulis and Farquharson, 1986). The pathogenic role of Th1 cells in T1D in NOD mice is well established and seems to be related to a genetically controlled alteration intrinsic to T cells (Koarada et al., 2002). Disruption of the STAT4 gene, whose product mediates IL-12 signaling and regulates Th1 differentiation (Rengarajan et al., 2000), completely prevented the development of spontaneous diabetes in NOD mice (Yang et al., 2004). Accordingly, systemic administration of IL-18, a cytokine involved in Th1 polarization, promotes T1D development in young NOD mice (Oikawa et al., 2003). The role of Th1 cytokines in the pathogenesis of T1D in NOD mice is more complex than initially thought. Indeed, IFN-γ can have either a pathogenic or protective role for T1D development in NOD mice, since its deletion can allow alternative pathways to accelerate the establishment of diabetes (Trembleau et al., 2003).

The inflammatory cell infiltrate in insulitis lesions in human T1D has not been fully characterized. However, it appears that it is composed of both lymphocytes and macrophages (ratio 7–9:1) and that high proportions of lymphocytes (about 40%) contain IFN-γ (Foulis et al., 1991), thus suggesting a predominant Th1 phenotype. Interestingly, T-cell clones from peripheral blood of patients with recent-onset T1D had a Th1 cytokine profile, while those from prediabetic patients were of the Th2 subtype (Chang et al., 1995). Moreover, overwhelming IFN-γ production has been found in high-risk first-degree relatives of children with T1D, suggesting a Th1-like immune deviation in the prediabetic phase (Karlsson et al., 2000). Likewise, serum concentrations of CXCL10, a chemokine induced by IFN-γ are increased in patients with T1D, but only during the early and subclinical stage of the disease (Nicoletti et al., 2002). Recent observations also suggest a possible deficiency of $CD4^+CD25^+$ Tregs in patients with T1D (Kukreja et al., 2002).

A hypothetical scheme illustrating the main immunologic events in patients with T1D is shown in Figure 7.7.

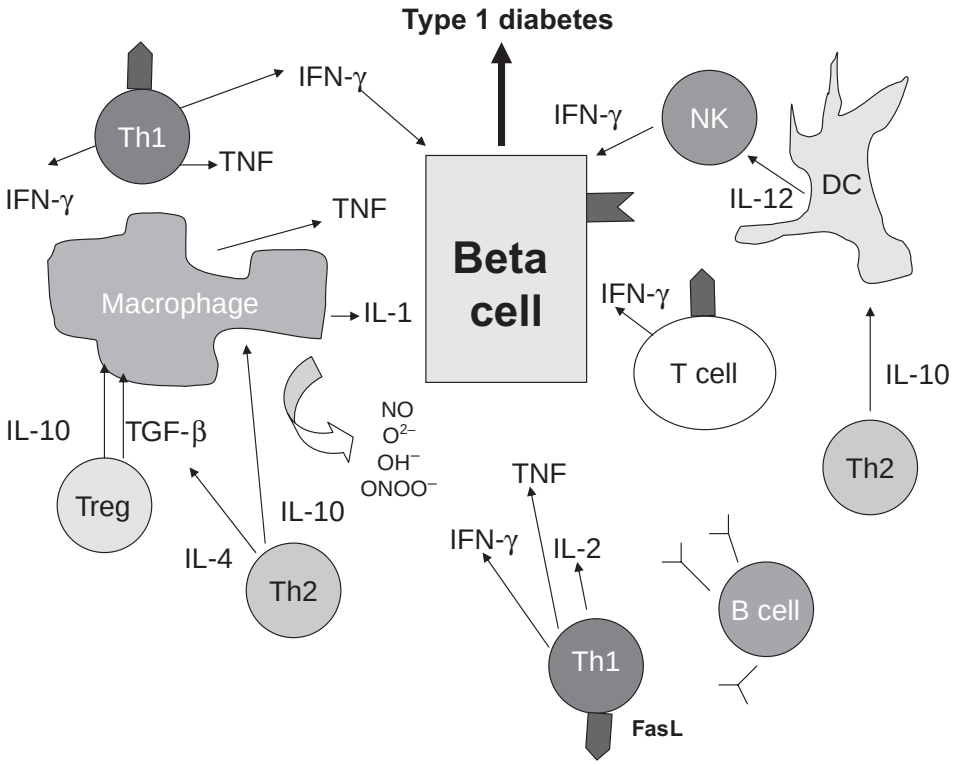


FIGURE 7.7 Possible events contributing to the development of type 1 diabetes (T1D). The hallmark is the selective destruction of insulin-producing cells (β cells) in the pancreas (insulitis). The islet tissue obtained by pancreatic biopsy from patients with recent-onset T1D shows insulitis, characterized by the infiltration of $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, and macrophages. An interaction between genetic and environmental factors is responsible for triggering an immune-mediated response, characterized by the appearance of autoantibodies as the first sign of β cell destruction by $CD4^+$ and $CD8^+$ T lymphocytes. A staged progression to overt diabetes through extensive beta cell destruction is related to various factors, including development of a more aggressive T-cell phenotype and a change in the Th1/Th2 balance towards a more proinflammatory milieu. The expression of FasL on cytotoxic T cells is also a marker of the progression to overt T1D. DC, dendritic cell; IFN, interferon; IL, interleukin; NK, natural killer cell; TGF, transforming growth factor; Treg, regulatory T cell.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by a chronic synovitis, which often leads to joint destruction (Harris, 1990) (see Chapter 32). Many studies have investigated the role of cytokines in the pathogenesis of RA. These studies have been performed both in patients and animals with collagen-induced arthritis (CIA), which is considered to be the best model of experimentally-induced RA. There is considerable and convincing experimental evidence for a dominant Th1 drive in murine CIA [reviewed by Schulze-Koops and Kalden (2001)]. When cytokine levels of cultured draining lymph node cells from mice with CIA are monitored during induction and throughout the time of clinical manifestation, IFN-γ can be detected very early, whereas production of Th2 cytokines is suppressed. Moreover, mice treated with IL-12 or IL-18, two powerful Th1-polarizing agents, develop a more severe disease. Finally, and most importantly, IL-12-deficient mice, as well as mice treated with an anti-IL-12 antibody, which have an impaired Th1 response and reduced IFN-γ production, manifest a significant reduction in both the incidence and severity of CIA. On the other hand, a beneficial effect of both Th2 and Treg cytokines, such as IL-4, IL-10, or TGF-β, has been reported (Schulze-Koops and Kalden, 2001).

The experimental evidence for a dominant Th1 drive in RA in humans is less clear, and categorization of a complex disease such as RA as "Th1" is probably too simplistic. It is well-established that pro-inflammatory cytokines, such as TNF-α, IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-6 (Di Giovine et al., 1988; Hirano et al., 1988; Bucala et al., 1991; Haworth et al., 1991; Deleuran et al., 1992; Wood et al., 1992), as well as chemokines, such as CXCL8, CCL2, and CCL5 (Hosaka et al., 1994), are produced by the synovial membrane in RA and are considered to be important in the pathophysiology of the disease. Indeed, they can both induce bone resorption and cartilage destruction, and can stimulate prostaglandin

E2 release and collagenase production (Feldman et al., 1992). In contrast to the abundance of monocyte-derived cytokines, T-cell-derived cytokines have often proven difficult to detect in RA synovium (Firestein et al., 1988; Brennan et al., 1990), despite the fact that synovial membrane-infiltrating T cells appear to be phenotypically activated (Hovdenes et al., 1989). This has led to the suggestion that the pathogenesis of RA is mediated solely by macrophages and their effector cytokines (Firestein and Zvaifler, 1993).

An alternative view is that T-cell cytokines may be important, but are expressed at levels too low for detection by conventional methods (Panayi et al., 1992). In support of this possibility is the demonstration, by either PCR or *in situ* hybridization, of mRNA for IFN-γ and IL-2 in RA synovial tissue (Buchan et al., 1988; Firestein et al., 1990; Simon et al., 1994). Moreover, the majority of circulating mononuclear cells from RA patients show elevated expression of mRNA for Th1 cytokines, while elevated mRNA levels for IL-4 are present only in a few cases, which supports a predominant activation of Th1/Th0 cells (Schulze-Koops et al., 1995). Finally, T-cell clones derived from RA synovium produce predominantly IL-2 and IFN-γ, which suggests a Th1 profile (Miltenburg et al., 1992; Quayle et al., 1993). However, some rheumatoid inflammatory T-cell clones also exhibit a Th0-like or even a Th2-like profile (Quayle et al., 1993), which may represent the result of a disease-dampening immune deviation. Moreover, the number of IFN-γ-producing $CD4^+$ T cells is significantly increased in the synovial fluid compared to the peripheral blood (Davis et al., 2001), resulting in a markedly elevated Th1/Th2 ratio that correlates with disease activity (van der Graaf et al., 1999). In the synovial fluid of RA patients, IL-18 expression associates with the high levels of both IL-1β and TNF-α (Joosten et al., 2003), and seems to promote joint inflammation and cartilage destruction through a separate pathway (Joosten et al., 2004). Of note, progenitor cells and soluble factors in the synovial fluid of RA patients yield a subset of myeloid DCs that preferentially activate Th1 responses (Santiago-Schwartz et al., 2001). Increased numbers of peripheral blood cells secreting IFN-γ and IL-2 were also found in patients with new-onset synovitis (of under 1-year duration) and, more interestingly, the frequencies of IFN-γ-secreting cells in early arthritis correlated with disease activity, emphasizing the role of Th1 cells in the initiation of the disease (Kanik et al., 1998). Accordingly, analysis of subcutaneous nodules in RA patients revealed a classic Th1-like granuloma pathway (Hessian et al., 2003). The possible role of CXCL10 in the recruitment of Th1 cells from the bloodstream into the synovial joints has been suggested (Hanaoka et al., 2002). On the other hand, in addition to proinflammatory cytokines, a compensatory anti-inflammatory response has also been observed in RA synovial membranes. Thus, high levels of IL-1RA, soluble TNF receptors (both the 55- and 75-kDa receptors), IL-10, and TGF-β have been found in RA synovial fluid (Brennan et al., 1990; Deleuran et al., 1992). IL-10 was also found at both the mRNA and protein level in RA synovial membranes (Katsikis et al.,1994). This suggests that homeostatic immune system mechanisms exist in the rheumatoid joint to contain inflammation and limit joint destruction. Indeed, blocking IL-10 in synovial membrane cultures resulted in an increase in both TNF-α and IL-1β (Katsikis et al., 1994). Because IL-10 is a *powerful* inhibitor of both Th1 and macrophage cytokine production (Fiorentino et al., 1989; Del Prete et al., 1993), it is also possible that IL-10 may represent the factor, or one of the factors, responsible for the "elusiveness" of Th1-derived cytokines in RA. However, the possibility of a more heterogenous Th cell response cannot be excluded.

The complex immunologic events in patients with RA are shown in Figure 7.8.

Sjögren Syndrome

Sjögren syndrome (SS) is a chronic autoimmune disease with a clinical spectrum defined by the simultaneous presence of keratoconjunctivitis sicca and xerostomia in patients not fulfilling criteria for any other chronic inflammatory connective tissue disease (Skopouli et al., 1986) (see Chapter 31). The disorder is characterized by immune system hyperactivity expressed as hypergammaglobulinemia, multiple organ and non-organ-specific autoantibodies, and focal lymphocytic infiltration of the exocrine glands (Fox and Kang, 1992). Although not yet conclusive, the results of different studies suggest a predominant activation of Th1 cells in patients with SS. This has been observed in the IqI/Jic mouse model of primary SS, where the expression of IL-12 and IFN-γ associates with the presence of CD86-expressing DCs in the early development of sialodacryoadenitis (Konno et al., 2003).

In the human disease, the cytokines IL-1, IL-6, TNF-α, and IFN-γ were identified in defined parts of labial salivary gland specimens (Rowe et al., 1987; Oxholm et al., 1992). Increased serum levels of IL-6 and IFN-γ have also been reported (Al-Janadi et al., 1993). It has been suggested that virus-induced IFN-γ production may shift the distribution of the nuclear autoantigen La (SSB) from the nucleus to the cytoplasm and membrane of salivary cells, thus favoring the autoimmunization process (Clark et al., 1994). Indeed, spontaneous IFN-γ mRNA has been observed in freshly isolated unstimulated T cells from SS patients (Villarreal et al., 1995). Increased production of IL-10 by stimulated peripheral blood mononuclear cells and spontaneous IL-10 mRNA expression by freshly isolated mononuclear cells from SS patients have also been reported (Liorente et al., 1994; Villarreal et al., 1995), which may reflect a homeostatic attempt to contain the inflammation induced by the Th1

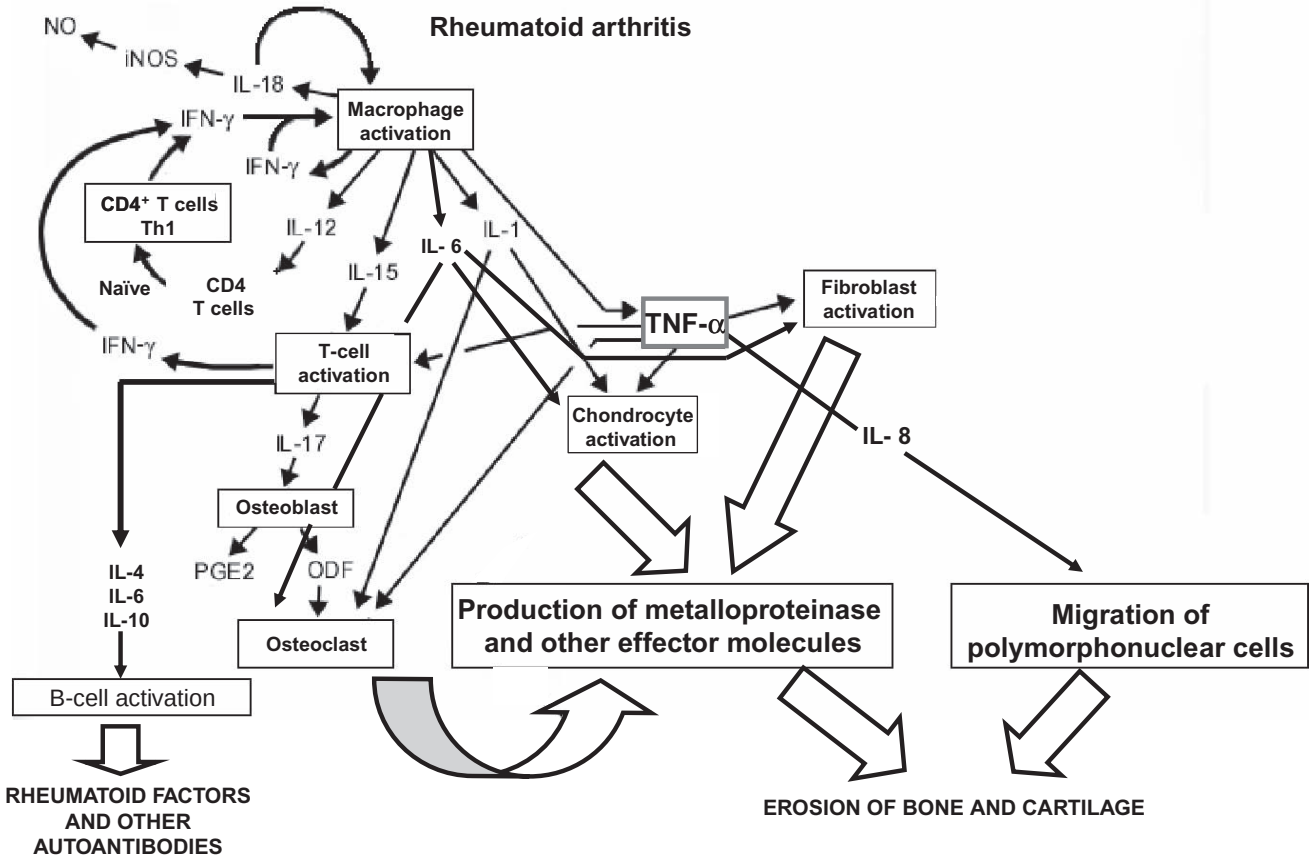


FIGURE 7.8 Possible events contributing to the development of rheumatoid arthritis (RA). Antigen-activated $CD4^+$ T cells stimulate macrophages and synovial fibroblasts to produce the cytokines interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and matrix metalloproteinases. Activated $CD4^+$ T cells also collaborate with B cells in the production of immunoglobulins, including rheumatoid factor and other autoantibodies, and stimulate osteoclastogenesis. IL-1, IL-6, and TNF-α are the key cytokines in RA. TNF-α and IL-1 are potent stimulators of synovial fibroblasts, osteoclasts, and chondrocytes, which release tissue-destroying matrix metalloproteinases. IL-1 and TNF-α also inhibit the production of tissue inhibitors of metalloproteinases by synovial fibroblast. These dual actions are thought to lead to joint damage. In addition, TNF-α stimulates the development of osteoclasts, which are responsible for bone degradation. NO, nitric oxide; iNOS, induced nitric oxide synthase; ODF, osteoclast differentiation factor; PGE2, prostaglandin E2.

cytokines (see RA above). The possible role of IL-15 produced by macrophages in recruiting and activating T-lymphocytes into synovial membrane has also been suggested, but the pathogenic meaning of this finding remains unclear (Mcinnes et al., 1996). More recent data demonstrate the existence of both Th1 and Th2 cytokines in labial salivary glands (LSGs) of SS patients, with the balance between the two shifting in favor of Th1 in patients with a high LSG infiltration score (Mitsias et al., 2002). The predominance of the Th1 drive in SS patients has also been recently confirmed by the observation of increased circulating levels and salivary gland expression of IL-18 (Bombardieri et al., 2004).

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by multiple immune abnormalities, culminating in the overproduction of a wide range of autoantibodies and a constellation of pathologic abnormalities involving the kidney, skin, brain, lungs, and other organs (Milis, 1994) (see Chapters 27 and 28). The imbalance between Th1 and Th2 cytokine production in SLE patients, which favors Th2 cytokines, may be critical to disease induction. It may contribute to the increased B-cell activation characteristic of SLE patients, and also to disease perpetuation. Moreover, the cytokine imbalance might underlie

the impaired self-tolerance, because several Th2 cytokines stimulate B cells. However, the immune alterations of SLE are likely much more complex than a Th1/Th2 imbalance alone, and the reasons for the defective Th1 responses in patients with SLE remain speculative. Downregulation by excessive Th2 cytokines, defective interaction between APCs, T cells, and NK cells, the presence of IL-2 inhibitors, and the downregulation of IL-2 receptors are possible mechanisms. Alternatively, the strong increase in IL-10 production may account for both the potent stimulation of B-cell proliferation and defective Th1 response (Csiszar et al., 2000). In contrast, IL-12 production was found to be inhibited in circulating mononuclear cells from SLE patients compared with matched controls (Horwitz et al., 1998). Thus, a dysregulation of the IL-10/IL-12 balance might play an important role in the impaired cellular immune responses seen in patients with SLE. However, multiple alterations in the immunoregulatory network (Figure 7.9) certainly play a more crucial role in SLE pathogenesis (Mok and Lau, 2003).

Systemic Sclerosis

Systemic sclerosis (SSc) is characterized by inflammatory, vascular, and fibrotic changes of the skin (scleroderma) and a variety of internal organs, most notably the gastrointestinal tract, lungs, heart, and kidney (see Chapter 29). In the skin, a thin epidermis overlies compact bundles of collagen, which lie parallel to the epidermis. Increased numbers of T cells may be present at the border of skin lesions, as well as in other organs in the early stages of the disease (Roumm et al., 1984). Furthermore, cellular autoimmunity to collagen and laminin (Fleischmajer et al., 1993), and the claimed development of scleroderma-like lesions after silicone mammoplasty (Endo et al., 1987) (see Chapter 23),

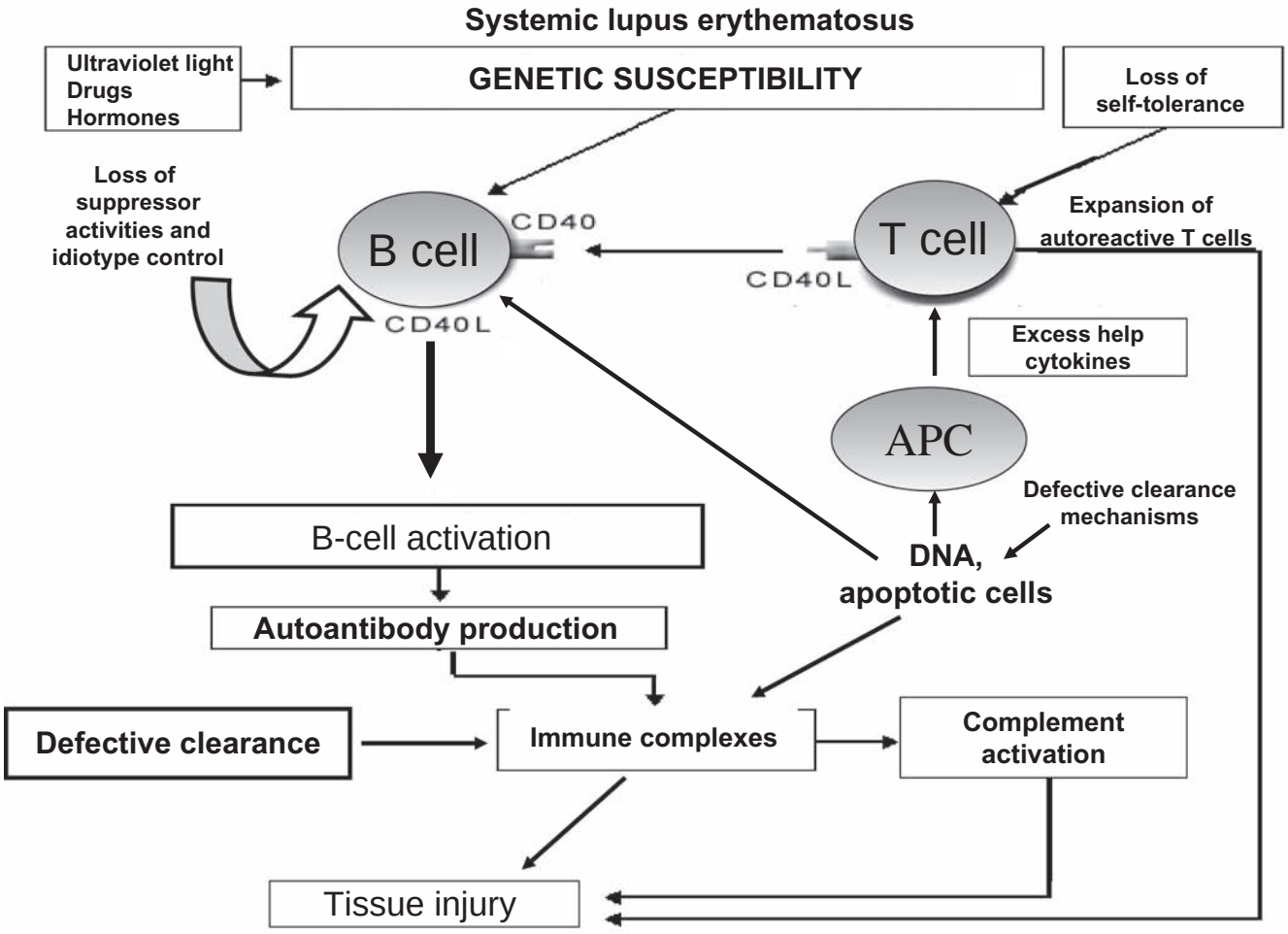


FIGURE 7.9 Possible events contributing to the development of systemic lupus erythematosus. Multiple genes confer susceptibility to the development of this disease. The interaction of sex-related factors, environmental factors, and defective immune regulation may predispose an individual to the expression of this susceptibility. Defective clearance mechanism, loss of immune tolerance, expansion of autoreactive T-cells, and defective B-cell suppression, lead to B-cell hyperactivity and the production of pathogenic autoantibodies. APC, antigen-presenting cell.

have suggested a role for activated T cells in the pathogenesis of SSc. Mice heterozygous for the tight-skin (Tsk) mutation develop skin fibrosis and are considered to be a good experimental animal model for the human disease. The administration of neutralizing anti-IL-4 antibodies to Tsk/$^{+}$ mice prevented the development of skin fibrosis in these mice, suggesting an important role for this Th2 cytokine in the pathogenesis of lesions. Accordingly, the development of skin fibrosis in Tsk/$^{+}$ mice was abrogated by the IL-4$^{-/-}$ or STAT6$^{-/-}$ mutation (Ong et al., 1999).

Interestingly, circulating mononuclear cells from patients with SSc produce higher amounts of IL-2 and IL-4 than controls (Famularo et al, 1990; Needleman et al, 1992; Sato et al., 1995). The great majority of SSc patients showed spontaneous IL-4, IL-5, and CD30 mRNA expression in peripheral blood T cells, and high numbers of CD4^{+} T cells in the perivascular infiltrates present in skin biopsy specimens obtained from SSc patients were CD30^{+} and expressed IL-4, but not IFN-γ, mRNA. These data strongly suggest predominant activation of Th2 cells in SSc and support the view that abnormal and persistent IL-4 production by the activated Th2 cells may play an important role in the induction of fibrosis and, therefore, in the pathogenesis of the disease.

A hypothetical scheme illustrating the possible immunologic events leading to the main pathophysiologic alterations characteristic of subjects with SSc is shown in Figure 7.10.

The origin of the Th2-dominated immune response in SSc is still unclear. The clinical features of SSc are similar to those of chronic graft-versus-host disease (GvHD) (Chosidow et al., 1992), a chimeric disorder in recipients of allogenic stem cell transplants and in whom Th2-type responses also predominate (de Wit et al., 1993). Identification of fetal DNA and cells in skin lesions and blood from women with SSc has been reported (Artlett et al., 1998), suggesting that a microchimerism established by fetal T

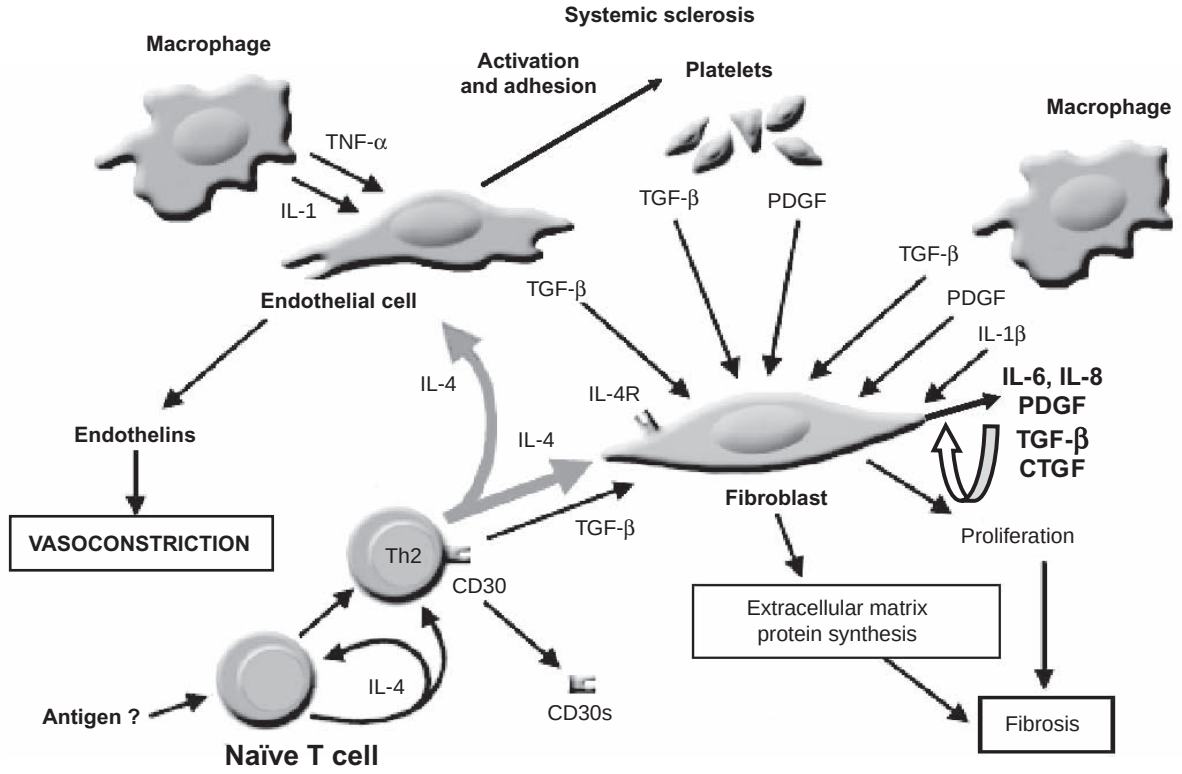


FIGURE 7.10 Possible events contributing to the development of systemic sclerosis (SSc). Extracellular matrix protein synthesis by fibroblasts is the hallmark of the disease. Collagen accumulation results from abnormal interactions between endothelial cells, lymphocytes, macrophages, and fibroblasts, leading to the production of fibrosis-inducing cytokines. Fibroblasts from subjects with SSc are activated and overproduce extracellular matrix proteins. Collagen synthesis is dependent upon tumor growth factor (TGF)-β, a cytokine produced by fibroblasts and endothelial cells, and connective tissue growth factor (CTGF), synthesized by fibroblasts under the influence of both TGF-β produced by fibroblasts and interleukin (IL)-4 produced by Th2 cells. PDGF, platelet-derived growth factor; TNF, tumor necrosis factor.

cells and the activation of such cells may induce a graft-versus-host response, manifesting as SSc. However, subsequent studies showed no difference in the frequency of microchimerism with fetal cells between healthy women and women with SSc; therefore, the casual link between microchimerism and disease pathogenesis remains uncertain (Evans et al., 1999). Seven maternal T-cell clones derived from women with SSc and one derived from a healthy woman proliferated in response to autologous non-T-cells and exhibited the Y chromosome, which demonstrated that they originated from male-offspring T cells. These data suggest that male-offspring T cells, which are present in blood and/or skin of women with SSc and are reactive against maternal MHC antigens, exhibit a Th2-oriented profile, thus supporting their possible role in the chronic GvHD occurring in women with SSc (Scaletti et al., 2002).

CONCLUDING REMARKS

It is now clear that the Th cell population contains functionally polarized subsets that are characterized by the patterns of cytokines they produce in response to different types of antigenic stimulation. Although Th1 and Th2 cells were first identified by *in vitro* analysis of murine T-cell clones, strong evidence now exists for similar subsets *in vivo* in mice, rats, and humans. Provided oversimplifications are avoided, these two extremely polarized forms of the specific cellular immune response, evoked by intracellular parasites and gastrointestinal nematodes, respectively, provide a useful model for explaining not only the different types of protection, but also the pathogenic mechanisms of several immunopathologic disorders.

Th1-dominated responses are very effective at eradicating infectious agents, including those "hidden" within the cell; however, if the Th1 response is not effective or is excessively prolonged, it may become dangerous for the host, due to both the activity of cytotoxic cytokines and the strong activation of phagocytic cells. In contrast, Th2 responses are not sufficiently protective against the majority of infectious agents, but they do provide an important downregulatory mechanism for exaggerated and/or excessively prolonged Th1 responses (Th1 to Th2 switch or immune deviation from Th1 to Th2).

Studies of autoimmune disorders in the context of Th1 and Th2 T-cell responses suggest the existence of a complex network of interactions that involves Th1 responses, counter-regulatory Th2 responses, and the defective intervention of Tregs. The majority of autoimmune diseases studied, especially organ-specific autoimmune diseases, appear mainly to be mediated by Th1-triggered inflammatory processes, the two clearest examples being MS and T1D. Interestingly, in these diseases, switching from a Th1 to a Th2 response can prevent Th1-mediated destruction of tissue in the experimental models of many conditions. However, a role for defective Treg activity is also emerging. In human SLE, a Thl/Th2 polarization is not apparent, and a more general imbalance of different immune mechanisms seems to be operating. In other autoimmune disorders, Th2 responses seem to predominate and can mediate tissue damage. Th2 cells are indeed involved in autoimmunity during chronic GvHD, in SLE induced by chemicals, and in SSc. It is of note that the chronic autoimmune diseases related to a prevalent Th2 response can in turn be prevented by switching from a Th2 to Th1 response. Taken together, these findings suggest that modulation of the relative contribution of Th1 or Th2 T cells to an autoimmune response, or suppression of the response that is prevalently involved in the inflammatory process, may allow clinical autoimmune disease to be regulated, thus opening the way for new therapeutic strategies.

References

Abbas, A.K., Murphy, K.M., and Sher, A. (1996). Functional diversity of helper T lymphocytes. *Nature* 383, 787–793.

Al-Janadi, M., Al-Balla, S., Al-Dalaan, A., and Raziuddin, S. (1993). Cytokine profile in systemic lupus erythematosus, rheumatoid arthritis, and other rheumatic diseases. *J. Clin. Immunol. 13*, 58–67.

Amsen, D., Blandr, J.M., Lee, G.R., Tanigaki, K., Honjo, T., and Flavell, R.A. (2004). Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell 117*, 515–526.

Artlett, C.M., Smith, J.B., and Jimenez, S.A. (1998). Identification of fetal DNA and cells in skin lesions from women with systemic sclerosis. *N. Engl. J. Med. 338*, 1186–1191.

Balashov, K.E., Rottman, J.B., Weiner, H.L., and Hancock, W.W. (1999). CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. U.S.A. 96*, 6873–6878.

Benvenuto, R., Paroli, M., Buttinelli, C., Franco, A., Bamaba, V., Fieschi, C., and Balsano, F. (1991). Tumor necrosis factor-alpha synthesis by cerebrospinal-fluid-derived T cell clones from patients with multiple sclerosis. *Clin. Exp. Immunol. 84*, 97–102.

Bird, J.J., Bown, D.R., Mullen, A.C., Moskowitz, N.H., Mahowald, M.A., Sid, J.R., Gajewski, T.F., Wang, C.R., and Reiner, S.L. (1998). Helper T cell differentiation is controlled by the cell cycle. *Immunity 9*, 229–237.

Bluestone, J.A. and Abbas, A.K. (2003). Natural versus adaptive regulatory T cells. *Nat. Rev. Immunol. 3*, 253–257.

Bombardieri, M., Barone, F., Pittoni, V., Alessandri, C., Conigliaro, P., Blades, M.C., Priori, R., McInnes, I.B., Varesini, G., and Pitzalis, C. (2004). Increased circulating levels and salivary gland expression of interleukin-18 in patients with Sjögren's syndrome: relationship with autoantibody production and lymphoid organization of the periductal inflammatory infiltrate. *Arthritis Res. Ther. 6*, 447–456.

Bottazzo, G.F., Dean, B.M., McNally, J.M., Mackay, E.H., Swift, P.G.F., and Gamble, D.R. (1985). *In situ* characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. *N. Engl. J. Med. 31*, 353–360.

Brennan, F.M., Chantry, D., Turner, M., Foxwell, B., Maini, R.N., and Feldmann, M. (1990). Detection of transforming growth factor-beta in rheumatoid arthritis synovial tissue: Lack of effect on spontaneous cytokine production in joint cell cultures. *Clin. Exp. Immunol. 81*, 278–285.

Brod, S.A., Benjamin, D., and Hafler, D.A. (1991). Restricted T cell expression of IL2/IFN-mRNA in human inflammatory disease. *J. Immunol. 147*, 810–815.

Bucala, R., Ritchiin, C., Winchester, R., and Cerami, A. (1991). Constitutive production of inflammatory and mitogenic cytokines by rheumatoid synovial fibroblasts. *J. Exp. Med. 173*, 569–574.

Buchan, G.K., Barrett, T., Fujita, T., Taniguchi, T., Maini, R.N., and Feldmann, M. (1988). Detection of activated T products in the rheumatoid joint using cDNA probes to interleukin 2 (IL-2), IL-2 receptor and IFN-gamma. *Clin. Exp. Immunol. 71*, 295–301.

Chang, J.C., Linarelli, L.G., Laxer, J.A., Froning, K.J., Caralli, L.L., Brostoff, S.W., and Carlo, D.J. (1995). Insulin-secretory-granule specific T cell clones in human insulin dependent diabetes mellitus. *J. Autoimmunity 8*, 221–234.

Chosidow, O., Bagot, M., Vernant, J.P., Roujeau, J.C., Cordonnier, C., Kuentz, M., Wechsler, J., Andre, C., Touraine, R., and Revuz, J. (1992). Sclerodermatous chronic graft-versus-host disease. Analysis of seven cases. *J. Am. Acad. Dermatol. 26*, 49–55.

Clark, D.A., Lamey, P.J., Jarrett, R.F., and Onions, D.E. (1994). A model to study viral and cytokine involvement in Sjögren's syndrome. *Autoimmunity 18*, 7–14.

Coles, A.J., Wing, M., Smith, S., Coraddu, F., Greer, S., Taylor, C., Weetman, A., Hale, G., Chatterjee, V.K., Waldmann, H., and Compston A. (1999). Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet 354*, 1691–1695.

Correale, J., Gilmore, W., McMillan, M., Li, S., McCarthy, K., Le, T., and Weiner, L.P. (1995). Patterns of cytokine secretion by autoreactive proteolipid protein-specific T cell clones during the course of multiple sclerosis. *J. Immunol. 154*, 2959–2968.

Csiszar, A., Nagy, G., Gergely, P., Pozsonyi, T., and Pocsik, E. (2000). Increased interferon-gamma (IFN-gamma), IL-10 and decreased IL-4 mRNA expression in peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol. 122*, 464–470.

Davis, L., Shulze-Koops, H., and Lipsky, P.E. (2001). Rheumatoid synovial $CD4^+$ T cells exhibit a reduced capacity to differentiate into IL-4-producing T-helper-2 effector cells. *Arthritis Rheumatol. 3*, 54–64.

De Carli, M., D'Elios, M.M., Mariotti, S., Marcocci, C., Pinchera, A., Ricci, M., Romagnani, S., and Del Prete, G.F. (1993). Cytolytic T cells with Th1-like cytokine profile predominate in retroorbital lymphocytic infiltrates of Graves' ophthalmopathy. *J. Clin. Endocrinol. Metab. 77*, 1120–1124.

Deleuran, B., Chu, C.-Q., Field, M., Brennan, F. M., Katsikis, P., Feldmann, M., and Maini, R. N. (1992). Localization of interleukin lα (IL-lα), type 1 IL-1 receptor and interleukin 1 receptor antagonist protein in the synovial membrane and cartilage/pannus junction in rheumatoid arthritis. *Br. J. Rheumatol. 91*, 801–809.

Del Prete, G.F., Tiri, A., Mariotti, S., Pinchera, A., Ricci, M., and Romagnani, S. (1987). Enhanced production of gamma-interferon by thyroid-derived T cell clones from patients with Hashimoto's thyroiditis. *Clin. Exp. Immunol. 69*, 323–331.

Del Prete, G.F., Tiri, A., De Carli, M., Mariotti, S., Pinchera, A., Chretien, I., Romagnani, S., and Ricci, M. (1989). High potential to tumor necrosis factor α (TNF-α) production of thyroid infiltrating T lymphocytes in Hashimoto's thyroiditis: A peculiar feature of destructive thryoid autoimmunity. *Autoimmunity 4*, 267–276.

Del Prete, G.F., De Carli, M., Almerigogna, F., Giudizi, M.-G., Biagiotti, R., and Romagnani, S. (1993). Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J. Immunol. 150*, 1–8.

de Wit, D., Van Mechelen, M., Zanin, C., Dautrelepont, J.-M., Velu, T., Gerard, C., Abramowicz, D., Scheerlink, J.-P., De Baetselier, P., Urbain, J., Leo, O., Goldman, M., and Moser, M. (1993). Preferential activation of Th2 cells in chronic graft-versus-host-reaction. *J. Immunol. 150*, 361–366.

Di Giovine, F.S., Nuki, G., and Duff, G.W. (1988). Tumor necrosis factor in synovial exudates. *Ann. Rheum. Dis. 47*, 768–772.

Eisenbarth, G.S. (1986). Type 1 diabetes mellitus. A chronic autoimmune disease. *N. Engl. J. Med. 314*, 1360–1368.

Endo, L.P., Edwards, N.L., Longley, S., Corman, L.C., and Panush, R.S. (1987). Silicon and rheumatic diseases. *Semin. Arthritis Rheum. 2*, 112–118.

Evans, P.C., Lambert, M., Maloney, S., Furst, D.E., Moore, J.M., and Nelson J.L. (1999). Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. *Blood 93*, 2033–2037.

Famularo, G., Procopio, A., Giacomelli, R., Danese, C., Sacchetti, S., Perego, M.A., Santoni, A., and Torneiti, G. (1990). Soluble interleukin-2 receptor, interleukin-2 and interleukin-4 in sera and suprenatants from patients with progressive systemic sclerosis. *Clin. Exp. Immunol. 81*, 368–372.

Feldmann, M., Brennan, F.M., Field, M., Maini, R.N. (1992). Pathogenesis of rheumatoid arthritis: Cellular and cytokine interactions. In J. Smolen, J. Kalden, and R.N. Maini (eds.), *Rheumatoid Arthritis* (pp. 41–54). Heidelberg: Springer-Verlag.

Finkelman, F.D. and Urban, A.F. (2001) The other side of the coin: the protective role of the Th2 cytokines. *J. Allergy Clin. Immunol. 107*, 772–780.

Fiorentino, D.F., Bond, M.W., and Mosmann, R.T. (1989). Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med. 170*, 2081–2095.

Firestein, G.S. and Zvaifler, N.J. (1993). The role of T cells in rheumatoid arthritis. *Ann. Rheum. Dis. 52*, 765–765.

Firestein, G.S., Xu, W.-D., Townsend, K., Broide, D., Alvaro-Garcia J., Glasebrook, A., and Zvaifler, N.J. (1988). Cytokines in chronic inflammatory arthritis. I. Failure to detect T cell lymphokines (interleukin 2 and interleukin 3) and presence of macrophage colony-stimulating factor (CSF-1) and a novel mast cell growth factor in rheumatoid synovitis. *J. Exp. Med. 168*, 1573–1586.

Firestein, G.S., Alvaro-Garcia, J.M., and Maki, R. (1990). Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J. Immunol. 144*, 3347–3353.

Fleischmajer, R., Perlish, R., and Duncan, M. (1983). Scleroderma. A model for fibrosis. *Arch. Dermatol. 119*, 957–962.

Fontenot A.D., Gavin M.A., and Rudennsky A.Y. (2003). Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol. 4*, 304–306.

Foulis, A.K. and Farquharson, M.A. (1986). Aberrant expression of HLA-DR antigens by insulin containing beta cells in recent onset type 1 (insulin-dependent) diabetes mellitus. *Diabetes 35*, 1215–1224.

Foulis, A.K., McGill, M., and Farquharson, M.A. (1991). Insulitis type 1 (insulin-dependent) diabetes mellitus in man. Macrophages, lymphocytes, and interferon-gamma containing cells. *J. Pathol. 165*, 97–103.

Fox, R.I. and Kang, H.-I. (1992). Pathogenesis of Sjögren's syndrome. *Rheum. Dis. Clin. North Am. 18*, 517–538.

Franciotta, D., Martino, G., Cardini, E., Furlan, R., Bergamaschi, R., Androni, L., and Cosi, V. (2001). Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. *J. Neuroimmunol. 115*, 192–198.

Grubeck-Loebenstein, B., Trieb, K., Sztankay, A., Holter, W., Anderi, H., and Wick, G. (1994). Retrobulbar T cells from patients with Graves' ophthalmopathy are $CD8^+$ and specifically recognize autologous fibroblasts. *J. Clin. Invest. 93*, 2738–2743.

Hanaoka, R., Kasama, T., Muramatsu, M., Yajima, N., Shiozawa, F., Miwa, Y., Negishi, M., Ide, H., Miyaoka, H., Uchida, H., and Adachi, M.

(2002). A novel mechanism for the regulation of IFN-γ inducible protein-10 expression in rheumatoid arthritis. *Arthritis Res. Ther. 5*, 74–81.

Harris, E.D., Jr. (1990). Rheumatoid arthritis: Pathophysiology and implications for therapy. *N. Engl. J. Med. 322*, 1277–1289.

Haworth, C., Brennan, F.M., Chantry, D., Turner, M., Maini, R.N., and Feldmann, M. (1991). Expression of granulocyte-macrophage colony-stimulating factor in rheumatoid arthritis: regulation by tumor necrosis factor-α. *Eur. J. Immunol. 21*, 2575–2579.

Hessian, P.A., Highton, J., Kean, A., Sun, C.K., and Chin, M. (2003). Cytokine profile of the rheumatoid nodule suggests that it is a Th1 granuloma. *Arthrtritis Rheum. 48*, 334–338.

Hidaka, Y., Kimura, M., Izumi, Y., Takano, T., Tatsumi, K.I., and Amino, N. (2003). Increased serum concentration of eosinophil-derived neurotoxin in patients with Graves' disease. *Thyroid 13*, 127–128.

Hirano, T., Matsuda, T., Turner, M., Miyasaka, N., Buchan, G., Tang, B., Sato, K., Shimizu, M., Maini, R.N., Feldmann, M., and Kishimoto, T. (1988). Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. *Eur. J. Immunol. 18*, 1797–1801.

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.

Hosaka, S., Akahoshi, T., Wada, C., and Kondo, H. (1994). Expression of the chemokine superfamily in rheumatoid arthritis. *Clin. Exp. Immunol. 97*, 451–457.

Hovdenes, J., Gaudernack, G., Kvien, T.K., and Egeland, T. (1989). Expression of activation markers on $CD4^+$ and $CD8^+$ cells from synovial fluid, synovial tissue and peripheral blood of patients with inflammatory arthritides. *Scand. J. Immunol. 29*, 631–639.

Joosten, L.A., Radstake, T.R., Lubberts, E., van den Bersselaar, L.A., van Riel, P., van Lent, P., Barrera, P., and van den Berg, W.B. (2003). Association of interleukin-18 expression with enhanced levels of both interleukin-1β and tumor necrosis factor α in knee synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum. 48*, 339–347.

Joosten, L.A., Smeets, R.L., Koenders, M.I, van den Bersselaar, L.A., Helsen, M.M.A., Oppers-Walgreen, B., Lubberts, E., Iwakura, Y., van de Loo, F.A.J., and van den Berg, W.B. (2004). Interleukin-18 promotes joint inflammation and induces interleukin-1-driven cartilage destruction. *Am. J. Pathol. 165*, 959–967.

Kanik, K.S., Hagiwara, E., Yarboro, C.H., Schumacher, H.R., Wilder, R.L., and Klinman, D.M. (1998). Distinct patterns of cytokine production characterize new onset synovitis versus chronic rheumatoid arthritis. *J. Rheumatol. 25*, 16–22.

Karlsson, M.G., Lawesson, S.S., and Ludvigsson, J. (2000). Th1-like dominance in high-risk first-degree relatives of type 1 diabetic patients. *Diabetologia 43*, 742–749.

Katsikis, P.D., Chu, C.-Q., Brennan, F.M, Maini, R.N., and Feldmann, M. (1994). Immunoregulatory role of interleukin 10 in rheumatoid arthritis. *Exp. Med. 179*, 1517–1527.

Koarada, S., Wu, Y., Olshansky, G., and Ridgway, W.M. (2002). Increased nonobese diabetic Th1:Th2 (IFN-gamma:IL-4) ratio is $CD4^+$ T cell intrinsic and independent of APC genetic background. *J. Immunol. 169*, 6580–6587.

Konno, A., Takada, K., Saegusa, J., and Takiguchi, M. (2003). Presence of $B7–2^+$ dendritic cells and expression of Th1 cytokines in the early development of sialodacryoadenitis in the IqI/Jic mouse model of primary Sjögren's syndrome. *Autoimmunity 36*, 247–254.

Kukreja, A., Cost, G., Marker, J., Zhang, C., Sun, Z., Lin, S.K., Ten, S., Sar Exley, M., Wilson, B., Porcelli, S., and Maclaren, N. (2002). Multiple immuno-regulatory defects in type-1 diabetes. *J Clin Invest. 109*, 131–140.

Lassmann, H. and Ransohoff, R.M. (2004). The CD4-Th1 model for multiple sclerosis: a crucial re-appraisal. *Trends Immunol. 25*, 132–137.

Leiter, E.H., Prochazka, M., and Coleman, D.L. (1987). The nonobese diabetic (NOD) mouse. *Am. J. Pathol. 128*, 380–393.

Liorente, L., Richaud-Patin, Y., Fior, R., Alcocer-Varela, J., Wijdenes, J., Morel Fourrier, B., Galanaud, P., and Emilie, D. (1994). *In vivo* production of interleukin 10 by non-T cells in rheumatoid arthritis, Sjögren's syndrome, and systemic lupus erythematosus. *Arthritis Rheum. 37*, 1647–1655.

Maggi, E., Parronchi, P., Manetti, R., Simonelli, C., Piccinni, M.-P., Santoni Rugiu, F., De Carli, M., Ricci, M., and Romagnani, S. (1992). Reciprocal regulatory role of IFN-γ and IL-4 on the *in vitro* development of human Thl and Th2 clones. *J. Immunol. 148*, 2142–2147.

Manetti, R., Parronchi, P., Giudizi, M.-G., Piccinni, M.-P., Maggi, E., Trinchieri, G., and Romagnani, S. (1993). Natural killer cell stimulatory factor (interleukin 12) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med. 7*, 1199–1204

Marliss, E.B., Nakhooda, A.F., and Poussier, P. (1983). Clinical forms and natural history of the diabetic syndrome and insulin and glucagon secretion in the BB rat. *Metabolism 32* (Suppl), 11–16.

Mcinnes, I.B., Al-Mughales, J., Field, M., Leung, B.P., Huang, F.-P., Dixon, R., Sturrock, R.D., Wilkinson, P.C., and Liew, F.Y. (1996). The role of interleukin-15 in T-cell migration and activation in rheumatoid arthritis. *Nat. Med. 2*, 175–182.

McLachlan, S.M., Prummel, M.F., and Rapoport, B. (1994). Cell-mediated or humoral immunity in Graves' ophthalmopathy? Profiles of T-cell cytokines amplified by polymerase chain reaction from orbital tissue. *Clin. Endocrinol. Metab. 78*, 1070–1074.

Milis, J.A. (1994). Systemic lupus erythematosus. *N. Engl. J. Med. 330*, 1871–1879.

Miltenburg, A.M.M., van Laar, J.M., De Kniper, R., Daha, M.R., and Breedveld, F.C. (1992). T cell clones from human rheumatoid synovial membrane functionally represent the Th1 subset. *Scand. J. Immunol. 35*, 603–610.

Misu, T., Onodera, H., Fujihara, K., Matsushima, K., Yoshie, O., Okita, N., Takase, S., and Itoyama, Y. (2001). Chemokine receptor expression on T cells in blood and cerebrospinal fluid at relapse and remission of multiple sclerosis: imbalance of Th1/Th2-associated chemokine signaling. *J. Neuroimmunol. 114*, 207–212.

Mitsias, D.I., Tzioufas, A.G., Veiopoulou, C., Zintzaras, E., Tassios, I.K., Kogopoulou, O., Moutsopoulos, H.M., and Thyphronitis, G. (2002). The Th1/Th2 cytokine balance changes with the progress of the immunopathological lesion of Sjögren's syndrome. *Clin. Exp. Immunol. 128*, 562–568.

Mok, C.C. and Lau, C.S. (2003). Pathogenesis of systemic lupus erythematosus. *J. Clin. Pathol. 56*, 481–490.

Mosmann, T.R. and Coffman, R.L. (1989). ThI and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol. 7*, 145–173.

Needleman, B.W., Wigley, F.M., and Stair, R.W. (1992). Interleukin 2, interleukin 4, interleukin 6, tumor necrosis factor α, and interferon γ levels in sera from scleroderma patients. *Arthritis Rheum. 35*, 67–72.

Nicoletti, F., Conget, I., Di Mauro, M., Di Marco, R., Mazzarino, M.C., Bendtzen, K., Messina, A., and Gomis, R. (2002). Serum concentrations of the interferon-gamma-inducible IP-10/CXCL10 are augmented in both newly diagnosed type 1 diabetes mellitus patients and subjects at risk of developing the disease. *Diabetologia 45*, 1107–1110.

Oikawa, Y., Shimada, A., Kasuga, A., Morimoto, J., Osaki, T., Tahara, H., Miyazaki, T., Tashiro, F., Yamato, E., Miyazaki, J., and Saruta, T. (2003). Systemic administration of IL-18 promotes diabetes development in young nonobese diabetic mice. *J. Immunol. 171*, 5865–5875.

Ong, C.J., Ip, S., Teh S.J., Wong, C., Jirik, F.R., Grusby, M.J., and The, H.S. (1999). A role for T helper 2 cells in mediating skin fibrosis in tight-skin mice. *Cell Immunol. 196*, 60–68.

Oxholm, P., Danieis, T.E., and Bendtzen, K. (1992). Cytokine expression in labial salivary glands from patients with primary Sjögren's syndrome. *Autoimmunity 12*, 185–191.

Panayi, G.S., Lanchbury, J.S., and Kingsiey, G.H. (1992). The importance of the T cell in initiating and maintaining the chronic synovitis of rheumatoid arthritis. *Arthritis Rheum. 35*, 729–735.

Paschke, R., Schuppert, E., Taton, M., and Velu, T. (1994). Intrathyroidal cytokine gene expression profiles in autoimmune thyroiditis. *J. Endocrinol. 141*, 309–315.

Pedotti, R., De Voss, J.J., Steinman, L., and Galli, S.J. (2003). Involvement of both allergic and autoimmune mechanisms in EAE, MS and other autoimmune diseases. *Trends Immunol. 24*, 479–484.

Quaratino, S., Badami, E., Pang, Y.Y., Bartok, I., Dyson, J., Kioussis, D., Londei, M., and Maiuri, L. (2004). Degenerate self-reactive human T cell receptor causes spontaneous autoimmune disease in mice. *Nat. Med. 10*, 920–926.

Quayle, A.J., Chomarat, P., Miossec, P., Kjeldsen-Kragh, J., Forre, O., and Natvig, J.B. (1993). Rheumatoid inflammatory T-cell clones express mostly Th1 but also Th2 and mixed (Th0-like) cytokine patterns. *Scand. J. Immunol.* 38, 75–82.

Rapoport, B. and McLachlan, S.M. (2001). Thyroid autoimmunity. *J. Clin. Invest. 108*, 1253–1259.

Rengarajan, J., Szabo, S.J., and Glimcher, L.H. (2000). Transcripional regulation of Th1/Th2 polarization. *Immunol. Today. 10*, 479–483.

Rieckmann, P., Aibrecht, M., Kitze, B., Weber, T., Tumani, H., Broocks, A., Luer, V., and Poser, S. (1994). Cytokine mRNA levels in mononuclear blood cells from patients with multiple sclerosis. *Neurology 44*, 1523–1526.

Rieckmann, P., Aibrecht, M., Kitze, B., Weber, T., Tumani, H., Broocks, A., Luer, V., Heiwig, A., and Poser, S. (1995). Tumor necrosis factor alpha messenger RNA expression in patients with relapsing remitting multiple sclerosis is associated with disease activity. *Ann. Neurol. 37*, 82–88.

Romagnani, S. (1995). Biology of human Th1 and Th2 cells. *J. Clin Immunol. 15*, 121–129.

Romagnani, S. (1997). The Thl/Th2 paradigm in disease. In R.G. Landes, Austin, Texas (ed), *The Th1/Th2 paradigm in disease*. Heidelberg: Springer Verlag.

Romagnani, P., Rotondi, M., Lazzeri, E., Lasagni, L., Francalanci, M., Buonamano, A., Milani, S., Vitti, P., Chiovato, L., Tonacchera, M., Bellaste, A., and Serio, M. (2002). Expression of IP-10/CXCL10 and MIG/CXCL9 in the thyroid and increased levels of IP-10/CXCL10 in the serum of patients with recent-onset Graves' disease. *Am. J. Pathol. 161*, 195–206.

Roumm, A.D., Whiteside, T.L., Medsger, T.A., and Rodnan, G.P. (1984). Lymphocytes in the skin of patients with progressive systemic sclerosis. Quantification, subtyping, and clinical correlations. *Arthritis Rheum. 6*, 645–653.

Roura-Mir, C., Catalfamo, M., Lucas-Martin, A., Sospedra, M., Pujol-Borrell, R., and Jaraquemada, D. (1996). IL-4/IFN-γ balance in Graves' disease contrasts with IFN-γ dominance in Hashimoto's thyroiditis. *Proceedings of the IV International Conference on Cytokines*, Florence, March 20–22 (abstract).

Rowe, D., Griffiths, M., Stewart, J., Novick, D., Beverley, P.C., and Isenberg, D.A. (1987). HLA class I and II, interferon, interleukin 2, and the interleukin 2 receptor expression on labial biopsy specimens from patients with Sjögren's syndrome. *Ann Rheum Dis 46*, 580–586.

Sabroe, I., Read, R.C., Whyte, M.K., Dockrell, D.H., Vogel, S.N., and Dower, S.K. (2003). Toll-like receptors in health and disease: complex questions remain. *J. Immunol. 171*, 1630–1635.

Santiago-Schwartz, F., Anand, P., Liu, S., and Carsons, S.E. (2001). Dendritic cells (DCs) in rheumatoid arthritis (RA): progenitor cells and soluble factors contained in RA synovial fluid yield a subset of myeloid DCs that preferentially activate Th1 inflammatory-type responses. *J. Immunol. 167*, 1758–1768.

Sato, I.H., Fujimoto, S., Kikuchi, M., and Takehara, K. (1995). Demonstration of IL-2, IL-4, IL-6 in sera from patients with scleroderma. *Arch. Dermatol. Res. 287*, 193–197.

Scaletti, C., Vultaggio, A., Bonifacio, S., Emmi, L., Torricelli, F., Maggi, E., Romagnani, S., and Piccinini, M.P. (2002). Th2 oriented profile of male offspring cells present in women with systemic sclerosis and reactive with maternal major histocompatibility complex antigens. *Arthritis Rheum. 46*, 445–450.

Schulze-Koops, H. and Kalden, J.R. (2001). The balance of Th1/Th2 cytokines in rheumatoid arthritis. *Best Pract. Res Clin Rheumatol 15*, 677–691.

Schulze-Koops, H., Lipsky, P.E., Kavanaugh, A.F., and Benefit, L.S. (1995). Elevated Th1- or Th0-like cytokine mRNA in peripheral circulation of patients with rheumatoid arthritis. *J. Immunol. 155*, 5029–5037.

Seder, R.A. and Paul, W.E. (1994). Acquisition of lymphokine-producing phenotype by $CD4^+$ T cells. *Annu. Rev. Immunol. 12*, 635–674.

Selmaj, K., Raine, C.S., Cannella, B., and Brosnan, C.F. (1991). Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. *J. Clin. Invest. 87*, 949–954.

Sharief, M.K. and Hentges, R. (1991). Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. *N. Engl. J. Med. 15*, 467–472.

Simon, A.K., Seipeit, E., and Sieper, J. (1994). Divergent T-cell cytokines patterns in inflammatory arthritis. *Proc. Nat. Acad. Sci. U.S.A. 91*, 8562–8566.

Skopouli, F.N., Dorsos, A.A., Papaioannu, T., and Moutsopoulos, H.M. (1986). Preliminary diagnostic criteria for Sjögren's syndrome. *Scand. J. Rheumatol. 61*(Suppl), 22–25.

Sørensen, T.L., Tani, M., Jensen, J., Pierce, V., Lucchinetti, C., Folcik, V.A., Qin, S., Rottman, J., Sellebjerg, F., Strieter, R.M., Frederiksen, J.L., and Ransohoff, R.M. (1999). Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest. 103*, 807–815.

Sørensen, T.L., Sellebjerg, F., Jensen, C.V., Strieter, R.M., and Ransohoff, R.M. (2001). Chemokines CXCL 10 and CCL 2: differential involvement in intrathecal inflammation in multiple sclerosis. *Eur. J. Neurol. 8*, 665–672.

Trembleau, S., Penna, G., Gregori, S., Giarratana, N., and Adorini, L. (2003). IL-12 administration accelerates autoimmune diabetes in both wild-type and IFN-gamma-deficient nonobese diabetic mice, revealing pathogenic and protective effects of IL-12 induced IFN-gamma. *J. Immunol. 170*, 5491–5501.

Tsukada, N., Miyagi, K., Matsuda, M., Yanagisawa, N., and Yone, K. (1991). Tumor necrosis factor and interleukin 1 in the CSF and sera of patients with multiple sclerosis. *Neurol. Sci. 102*, 230–234.

van der Graaf, W.L., Prins, A.P., Niers, T.M., Dijkmans, B.A., and van Lier, R.A. (1999). Quantitation of interferon-gamma- and interleukin-4-producing T cells in synovial fluid and peripheral blood of arthritis patients. *Rheumatology 38*, 214–220.

Viglietta, V., Baecher-Allan, C., Weiner, H.L., and Hafler, D.A. (2004). Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med. 199*, 971–979.

Villarreal, G.M., Alcocer-Varela, J., and Fiorente, L. (1995). Cytokine gene and CD25 antigen expression by peripheral blood T cells from patients with primary Sjögren's syndrome. *Autoimmunity 20*, 223–229.

Watson, P.F., Pickerill, A.P., Davies, R., and Weetman, A.P. (1994). Analysis of cytokine gene expression in Graves' disease and multinodular goiter. *J. Clin. Endocrinol. Metab. 79*, 355–360.

Weber, A, Wandinger, K.P., Mueller, W., Aktas, O., Wengert, O., Grundst, E., Ehrlich, S., Windemuth, C., Kuhlmann, T., Wienker, T., and Bruck, W. (2004). Identification and functional characterization of a highly

polymorphic region in the human TRAIL promotor in multiple sclerosis. *J. Neuroimmunol. 149*, 195–201.

Windhagen, A., Newcombe, J., Dangond, F., Strand, C., Woodroofe, M.N., Cuzner, M.L., and Hafler, D.A. (1995). Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin-12 cytokine in multiple sclerosis lesions. *J. Exp. Med.182*, 1985–1996.

Wood, N.C., Symons, J.A., Dickens, E., and Duff, G.W. (1992). *In situ* hybridization of IL-6 in rheumatoid arthritis. *Clin. Exp. Immunol. 87*, 183–189.

Yang, Z., Chen M., Ellett, J.D., Fialkow, L.B., Carter, J.D., McDuffle, M., and Nadler, J.L. (2004). Autoimmune diabetes is blocked in STAT-deficient mice. *J. Autoimmun. 22*, 191–200.

CHAPTER

8

Tolerance and Autoimmunity: T Cells

MATTHEW A. GRONSKI AND PAMELA S. OHASHI

University Health Network, Ontario Cancer Institute, Departments of Medical Biophysics and Immunology, University of Toronto, Canada

Autoimmune diseases generally develop as a result of an immune response against self-molecules. Insulin-dependent diabetes, multiple sclerosis, and myasthenia gravis are all examples of diseases that are believed to result from such a misdirected response. Immune tolerance mechanisms are normally in place to limit potential autoimmune responses. Self-reactive T cells, which often mediate autoimmune disease, are controlled through mechanisms of central tolerance that occur in the thymus during T cell development and peripheral tolerance that act on mature T cells after they leave the thymus.

THYMIC TOLERANCE

T-Cell Receptor Interactions Determine Thymocyte Fate

Differentiation of thymocytes is characterized by the cell-surface expression of proteins such as CD4 and CD8. Bone marrow progenitors that enter the thymus initially do not express CD4 or CD8 and are referred to as double-negative (DN) $CD4^-CD8^-$ thymocytes (Figure 8.1). Following T-cell receptor (TCR) β chain rearrangement, only thymocytes expressing a functionally rearranged TCR β chain are selected to continue maturation and upregulate both CD4 and CD8. At this $CD4^+CD8^+$ double-positive (DP) stage, TCR α chain rearrangement is initiated and functionally rearranged TCR α chains are expressed on the cell surface with the TCR β chain. Interactions between the TCR expressed by DP thymocytes and self-major histocompatibility complex (MHC) molecules mediate survival or death (Figure 8.1). Thymocytes expressing TCRs that do not interact with self-MHC molecules die within a few days. Thymocytes expressing TCRs that are able to interact with intermediate affinity with self-MHC molecules are rescued from death by a process called positive selection. Positive selection ensures that only T cells that are able to recognize peptides presented on self-MHC molecules are exported to the periphery. Thymocytes expressing TCRs recognizing class I MHC molecules downregulate CD4 and become cytotoxic $CD8^+$ single positive (SP) cells, while $CD4^+$ helper T (Th) cells arise as a result of interactions between TCRs and class II MHC molecules. A strong interaction between TCR and self-peptide–MHC leads to the elimination of thymocytes by a process called negative selection (Palmer, 2003; Starr et al., 2003).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

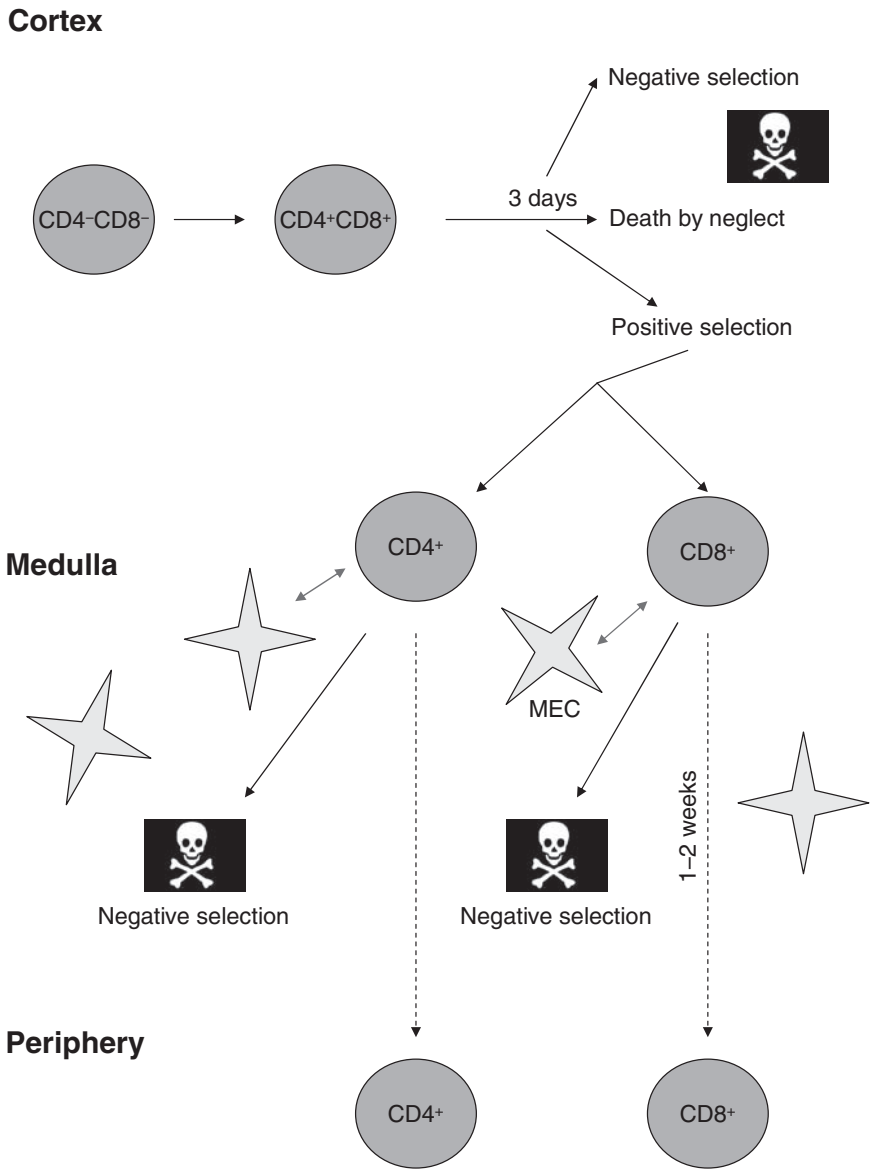


FIGURE 8.1 Thymocyte development and selection. Developing thymocytes follow a defined differentiation program in the thymus. Early progenitors are found in the cortex of the thymus as $CD4^-CD8^-$ double-negative (DN) thymocytes. Subsequently they upregulate the expression of the coreceptors CD4 and CD8 and become double-positive (DP) T cells. The DP thymocytes survive for approximately 3 days before undergoing programmed cell death. Intermediate-affinity interactions between the T-cell receptor (TCR) and self-peptide–MHC trigger differentiation and survival through a process called positive selection. Positive selection leads to the maturation of single-positive $CD4^+$ or $CD8^+$ thymocytes that transit into the medulla. A high-affinity/-avidity interaction between the TCR and self-peptide/MHC results in negative selection and central tolerance. Medullary epithelial cells (MECs) and other antigen-presenting cells contribute to negative selection.

Deletion of Autoreactive Thymocytes

What happens to the autoreactive thymocytes when they receive a strong signal through the TCR? Clonal deletion has been shown to be one of the main mechanisms that eliminate autoreactive cells (Kappler et al., 1987; 1988; MacDonald et al., 1988). This was first demonstrated by Kappler et al. (1987) using a monoclonal antibody that recognized the Vβ17 segment of the TCR. They found expression of Vβ17 in immature but not mature thymocytes of mice that expressed I-E, suggesting that the $V\beta17^+$ I-E reactive cells are deleted. Further evidence for clonal deletion as a

mechanism of negative selection was provided by TCR transgenic mice. H-Y TCR transgenic mice express TCRs that recognize the male H-Y antigen presented by MHC class I molecules. In male TCR transgenic mice, a striking reduction of the DP thymocytes and $CD8^+$ transgenic T cells was observed. In contrast, in female mice the H-Y TCR transgenic T cells were positively selected to the CD8 lineage (Kisielow et al., 1988; Teh et al., 1988). Together, these studies clearly demonstrate that thymocytes expressing self-reactive TCRs are clonally eliminated during development.

As an added level of protection from autoimmunity, thymic deletion appears to be more sensitive than mature T-cell activation. That is, stronger interactions with peptide–MHC are required for the activation of T cells in the periphery compared to deletion of immature thymocytes (Yagi and Janeway, 1990; Pircher et al., 1991; Vasquez et al., 1992; Davey et al., 1998; Lucas et al., 1999). The higher sensitivity in thymic selection creates a margin of safety by deleting T cells that would probably not receive strong enough signals to be activated by self-peptide–MHC in the periphery.

Clonal Inactivation of Autoreactive Thymocytes

In addition to thymic deletion, T-cell inactivation has also been proposed as a mechanism of thymic tolerance (Ramsdell et al., 1989; Speiser et al., 1990; Rellahan et al., 1990). This was originally shown by Ramsdell et al. (1989) using a chimeric model. They generated chimeric mice by transferring bone marrow cells from one mouse to an irradiated recipient mouse. Bone marrow cells are sensitive to irradiation while the thymic epithelial cells are resistant, allowing the selective depletion of bone marrow-derived cells. In this way, they generated animals that expressed the tolerizing superantigen Mls-1^a in the bone marrow cells or thymic stroma to study the effect on T cell development and tolerance. If Mls-1^a was expressed on bone marrow cells, deletion of $V\beta6^+$ Mls-1^a reactive T cells occurred. However, if the host expressed Mls-1^a and the donor bone marrow cells did not, clonal deletion did not occur. Instead, nondeleted mature $V\beta6^+$ T-cells were unresponsive to Mls-1^a or stimulation by monoclonal antibodies. Thus, it appears that under some circumstances thymocytes are inactivated rather than deleted.

Regulatory T-Cell Development

Some of the T cells that receive a relatively strong signal in the thymus, which is insufficient for negative selection, can develop immunosuppressive functions and negatively regulate responses by other T cells. Although the location and signals involved in the development of these regulatory T cells (Tregs) are unclear, evidence suggests that they are the result of relatively high-affinity interactions in the thymus (Itoh et al., 1999). At least one subset of $CD4^+CD25^+$ Tregs that express TCRs with self-reactive properties is produced in the thymus [reviewed in Fehervari and Sakaguchi (2004)]. Glucocorticoid signaling blockers, which effectively decrease the threshold for T-cell activation, result in the generation of more $CD4^+CD25^+$ Tregs in the thymus. Similarly, studies using double transgenic mice that express an ovalbumin (OVA)-specific TCR and a transgene-encoding OVA in the thymus, suggest that Tregs are the product of selection signals that are higher than those for positive, but lower than those for negative, selection (Jordan et al., 2001). However, a recent study has questioned the conclusions of these studies, suggesting that Tregs do not differentiate in the thymus as a consequence of high-affinity interactions. Rather, the observed increase in the percentage of these cells is a result of preferential deletion of $CD4^+CD25^-$ T cells (van Santen et al., 2004). Nonetheless, development of the Treg population in the thymus plays a key role in maintaining tolerance in the periphery.

Tissue-Specific Antigens and Negative Selection

A potential problem with central tolerance is the limited expression of peripheral tissue-specific antigens in the thymus. It has become clear, however, that certain cells of the thymic stroma express peripheral tissue-specific antigens that promote the elimination of self-reactive T cells (Smith et al., 1997; Kyewski et al., 2002). Studies have suggested that a putative transcription factor—the autoimmune regulator (AIRE) protein—is expressed in rare specialized cells called medullary epithelial cells (MECs). Interestingly, it was found that a mutation in this protein in humans led to the development of the multiorgan autoimmune endocrine disease, autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) (The Finnish–German APECED Consortium, 1997; Nagamine et al., 1997). Since both AIRE and peripheral antigens are expressed in MECs, it was speculated, and later confirmed by microarray technology, that AIRE controls the expression of these antigens (Anderson et al., 2002). To study the role of AIRE, AIRE-deficient mice were generated by two independent groups (Anderson et al., 2002; Ramsey et al., 2002). These mice developed lymphocytic infiltrates and autoantibodies directed against a number of peripheral organs and tissues, such as the salivary gland, retina, pancreas, ovary, stomach, and thyroid. Further analysis using bone marrow chimeras and thymic transplant experiments found that to prevent disease AIRE expression was only crucial in the non-hematopoietic cells of the thymic stroma (Anderson et al., 2002). Therefore, central tolerance includes mechanisms to

induce tolerance of T cells specific for tissue antigens expressed outside the thymus.

Other cell populations found in the thymus include dendritic cells (DCs) and macrophages. This is important because they expose thymocytes to antigens expressed on antigen-presenting cells (APCs) that activate T cells in the periphery. In this way, thymocytes become tolerant to "self"-antigens expressed by APCs, biasing the repertoire to respond to foreign antigens presented by these cells.

Negative Selection and Autoimmunity

If central tolerance were critical for eliminating autoreactive cells and preventing autoimmunity, it would be predicted that autoimmunity would develop if negative selection were impaired. Currently, the association between the loss of AIRE and autoimmunity in both humans and mouse models provides the best evidence for the importance of negative selection (The Finnish-German APECED Consortium, 1997; Nagamine et al., 1997; Ramsey et al., 2002; Anderson et al., 2002; Liston et al., 2003).

Other animal models of autoimmunity, however, have also been associated with impaired negative selection (Kishimoto and Sprent, 2001; Lesage et al., 2002). Since negative selection involves the death of self-reactive thymocytes, genes involved in apoptosis may be important for this process. As a result, mutations in apoptosis-related genes often result in autoimmune disease. An example of this is the proapoptotic molecule Bim, a Bcl-2 homology domain 3 (BH3)-only containing protein (Bouillet et al., 1999). Mice deficient in Bim accumulate lymphoid and myeloid cells, while older mice accumulate plasma cells and develop autoimmune kidney disease. Studies have suggested that defects in both central and peripheral tolerance contribute to these abnormalities (Bouillet et al., 2002; Davey et al., 2002; Hildeman et al., 2002).

This is not the only evidence for the importance of central tolerance in preventing autoimmunity. Many studies also support the corollary prediction: if central tolerance (clonal deletion) is induced to tissue-specific antigens, autoimmunity can be prevented (Herold et al., 1992; Posselt et al., 1992; Ally et al., 1995).

Escape of Autoreactive T Cells from Central Tolerance

Evidence suggests that central tolerance is incomplete because autoreactive cells can be found in the peripheral repertoire and autoimmune diseases do arise in humans and animal models (Haskins and McDuffie, 1990; Nagata et al., 1994; Verdaguer et al., 1997; Wong et al., 1999). Several factors have been identified to explain why self-reactive T cells may escape thymic deletion. Although the AIRE protein promotes expression of tissue-specific antigens in the thymus, it is likely that many are still not expressed at levels sufficient to induce T-cell tolerance. Models have suggested that many tissue-specific antigens are not expressed at sufficient levels for negative selection (Ohashi et al., 1991; Goverman et al., 1993; Katz et al., 1993; Pugliese et al., 1997; Vafiadis et al., 1997). Low levels of self-antigen expressed in the thymus would lead to deletion of only the high-affinity/-avidity autoreactive thymocytes, resulting in incomplete tolerance and allowing lower-affinity/-avidity, potentially self-reactive thymocytes to leave the thymus (von Herrath et al., 1994; Oehen et al., 1994). In addition, peptides that have a low affinity for MHC cannot induce negative selection of thymocytes expressing cognate TCRs (Liu et al., 1995). Weak binding to the MHC results in lower effective avidity that allows escape from deletion. T cells expressing TCRs with low affinity/avidity for peptide–MHC may also avoid negative selection (Morgan et al., 1998). These T cells could be activated in the periphery as a result of possibly higher levels of antigen expression. Finally, particular splice variants of proteins may not be expressed in the thymus and therefore specific T cells will not be tolerized (Anderton et al., 2002; Manoury et al., 2002). Thus, multiple factors alter the ability of self-peptides to be effectively presented in the thymus, limiting the efficiency of negative selection.

PERIPHERAL TOLERANCE

Because not all autoreactive thymocytes are deleted during development, peripheral tolerance mechanisms have been postulated to induce unresponsiveness in mature T cells that are specific for peripheral self-antigens. Four main mechanisms of peripheral tolerance play an important role in maintaining self-tolerance and preventing autoimmunity: deletion, anergy, ignorance, and regulatory cells.

Deletion

The mechanism of tolerance of mature T cells by clonal elimination was demonstrated *in vivo* using different approaches (Webb et al., 1990; Kawabe and Ochi, 1991; Rocha and von Boehmer, 1991). In the first report, injection of cells expressing the superantigen Mls-1^a into thymectomized Mls-1^b mice resulted in the expansion of Mls-1^a reactive $V\beta6^+CD4^+$ T cells, followed by deletion of these cells (Webb et al., 1990).

The relevance of clonal deletion as a mechanism of peripheral tolerance to tissue specific antigens was provided using transgenic mouse models (Chen et al., 1995; Kurts et al., 1996; Liblau et al., 1996). Kurts et al. (1996) utilized mice that expressed a membrane-bound form of OVA in the pancreas [rat insulin promotor (RIP)–OVA mice] and kidneys. Adoptive transfer of OVA-specific $CD8^+$ T cells

into RIP–OVA mice showed that in the lymph nodes draining the organs where the OVA antigen was expressed, the OVA-specific T cells divided and displayed an activated phenotype. The OVA-specific T cells detected antigen on bone marrow-derived APCs that picked up antigen and presented it in the context of MHC class I via an exogenous processing pathway. This activation and expansion in the draining lymph nodes, however, was only transient and was followed by deletion of the OVA-specific T cells (Kurts et al., 1997).

The importance of deletion as a mechanism of peripheral tolerance has also been suggested in mice deficient for genes involved in apoptosis, such as Fas and FasL. The natural mutant lpr mouse (lymphoproliferative), which carries a mutation in the TNF-family receptor Fas, and the FasL mutant gld mouse (generalized lymphoproliferative disorder), develop lymphadenopathy, splenomegaly, and spontaneous autoimmunity (Watanabe-Fukunaga et al., 1992; Takahashi et al., 1994). A similar phenotype and autoimmunity is seen in autoimmune lymphoproliferative syndrome (ALPS) patients (also known as Canale Smith syndrome), most of whom have a mutation in Fas (Fisher et al., 1995; Rieux-Laucat et al., 1995; Lenardo et al., 1999). However, the role of Fas in peripheral clonal deletion and autoimmunity remains controversial. While some studies have pointed to a role for Fas/FasL in activation-induced cell death of mature T cells but not thymocytes (Russell et al., 1993; Singer and Abbas, 1994), others have found Fas to be dispensable for peripheral deletion (Zhou et al., 1992; Sytwu et al., 1996; Hildeman et al., 1999; Nguyen et al., 2000; Reich et al., 2000). Numerous other examples exist of molecules involved in apoptosis whose dysregulation can result in autoimmunity (Field et al., 1996; Murga et al., 2001; Zhang et al., 2002).

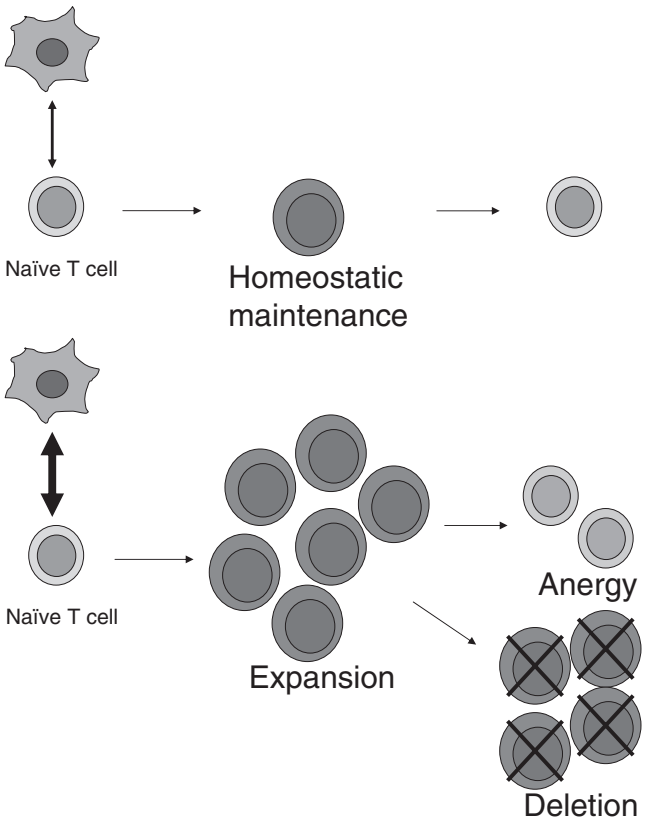


FIGURE 8.2 T-cell receptor (TCR) and peptide–MHC interactions govern homeostasis and peripheral T-cell tolerance. Weak-affinity/-avidity interactions with self-peptide–MHC are required for normal homeostatic T-cell survival as T cells that fail to receive TCR signals undergo programmed cell death. Strong-affinity/-avidity interactions with peptide–MHC on immature antigen-presenting cells result in T-cell expansion, followed by deletion of most of the clones, with the remaining ones being unresponsive to TCR stimuli (anergic).

T-Cell Inactivation

In some models, T-cell interaction with tolerizing antigen results in transient activation and eventual deletion. However, in some circumstances not all antigen-specific T cells are deleted; instead they become functionally inactivated, remaining alive for an extended period of time in a hyporesponsive state referred to as anergy (Figure 8.2) (Schwartz, 1990; 2003). Initial characterization showed that anergic cells were unable to proliferate or produce interleukin (IL)-2 upon stimulation.

The anergic state was first identified *in vitro* following activation of T cells with chemically fixed APCs (Jenkins and Schwartz, 1987; Mueller et al., 1989). Subsequently, other models have shown anergy induction *in vivo* (Lo et al., 1988; Burkly et al., 1989; Rammensee et al., 1989; Rellahan et al., 1990). *In vivo* anergy was first clearly shown by Rammensee et al. (1989) using superantigen Mls-1^a-positive cells that were injected into Mls-1^b mice. After the initial T-cell expansion and deletion of Mls-1^a-specific V$\beta6^+$ T cells, a population of V$\beta6^+$ T cells remained. These cells were unable to proliferate in response to Mls-1^a (Rammensee et al., 1989). Using a TCR transgenic mouse model, Rocha and von Boehmer (1991) have transferred H-Y specific transgenic T cells into nude male mice. This led to a rapid expansion and eventual deletion of the majority of T cells. The remaining T cells failed to proliferate in response to male cells and/or anti-TCR antibody.

Interestingly, there appears to be more than one type of anergy. Schwartz (2003) suggests that the *in vitro* induced clonal anergy is different from a state that is referred to as adaptive *in vivo* induced tolerance (Schwartz, 2003). Whereas clonal anergy can be reversed by the addition of IL-2, adaptive anergy cannot. Furthermore, adaptive anergy appears to depend on the continual presence of antigen. In the Rocha experiments with H-Y-specific T cells, if the anergic T cells were transferred into female mice that lacked the antigen, they recovered their ability to proliferate to

antigen (Rocha et al., 1993). This was also demonstrated in other models (Ramsdell and Fowlkes 1992; Migita and Ochi 1993). Further models have suggested that T-cell activation thresholds can be modulated; that is, the ability of T cells to be activated by antigens is affected by interactions with self-peptide–MHC encountered during development or in the periphery (Kawai and Ohashi, 1995; Sebzda et al., 1996; Mariathasan et al., 1998; Grossman and Paul, 2001). In addition, Tregs are generally believed to be anergic (Sakaguchi, 2004). Therefore, it appears that T lymphocyte unresponsiveness or anergy can be induced in multiple ways.

T-Cell Peripheral Tolerance Versus Activation

If mature T cells expressing TCRs that recognize self-peptide–MHC found in the periphery undergo clonal deletion and anergy, how is normal T-cell activation programmed to occur? Traditional models have suggested that T cells that are stimulated through the TCR (signal 1) together with a costimulatory signal 2 become fully activated effector T cells. In contrast, stimulation of T cells through the TCR in the absence of costimulation leads to the induction of anergy (Bretscher and Cohn, 1970; Lafferty and Cunningham, 1975; Schwartz, 1992). Costimulatory signals such as CD80 (B7.1) and CD86 (B7.2) are received from mature APCs. CD80 (B7.1) and CD86 (B7.2) bind to the costimulatory molecule CD28 that is constitutively expressed on the majority of T cells. Cytokines and chemokines also provide important signals that promote T-cell function and inflammation (Vella et al., 1997b; Curtsinger et al., 2003). Current models propose that the maturation state of the DC determines the outcome of T-cell tolerance versus activation. TCR-specific interactions between T cells and resting DCs result in tolerance (anergy and/or deletion), while interactions with activated DCs promote immunity (Steinman and Nussenzweig, 2002). Key signals that promote DC maturation *in vivo* include signals through Toll-like receptors (TLRs), which are triggered by various structural components of pathogens (Janeway and Medzhitov, 2002; Takeda et al., 2003).

T-cell function is also controlled through the actions of inhibitory receptors such as CTLA-4 and PD-1 on T cells (Greenwald et al., 2002). CTLA-4 is a molecule expressed on T cells that binds to CD80 (B7.1) and CD86 (B7.2) and is believed to be involved in the downregulation of T-cell responses, counteracting CD28 costimulation. CTLA-4 could do this by outcompeting CD28 for B7 ligands, antagonizing CD28-mediated signals, and/or antagonizing TCR-mediated signals; however, at this time the exact mechanism is unclear. CTLA-$4^{-/-}$ mice develop severe lymphoproliferative disorders ultimately resulting in death (Tivol et al. 1995; Waterhouse et al. 1995). Recently, a ligand-independent splice variant form of CTLA-4 (liCTLA-4) has been found and characterized as a negative regulator of signaling downstream of the TCR (Vijayakrishnan et al., 2004). It is more potent than the full-length CTLA-4 in inhibiting T-cell responses, and decreased levels of liCTLA-4 have been linked to susceptibility to diabetes, Graves' disease, and autoimmune hypothyroidism (Ueda et al., 2003).

Similarly, PD-1 (programmed death gene-1), which interacts with CD80/86 (B7) family members PD-L1 (B7-H1) and PD-L2 (B7-DC), has negative regulatory functions. PD-$1^{-/-}$ mice develop an autoimmune-like phenotype, although with delayed onset compared to CTLA-$4^{-/-}$ mice, with lupus-like glomerulonephritis and progressive arthritis associated with a high level of IgG_3 deposition, and increased numbers of myeloid and B cells (Nishimura et al., 1998; 1999). Thus, both the activation state of the APC and the inhibitory receptors affect T-cell tolerance and function, and regulate autoimmunity.

Ignorance

Many autoreactive T cells are not deleted or inactivated in the thymus or in the periphery. If tissue-specific self-antigens are not detectable by the immune system, potentially self-reactive "ignorant" T cells remain in the peripheral repertoire.

Ignorance was first demonstrated in an experimental model of diabetes (Ohashi et al., 1991; Oldstone et al., 1991). RIP-gp transgenic mice expressed the lymphocytic choriomeningitis virus (LCMV) glycoprotein under the control of the RIP, resulting in expression in the β cells of the pancreas. The LCMV-gp specific T cells from RIP-gp mice were not tolerized and yet, RIP-gp mice did not develop spontaneous diabetes. Lymphocytic choriomeningitis virus (LCMV) infection, however, resulted in the activation of gp-specific T cells that infiltrated and destroyed the insulin-producing islet β cells. Numerous examples exist where autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE), can be induced by immunizing animals with peptide in adjuvant, leading to the activation of self-specific T cells (Kuchroo et al., 2002). These studies suggest that T cells are not tolerized in the presence of antigen; rather they remain "ignorant" of the antigen expressed in the tissues.

Regulation by Other Cells

The existence of regulatory (suppressor) cells was postulated over 30 years ago following experiments using thymectomized mice and rats. These animals developed autoimmune destruction of the ovaries or thyroid gland, respectively, which could be prevented by adoptive transfer of normal $CD4^+$ cells (Nishizuka and Sakakura, 1969; Penhale et al., 1973). Sakaguchi et al. (1995) showed that the autoimmune disease induced by the transfer of $CD4^+CD25^-$ T cells into athymic nude mice could be pre-

vented by the cotransfer of $CD4^+CD25^+$ T cells. These $CD4^+CD25^+$ T cells occur naturally and act by suppressing immune responses. The molecular basis of $CD25^+CD4^+$ suppression remains controversial. *In vivo* studies with knockout mice and blocking antibodies suggested a role for the cytokines IL-10 and transforming growth factor (TGF)-β in some models [reviewed in Sakaguchi (2004)]. *In vitro* suppression requires direct cell–cell interactions but it is not clear if this is also true *in vivo* (Read et al., 1998; Takahashi et al. 1998; Thornton and Shevach, 1998). Interestingly, $CD4^+CD25^+$ T cells constitutively express CTLA-4 and its blockade abrogates Treg function *in vivo* (Read et al. 2000; Takahashi et al., 2000). Thus, it is possible that more than one mechanism of suppression exists.

Further evidence for the importance of Tregs is provided by both human and animal models. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome is an X-linked immunodeficiency syndrome associated with autoimmunity in multiple organs. The Scurfy strain of mice is an analogous disease model in mice displaying hyperactivation of $CD4^+$ T cells and overproduction of proinflammatory cytokines. The defective gene has been identified as Foxp3, a member of the forkhead/winged-helix family of transcription factors (Bennett et al., 2001; Brunkow et al., 2001; Wildin et al., 2001). Further studies have suggested a role for Foxp3 in the development and function of natural $CD4^+CD25^+$ Tregs (Fontenot et al., 2003; Hori et al. 2003; Khattri et al., 2003). For example, $CD4^+CD25^-$ T cells that were transduced with Foxp3 could suppress proliferation by other T cells *in vitro* and inflammatory bowel disease *in vivo* (Hori et al., 2003). Thus, Tregs appear to play an important role in the control of immune responses and autoimmunity.

AUTOIMMUNITY

In spite of the different tolerance mechanisms that exist, occasionally tolerance fails. This alone may not be sufficient to cause autoimmunity, but a variety of background genes may modulate disease susceptibility and contribute to the onset of autoimmunity in different ways. In the following section, we highlight potential mechanisms and discuss the requirements for the induction of autoimmune disease (Ohashi, 2002).

Aberrant T-Cell Homeostasis

T-cell homeostasis and survival in the periphery is controlled by several signals. The recognition of self-MHC ligand by naïve T cells is required for survival and thus is important in normal T-cell homeostasis (Figure 8.2) (Takeda et al., 1996; Tanchot et al., 1997; Jameson, 2002). These relatively weak interactions lead to partial tyrosine phosphorylation of the TCR ζ chain and upregulation of a subset of T-cell activation or memory markers, such as CD44 and CD122, respectively (Goldrath et al., 2002; Stefanova et al., 2003). Cytokines also play a crucial role in T-cell survival and homeostasis, particularly those that signal through the common γ chain. IL-4, IL-7, and IL-15 support homeostatic T-cell expansion *in vitro*, although only IL-7 has been shown to be crucial *in vivo* (Schluns et al., 2000; Goldrath et al., 2002). We suggest that dysregulation of lymphocyte survival may result in autoimmunity (Figure 8.3). Enhanced lymphocyte survival may occur as a result of altered lymphocyte cell survival signals. Semi-activated T-lymphocytes may constantly relay signals to APCs or B cells, promoting increased cytokine production and leading to the accumulation of lymphocytes in the secondary organs (splenomegaly and lymphadenopathy) and the production of antiself antibodies. Alternatively, enhanced TCR signaling could also lead to aberrant homeostasis. Weak interactions with self-peptide–MHC that normally lead to T-cell maintenance may actually lead to limited proliferation of certain clones, resulting in expansion and lymphoid hyperplasia (Figure 8.3).

Animal models have shown that altering the expression or regulation of cytokines important in homeostatic survival leads to autoimmunity. IL-7 functions as a crucial regulator of peripheral T-cell homeostasis by modulating the expansion of peripheral T-cell populations in lymphopenic animals (Schluns et al., 2000; Tan et al., 2001). It functions by increasing the proliferation of peripheral T cells in response to both low- and high-affinity antigens, in effect increasing peripheral homeostatic expansion. IL-7 potently inhibits programmed cell death by the upregulation of anti-apoptotic bcl-2 family members (Vella et al., 1997a; Fry and Mackall, 2001). Transgenic mice that expressed IL-7 under the control of the MHC class II promotor had a 10–20-fold increase in total T-cell numbers and $CD8^+$ T cells displayed a memory ($CD44^{hi}$ and $CD122^{hi}$) phenotype with enhanced production of IFN-γ after stimulation (Mertsching et al., 1995; Kieper et al., 2002). Some IL-7 transgenic lines developed dermatitis characterized by massive infiltration of mononuclear cells (Uehira et al., 1993). IL-7 administration also resulted in T-cell proliferation, although the upregulation of activation markers was not detected (Geiselhart et al., 2001).

IL-6 is a cytokine that regulates the immune response, hematopoiesis, and inflammation (Teague et al., 1997; Hirano, 1998). IL-6 transgenic mice developed a massive polyclonal plasmacytosis with autoantibody production and mesangial cell proliferative glomerulonephritis that resembled the autoimmune disease observed in systemic lupus erythematosus (SLE) patients (Suematsu et al., 1989). Hypergammaglobulinemia was also observed in mice who received a transplant of bone marrow cells infected with a retroviral vector expressing murine IL-6 (Brandt et al.,

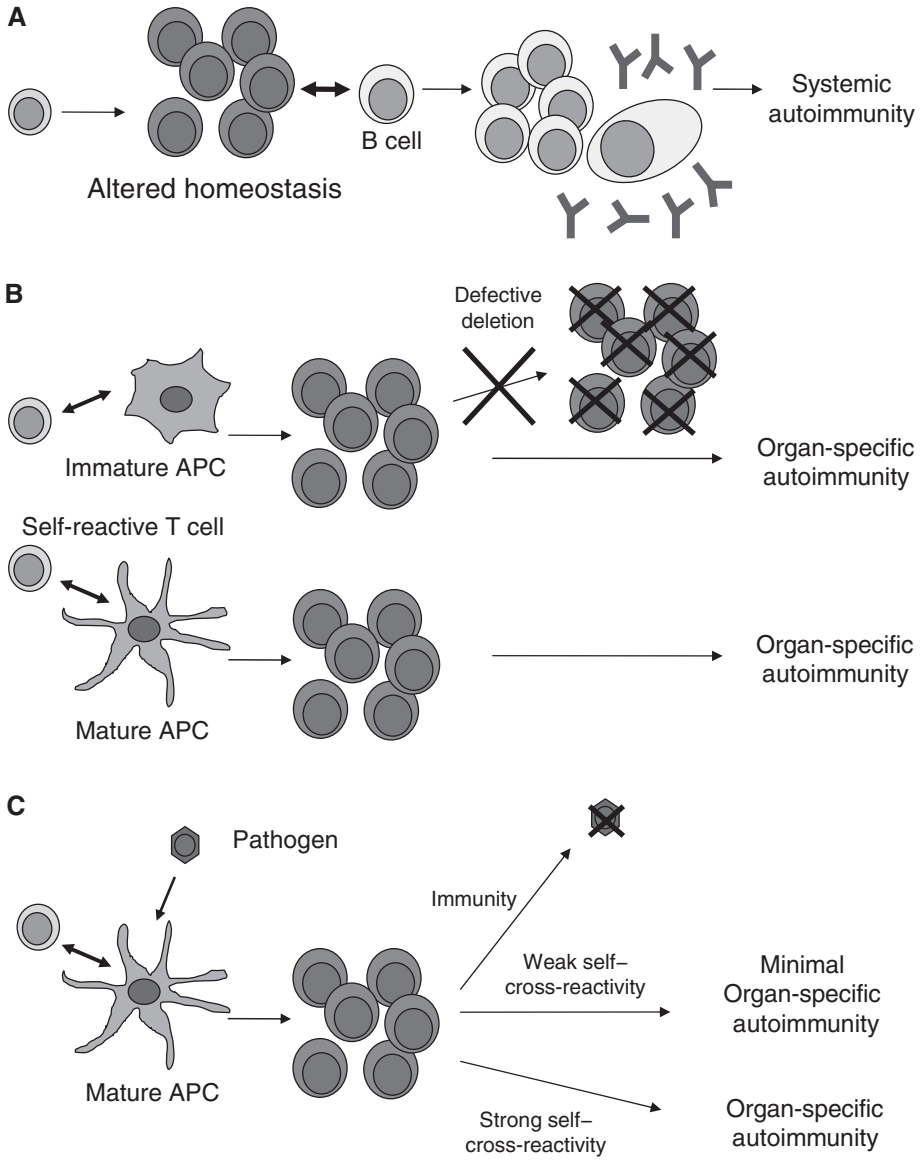


FIGURE 8.3 Potential mechanisms of autoimmunity. *A*, Weak interactions with self-peptide–MHC normally lead to homeostatic maintenance of T cells. If this process is disturbed by altering T-cell survival this can result in splenomegaly and lymphadenopathy. Over time, interactions between these partially activated T cells (dark cells) and B cells (light cells) can lead to systemic autoimmunity characterized by autoantibody production. *B*, T cells that encounter high-affinity/-avidity antigen on resting APCs would normally undergo a process of expansion followed by deletion or anergy. However, defects in apoptosis might lead to defective peripheral tolerance induction, either to the inability to directly delete the self-reactive cell or to the lack of cross-presentation of self-antigen, and result in autoimmunity. Alternatively, interactions with hyperactivated mature APCs may promote full activation of self-antigen-specific T cells instead of tolerance, and predispose individuals towards autoimmunity. *C*, Activation by pathogens results in the activation of a variety of T-cell clones specific for the foreign antigen. These clones proliferate and eliminate the pathogen. Weak cross-reactivity of these activated T cells with self-peptides may result in transient or minimal autoimmunity, i.e., organ infiltration but no destruction. On the other hand, T cells strongly reactive against self-antigen will cause organ-specific autoimmunity. It is not yet clear at which stage and by which of these mechanisms regulatory T cells act to prevent autoimmunity.

1990). Furthermore, high levels of IL-6 have been associated with a number of autoimmune diseases, like diabetes, inflammatory bowel disease, EAE, and rheumatoid arthritis (Ishihara and Hirano, 2002).

Cells have evolved important mechanisms to prevent excessive responses to cytokines. Suppressor of cytokine signaling (SOCS) proteins inhibit components of the cytokine signaling cascade via direct binding or by preventing access to the signaling complex. There are eight members of the SOCS family: CIS and SOCS1–SOCS7 (Alexander and Hilton, 2004). SOCS1 inhibits JAK-STAT signaling by many cytokines, including IL-2, IL-6, IL-7, and interferons. $SOCS1^{-/-}$ mice die by 3 weeks of age with monocytic infiltration, fatty necrosis of the liver, and inflammation of the pancreas and heart (Starr et al., 1998). The disease is thought to result from hypersensitivity to IFN-γ (Alexander et al., 1999). T cells in $SOCS1^{-/-}$ mice displayed an activated phenotype with increased cell size and expression of activation markers CD44, CD25, and CD69 (Marine et al., 1999). OT-I OVA-specific TCR transgenic $SOCS1^{-/-}$ $Rag1^{-/-}$ mice were used to determine if T cells would remain naïve in the absence of their antigen. These mice, however, still succumbed to disease in early adulthood and their T cells exhibited a blast-like activated phenotype with high levels of CD44 on the cell surface (Cornish et al., 2003). Studies by Hanada et al. (2003) examined the role of SOCS1 specifically in APCs by reintroducing SOCS1 as a transgene in lymphocytes. They provided evidence that SOCS1-deficient DCs are activated and promote autoimmunity. These experiments outline an important role for SOCS1 in directly regulating homeostasis of DCs, subsequently altering the homeostasis of lymphocytes.

Experimental evidence also suggests that altered T-cell survival via the phosphatidylinositol-3-kinase (PI3K) pathway can promote autoimmunity. Activation of PI3K catalyzes the formation of phosphatidylinositol-3,4-bisphosphate and phosphatidylinositol-3,4,5-triphosphate (PIP_3), which regulates many cellular events, including cell survival, division, and migration (Fruman et al., 1998; Scheid and Woodgett, 2001). The amount of PIP_3 is regulated by a lipid phosphatase known as PTEN (phosphatase and tensin homolog found on chromosome 10). The presence of PIP_3 on the membrane leads to recruitment and activation of a serine threonine kinase, protein kinase B (PKB/Akt). Perturbations in the activity of PI3K, PTEN or PKB lead to an autoimmune phenotype in mice characterized by activated $CD4^+$ cells, lymphadenopathy, a defect in Fas-mediated apopotosis, inflammation, and autoantibody production (Di Cristofano et al., 1999; Borlado et al., 2000; Parsons et al., 2001). Studies have shown that active PKB prevents Fas-induced death by inhibiting the formation of the death-inducing signaling complex, which is essential for the induction of apoptosis (Jones et al., 2002). These and other studies suggest that the primary role of Fas may be associated with homeostasis and not peripheral deletion (Markiewicz et al., 2003). Thus, evidence suggests that events that alter homeostasis or survival of lymphocytes may contribute to autoimmunity.

Defects in Peripheral T-Cell Tolerance

Strong stimulatory TCR signals that result from T-cell encounter with self-antigen on resting APCs generally lead to T-cell proliferation followed by deletion or anergy (Figure 8.2) (Kurts et al., 1997; Hawiger et al., 2001; Steinman et al., 2003). As discussed above, if the deletion of autoreactive clones is perturbed, autoimmunity might occur due to the presence of autoreactive cells (Figure 8.3). Aberrant peripheral tolerance may also occur if self-antigen is presented by activated APCs or if T cells circumvent the need for activated APCs to be fully stimulated (Diehl et al., 1999; Sotomayor et al., 1999; Garza et al., 2000).

Interaction of T cells with immature APCs normally results in tolerance. However, examples from genetically manipulated mice have shown that there are genes that influence the ability of APCs to induce tolerance versus activation. STAT3 is one of a family of cytoplasmic transcription factors that are key mediators of cytokine and growth factor signaling pathways. Disruption of STAT3 in mice resulted in generation of APCs that effectively primed rather than tolerized naïve T cells. Moreover, $STAT3^{-/-}$ DCs could restore the unresponsiveness of anergic $CD4^+$ T cells (Cheng et al., 2003). Studies have suggested that the absence of STAT3 leads to a proinflammatory environment, promoting inflammatory bowel disease (Takeda et al., 1999; Welte et al., 2003).

Tyro 3, Axl, and Mer (TAM) are receptor tyrosine kinases that are expressed by macrophages and DCs, but not T- or B cells. Upon receptor engagement, the Src family kinases are activated and signaling pathways downstream of Grb2 promote cell proliferation and protect against cell death (Schwartzberg, 2001). The Tyro 3, Axl, and Mer triple knockout mice contain large numbers of apoptotic cells in many tissues and have features associated with autoimmunity (Lu and Lemke, 2001). Beginning at 4 weeks of age, the spleen and lymph nodes of these mice expanded by up to 10 times those of wild-type mice. The aberrant growth of peripheral lymphoid organs was primarily due to hyperproliferation of B and T cells. All triple mutant TAM-deficient mice developed autoimmune disorders displaying lymphocytic invasion of multiple organs, autoantibodies to dsDNA, various collagens and phospholipids, as well as deposition of immune complexes in tissues. The triple knockout mice, as well as Mer^{kd} mutant mice that lack the cytoplasmic region of the molecule, have delayed clearance of apoptotic cells (Lu and Lemke, 2001; Scott et al., 2001; Cohen et al., 2002). This may result in failed tolerance, since there may be inefficient presentation of self-antigens from apoptotic

cells. $CD11c^+$ cells (DCs) from the knockout mice have upregulated levels of MHC II and CD86 (B7.2), markers of APC activation, while macrophages in these mice release larger amounts of TNF-α, a proinflammatory cytokine (Camenisch et al., 1999; Lu and Lemke, 2001). Therefore, the combination of an increase in tissue-specific T cells (due to the lack of peripheral tolerance) together with increased APC activation possibly results in autoimmunization and autoimmunity in these mice.

Although hyperactivated APCs can provide costimulation to T cells and predispose animals towards autoimmunity, studies have identified a genetic mutation that circumvents the requirement for costimulatory signals for full T-cell activation. Cbl, a family of E3 ubiquitin ligases, directs the ubiquitination of tyrosine kinases involved in signaling downstream of the TCR. Cbl-b-deficient mice have enhanced IL-2 production after TCR stimulation (Bachmaier et al., 2000; Chiang et al., 2000). The Penninger group described autoimmunity in the absence of Cbl-b characterized by multiorgan inflammation and autoantibody production (Bachmaier et al., 2000). Gu and colleagues showed that $Cbl\text{-}b^{-/-}$ mice were also more susceptible to antigen-induced EAE (Chiang et al., 2000). Both groups suggested that Cbl-b acts primarily by regulating the requirement for CD28 costimulation. Further studies have shown that T cells from Cbl-b-deficient animals cannot be anergized (Heissmeyer et al., 2004). Interestingly, Yokoi et al. (2002) mapped a diabetes-susceptibility locus in the Komeda rats to Cbl-b. These rats had a nonsense mutation that resulted in the transcription of a truncated form of the molecule. Transgenic expression of a full-length Cbl-b prevented diabetes in the rat model.

Other E3 ubiquitin ligases, such as Itch and Grail, have also recently been shown to play a role in the induction of T-cell tolerance. The expression of Itch, Cbl-b, and GRAIL is upregulated in anergic T cells and is crucial for the induction of anergy, suggesting the importance of these molecules in peripheral tolerance (Heissmeyer et al., 2004; Jeon et al., 2004). Like mice lacking Cbl-b, those lacking Itch also develop spontaneous autoimmunity characterized by inflammation of numerous tissues (Perry et al., 1998; Fang et al., 2002). Thus, in addition to Cbl-b, other E3 ubiquitin ligases are also crucial for the induction of anergy and tolerance by ubiquitinating downstream signaling molecules.

Collectively these studies suggest that the impaired induction of anergy due to the absence of E3 ubiquitin ligases, such as Cbl-b or Itch, promotes autoimmunity.

Molecular Mimicry

Potentially autoreactive T cells exist in the peripheral T-cell repertoire, but are not usually activated because APCs are normally not activated. Infections with pathogens usually lead to the generation of an effective immune response, because pathogens provide the necessary signals for APC maturation. If pathogens express antigens that are similar to host antigens, cross-reactive responses can lead to the destruction of host tissues. This process is called molecular mimicry and studies in various experimental models have shown molecular mimicry as a mechanism triggering autoimmunity (Damian, 1964; Fujinami and Oldstone, 1985; Ohashi et al., 1991; Oldstone et al., 1991; Jahnke et al., 1995; Oldstone, 1998; Olson et al., 2001). Examples of autoimmune diseases possibly induced by molecular mimicry are shown in Table 8.1. Although a number of studies suggest molecular mimicry as a mechanism for clinical autoimmune disease, it is still unclear whether this is a common way in which tolerance is broken (Benoist and Mathis, 2001).

Pathogenic infections have been associated with autoimmune diseases, which are believed to occur as a result of molecular mimicry (Table 8.1). Lyme arthritis results from an infection by the tick-borne spirochete *Borrelia burgdorferi*. In approximately 10% of patients with Lyme arthritis, joint inflammation continues even after bacterial DNA can no longer be detected in the joints of patients. The majority

TABLE 8.1 Autoimmune diseases that are associated with a pathogenic infection and where a candidate mimic and/or self-protein have been identified

Disease	Pathogen	Mimic protein	Self-protein	Reference
Antibiotic-resistant Lyme arthritis	*Borrelia burgdorferi*	OspA	hLFA-1	Gross et al. (1998)
Herpes stromal keratitis	HSV-1	UL6	Unknown (eye protein)	Zhao et al. (1998)
HTLV-1-associated myelopathy/tropical spastic paraparesis	HTLV-1	Unknown	hnRNP-A1	Levin et al. (2002)
Autoimmune gastritis	*Helicobacter pylori*	LPS biosynthesis protein	H+K+-adenosine triphosphate	Amedei et al. (2003)
Reactive arthritis	*S. typhimurium*	GroEL presented by class Ib molecule Qa1	mHSP60	Lo et al. (2000)

of patients with Lyme arthritis has HLA-DRB1*0401 or HLA-DRB1*0101. Patients also show immune reactivity to the outer surface protein A (OspA) of the spirochete. A study by Huber and colleagues identified the immunodominant HLA-DRB1*0401-restricted peptide of OspA (Gross et al., 1998). From this they identified human LFA-1 as a candidate autoantigen with homology to the OspA peptide. They also showed that synovial fluid T cells could respond to both the OspA and LFA-1 peptides that were identified. Although Lyme arthritis links human autoimmunity and molecular mimicry, whether molecular mimicry is truly the cause of Lyme arthritis remains controversial (See Chapter 33). The LFA-1 peptide acts only as a weak agonist in Lyme-resistant arthritis patients, mainly producing the Th2 cytokine IL-13 (Trollmo et al., 2001). Furthermore, a number of postulates have not been fulfilled to implicate molecular mimicry as the true cause of Lyme arthritis (Benoist and Mathis, 2001; Steere and Glickstein, 2004).

CONCLUDING REMARKS

Research over the past several decades has revealed a detailed understanding of T-cell tolerance mechanisms in the thymus and periphery. New insights into the mechanisms of breaking tolerance and factors that contribute to the induction of autoimmune disease are being unveiled with the generation of many different gene-deficient strains of mice. Ideally, the next few decades will bring a greater understanding regarding the molecular pathways and mechanisms that contribute to the different spectra of autoimmune diseases. This should provide insights and directions for specific therapy, targeting different autoimmune diseases.

Acknowledgments

We would like to thank Elissa Deenick, Nicole Liadis, Kiichi Murakami, Nobuyuki Ono, and Janice Sarmiento for critical reading of this manuscript.

References

Alexander, W.S. and Hilton, D.J. (2004). The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. *Annu. Rev. Immunol. 22*, 503–529.

Alexander, W.S., Starr, R., Fenner, J.E., Scott, C.L., Handman, E., Sprigg, N.S., Corbin, J.E., Cornish, A.L., Darwiche, R., Owczarek, C.M., Kay, T.W., Nicola, N.A., Hertzog, P.J., Metcalf, D., and Hilton, D.J. (1999). SOCS1 is a critical inhibitor of interferon gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell 98*, 597–608.

Ally, B.A., Hawley, T.S., MacKall-Faienza, K., Kündig, T.M., Oehen, S.U., Pircher, H., Hawley, R.G., and Ohashi, P.S. (1995). Prevention of autoimmune disease by retroviral-mediated gene therapy. *J. Immunol. 155*, 5404–5408.

Amedel, A., Bergman, M.P., Appelmelk, B.J. Azzurri, A., Benagiano, M., Tamburini, C., Van der Zee, R., Telford, J.L., Vandenbroucke-Grauls, C.M.J.F., D'Elios, M.M., and Del Prete, G. (2003). Molecular mimicry between *Helicobacter pylori* antigens and H+K+-adenosine triphosphatase in human gastric autoimmunity. *J. Exp. Med. 198*, 1147–1156.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298, 1395–1401.

Anderton, S.M., Viner, N.J., Matharu, P., Lowrey, P.A., and Wraith, D.C. (2002). Influence of a dominant cryptic epitope on autoimmune T cell tolerance. *Nat. Immunol. 3*, 175–181.

Bachmaier, K., Krawczyk, C., Kozieradzki, I., Kong, Y.-Y., Sasaki, T., Oliveira-dos-Santos, A.J., 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*, 211–216.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F., and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of *FOXP3*. *Nat. Genet. 27*, 20–21.

Benoist, C. and Mathis, D. (2001). Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat. Immunol. 2*, 797–801.

Borlado, L.R., Redondo, C., Alverez, B., Jimenez, C., Criado, L.M., Flores, J., Marcos, M.A., Martinez A.C., Balomenos, D., and Carrera, A.C. (2000). Increased phosphoinositide 3-kinase activity induces a lymphoproliferative disorder and contributes to tumor generation *in vivo*. *FASEB J. 14*, 895–903.

Bouillet, P., Metcalf, D., Huang, D.C.S., Tarlinton, D.M., Kay, T.W.H., Köntgen, 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*, 1735–1738.

Bouillet, P., Purton, J.F., Godfrey, D.I., Zhang, L.-C., Coultas, L., Puthalakath, H., Pellegrini, M., Cory, S., Adams, J.M., and Strasser, A. (2002). BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature 415*, 922–926.

Brandt, S.J., Bodine, D.M., Dunbar, C.E., and Nienhuis, A.W. (1990). Dysregulated interleukin 6 expression produces a syndrome resembling Castleman's disease in mice. *J. Clin. Invest. 86*, 592–599.

Bretscher, P. and Cohn, M. (1970). A theory of self-nonself discrimination. *Science 169*, 1042–1049.

Brunkow, M.E., Jeffery, E.W., Hjerrild, K.A., Paeper, B., Clark, L.B., Yasayko, S.A., Wilkinson, J.E., Galas, D., Ziegler, S.F., and Ramsdell, F. (2001). Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet. 27*, 68–73.

Burkly, L.C., Lo, D., Kanagawa, O., Brinster, R.L., and Flavell, R.A. (1989). T-cell tolerance by clonal anergy in transgenic mice with non-lymphoid expression of MHC class II I-E. *Nature 342*, 564–566.

Camenisch, T.D., Koller, B.H., Earp, H.S., and Matsushima, G.K. (1999). A novel receptor tyrosine kinase, Mer, inhibits TNF-alpha production and lipopolysaccharide-induced endotoxic shock. *J. Immunol. 162*, 3498–3503.

Chen, Y., Inobe, J., Marks, R., Gonnella, P., Kuchroo, V.K., and Weiner, H.L. (1995). Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature 376*, 177–180.

Cheng, F., Wang, H.-W., Cuenca, A., Huang, M., Ghansah, T., Brayer, J., Kerr, W.G., Takeda, K., Akira, S., Schoenberger, S.P., Yu, H., Jove, R., and Sotomayor, E.M. (2003). A critical role for Stat3 signaling in immune tolerance. *Immunity 19*, 425–436.

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*, 216–220.

Cohen, P.L., Caricchio, R., Abraham, V., Camenisch, T.D., Jennette, J.C., Roubey, R.A.S., 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*, 135–140.

Cornish, A.L., Davey, G.M., Metcalf, D., Purton, J.F., Corbin, J.E., Greenhalgh, C.J., Darwiche, R., Wu, L., Nicola, N.A., Godfrey, D.I., Heath, W.R., Hilton, D.J., Alexander, W.S., and Starr, R. (2003). Suppressor of cytokine signaling-1 has IFN-gamma-independent actions in T cell homeostasis. *J. Immunol. 170*, 878–886.

Curtsinger, J.M., Lins, D.C., and Mescher, M.F. (2003). Signal 3 determines tolerance versus full activation of naive CD8 T cells: dissociating proliferation and development of effector function. *J. Exp. Med. 197*, 1141–1151.

Damian, R.T. (1964). Molecular mimicry: antigen sharing by parasite and host and its consequences. *Am. Naturalist XCVIII*, 129–149.

Davey, G.M., Kurts, C., Miller, J.F.A.P., Bouillet, P., Strasser, A., Brooks, A.G., Carbone, F.R., and Heath, W.R. (2002). Peripheral deletion of autoreactive CD8 T cells by cross presentation of self-antigen occurs by a Bcl-2-inhibitable pathway mediated by Bim. *J. Exp. Med. 196*, 947–955.

Davey, G.M., Schober, S.L., Endrizzi, B.T., Dutcher, A.K., Jameson, S.C., and Hogquist, K.A. (1998). Preselection thymocytes are more sensitive to T cell receptor stimulation than mature T cells. *J. Exp. Med. 188*, 1867–1874.

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*, 2122–2125.

Diehl, L., den Boer, A., Schoenberger, S., van der Voort, E.I.H., Schumacher, T.N.M., Melief, C.J.M., Offringa, R., and Toes, R.E.M. (1999). CD40 activation *in vivo* overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. *Nat. Med. 5*, 774–779.

Fang, D., Elly, C., Gao, B., Fang, N., Altman, Y., Joazeiro, C., Hunter, T., Copeland, N., Jenkins, N., and Liu, Y.C. (2002). Dysregulation of T lymphocyte function in itchy mice: a role for Itch in TH2 differentiation. *Nat. Immunol. 3*, 281–287.

Fehervari, Z. and Sakaguchi, S. (2004). Development and function of $CD25^{+}CD4^{+}$ regulatory T cells. *Curr. Opin. Immunol. 16*, 203–208.

Field, S.J., Tsai, F.Y., Kuo, F., Zubiaga, A.M., Kaelin, W.G. Jr., Livingston, D.M., Orkin, S.H., and Greenberg, M.E. (1996). E2F-1 functions in mice to promote apoptosis and suppress proliferation. *Cell 85*, 549–561.

Fisher, G.H., Rosenberg, F.J., Straus, S.E., Dale, J.K., Middelton, L.A., Lin, A.Y., Strober, W., Lenardo, M.J., and Puck, J.M. (1995). Dominant interfering Fas gene mutations. *Cell 81*, 935–946.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol. 4*, 330–336.

Fruman, D.A., Meyers, R.E., and Cantley, L.C. (1998). Phosphoinositide kinases. *Annu. Rev. Biochem. 67*, 481–507.

Fry, T.J. and Mackall, C.L. (2001). Interleukin-7: master regulator of peripheral T-cell homeostasis? *Trends Immunol. 22*, 564–571.

Fujinami, R.S. and Oldstone, M.B.A. (1985). Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science 230*, 1043–1045.

Garza, K.M., Chan, S.M., Suri, R., Nguyen, L.T., Odermatt, B., Schoenberger, S.P., and Ohashi, P.S. (2000). Role of antigen presenting cells in mediating tolerance and autoimmunity. *J. Exp. Med. 191*, 2021–2027.

Geiselhart, L.A., Humphries, C.A., Gregorio, T.A., Mou, S., Subleski, J., and Komschlies, K.L. (2001). IL-7 administration alters the CD4:CD8 ratio, increases T cell numbers, and increases T cell function in the absence of activation. *J. Immunol. 166*, 3019–3027.

Goldrath, A.W., Sivakumar, P.V., Glaccum, M., Kennedy, M.K., Bevan, M.J., Benoist, C., Mathis, D., and Butz, E.A. (2002). Cytokine requirements for acute and Basal homeostatic proliferation of naive and memory $CD8^{+}$ T cells. *J. Exp. Med. 195*, 1515–1522.

Goverman, J., Woods, A., Larson, L., Weiner, L.P., Hood, L., and Zaller, D.M. (1993). Transgenic mice that express a myelin basic protein specific T cell receptor develop spontaneous autoimmunity. *Cell 72*, 551–560.

Greenwald, R.J., Latchman, Y.E., and Sharpe, A.H. (2002). Negative co-receptors on lymphocytes. *Curr. Opin. Immunol. 14*, 391–396.

Gross, D.M., Forsthuber, T., Tary-Lehmann, M., Etling, C., Ito, K., Nagy, Z.A., Field, J.A., Steere, A.C., and Huber, B.T. (1998). Identification of LFA-1 as a candidate autoantigen in treatment-resistant lyme arthritis. *Science 281*, 703–706.

Grossman, Z. and Paul, W.E. (2001). Autoreactivity, dynamic tuning and selectivity. *Curr. Opin. Immunol. 13*, 687–698.

Hanada, T., Yoshida, H., Kato, S., Tanaka, K., Masutani, K., Tsukada, J., Nomura, Y., Mimata, H., Kubo, M., and Yoshimura, A. (2003). Suppressor of cytokine signaling-1 is essential for suppressing dendritic cell activation and systemic autoimmunity. *Immunity 19*, 437–450.

Haskins, K. and McDuffie, M. (1990). Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T cell clone. *Science 249*, 1433–1438.

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.

Heissmeyer, V., Macian, F., Im, S.H., Varma, R., Feske, S., Venuprasad, K., Gu, H., Liu, Y.C., Dustin, M.L., and Rao, A. (2004). Calcineurin imposes T cell unresponsiveness through targeted proteolysis of signaling proteins. *Nat. Immunol. 5*, 255–265.

Herold, K.C., Montag, A.G., and Buckingham, F. (1992). Induction of tolerance to autoimmune diabetes with islet antigens. *J. Exp. Med. 176*, 1107–1114.

Hildeman, D.A., Mitchell, T., Teague, T.K., Henson, P., Day, B.J., Kappler, J., and Marrack, P.C. (1999). Reactive oxygen species regulate activation-induced T cell apoptosis. *Immunity 10*, 735–744.

Hildeman, D.A., Zhu, Y., Mitchell, T.C., Bouillet, P., Strasser, A., Kappler, J., and Marrack, P. (2002). Activated T-cell death *in vivo* mediated by proapoptotic Bcl-2 family member bim. *Immunity 16*, 759–767.

Hirano, T. (1998). Interleukin 6 and its receptor: ten years later. *Int. Rev. Immunol. 16*, 249–284.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science 299*, 1057–1061.

Ishihara, K. and Hirano, T. (2002). IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev. 13*, 357–368.

Itoh, M., Takahashi, T., Sakaguchi, N., Kuniyasu, Y.S.J., Otsuka, F., and Sakaguchi, K. (1999). Thymus and autoimmunity: production of $CD25^{+}CD4^{+}$ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunological self-tolerance. *J. Immunol. 162*, 5317–5326.

Jahnke, U.K., Fischer, E.H., and Alvord, E.C. Jr. (1995). Sequence homology between certain viral proteins and proteins related to encephalomyelitis and neuritis. *Science 229*, 282–284.

Jameson, S.C. (2002). Maintaining the norm: T-cell homeostasis. *Nat. Rev. Immunol. 2*, 547–556.

Janeway, C.A. Jr. and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol. 20*, 197–216.

Jenkins, M.K. and Schwartz, R.H. (1987). Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. *J. Exp. Med. 165*, 302–319.

Jeon, M.-S., Atfield, A., Venuprasad, K., Krawczyk, C., Sarao, R., Elly, C., Yang, C., Arya, S., Bachmaier, K., Su, L., Bouchard, D., Jones, R., Gronski, M., Ohashi, P., Wada, T., Bloom, D., Fathman, C.G., Liu, Y.-C., and Penninger, J.M. (2004). Essential role of the E3 ubiquitin ligase Cbl-b in T cell anergy induction. *Immunity 21*, 167–177.

Jones, R.G., Elford, A.R., Parsons, M.J., Wu, L., Krawczyk, C.M., Yeh, W.-C., Hakem, R., Rottapel, R., Woodgett, J.R., and Ohashi, P.S. (2002). CD28-dependent activation of protein kinase B/Akt blocks Fas-mediated apoptosis by preventing death-inducing signaling complex assembly. *J. Exp. Med. 196*, 335–348.

Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of $CD4^+CD25^+$ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol. 2*, 301–306.

Kappler, J.W., Roehm, N., and Marrack, P. (1987). T cell tolerance by clonal elimination in the thymus. *Cell 49*, 273–280.

Kappler, J.W., Staerz, U.D., White, J., and Marrack, P. (1988). Self tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature 332*, 35–40.

Katz, J.D., Wang, B., Haskins, K., Benoist, C., and Mathis, D. (1993). Following a diabetogenic T cell from genesis through pathogenesis. *Cell 74*, 1089–1100.

Kawabe, Y. and Ochi, A. (1991). Programmed cell death and extrathymic reduction of $V\beta8^+$ $CD4^+$ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. *Nature 349*, 245–247.

Kawai, K. and Ohashi, P.S. (1995). Immunological function of a defined T-cell population tolerized to low-affinity self antigens. *Nature 374*, 68–69.

Khattri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003). An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. *Nat. Immunol. 4*, 337–342.

Kieper, W.C., Tan, J.T., Bondi-Boyd, B., Gapin, L., Sprent, J., Ceredig, R., and Surh, C.D. (2002). Overexpression of interleukin (IL)–7 leads to IL-15-independent generation of memory phenotype $CD8^+$ T cells. *J. Exp. Med. 195*, 1533–1539.

Kishimoto, H. and Sprent, J. (2001). A defect in central tolerance in NOD mice. *Nat. Immunol. 2*, 1025–1031.

Kisielow, P., Blüthmann, H., Staerz, U.D., Steinmetz, M., and von Boehmer, H. (1988). Tolerance in T cell receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature 333*, 742–746.

Kuchroo, V.K., Anderson, A.C., Waldner, H., Munder, M., Bettelli, E., and Nicholson, L.B. (2002). T cell response in experimental autoimmune encephalomyelitis (EAE): Role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annu. Rev. Immunol. 20*, 101–123.

Kurts, C., Heath, W.R., Carbone, F.R., Allison, J., Miller, J.F.A.P., and Kosaka, H. (1996). Constitutive class I-restricted exogenous presentation of self antigens *in vivo*. *J. Exp. Med. 184*, 923–930.

Kurts, C., Kosaka, H., Carbone, F.R., Miller, J.F.A.P., and Heath, W.R. (1997). Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive $CD8^+$ T cells. *J. Exp. Med. 186*, 239–245.

Kyewski, B., Derbinski, J., Gotter, J., and Klein, L. (2002). Promiscuous gene expression and central T-cell tolerance: more than meets the eye. *Trends Immunol. 23*, 364–371.

Lafferty, K.J. and Cunningham, A.J. (1975). A new analysis of allogeneic interactions. *Austr. J. Exp. Biol. Med. Sci. 53*, 27.

Lenardo, M.J., Chan, F.K.M., Hornung, F., McFarland, H., Siegel, R., Wang, J., and Zheng, L. (1999). Mature T lymphocyte apoptosis—immune regulation in a dynamic and unpredictable environment. *Annu. Rev. Immunol. 17*, 221–253.

Lesage, S., Hartley, S.B., Akkaraju, S., Wilson, J., Townsend, M., and Goodnow, C.C. (2002). Failure to censor forbidden clones of CD4 T cells in autoimmune diabetes. *J. Exp. Med. 196*, 1175–1188.

Levin, M.C., Lee, S.M., Kalume, F., Morcos, Y., Dohan, F.C., Jr., Hasty, K.A., Callaway, J.C., Zunt, J., Desiderio, D.M., and Stuart, J.M. (2002). Autoimmunity due to molecular mimicry as a cause of neurological disease. *Nat. Med. 8*, 509–513.

Liblau, R.S., Tisch, R., Shokat, K., Yang, X.-D., Dumont, N., Goodnow, C.C., and McDevitt, H.O. (1996). Intravenous injection of soluble antigen induces thymic and peripheral T-cell apoptosis. *Proc. Natl. Acad. Sci. USA 93*, 3031–3036.

Liston, A., Lesage, S., Wilson, J., Peltonen, L., and Goodnow, C.C. (2003). Aire regulates negative selection of organ-specific T cells. *Nat. Immunol. 4*, 350–354.

Liu, G.Y., Fairchild, P.J., Smith, R.M., Prowle, J.R., Kioussis, D., and Wraith, D.C. (1995). Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity 3*, 407–415.

Lo, D., Burkly, L.C., Widera, G., Cowing, C., Flavell, R.A., Palmiter, R.D., and Brinster, R.L. (1988). Diabetes and tolerance in transgenic mice expressing class II MHC molecules in pancreatic beta cells. *Cell 53*, 159–168.

Lo, W.F., Woods, A.S., DeCloux, A., Cotter, R.J., Metcalf, E.S., and Soloski, M.J. (2000). Molecular mimicry mediated by MHC class Ib molecules after infection with gram-negative pathogens. *Nat. Med. 6*, 215–218.

Lu, Q. and Lemke, G. (2001). Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science 293*, 306–311.

Lucas, B., Stefanova, I., Yasutomo, K., Dautigny, N., and Germain, R.N. (1999). Divergent changes in the sensitivity of maturing T cells to structurally related ligands underlies formation of a useful T cell repertoire. *Immunity 10*, 367–376.

MacDonald, H.R., Schneider, R., Lees, R.K., Howe, R.C., Acha-Orbea, H., Festenstein, H., Zinkernagel, R.M., and Hengartner, H. (1988). T cell receptor Vβ use predicts reactivity and tolerance to Mls^a-encoded antigens. *Nature 332*, 40–45.

Manoury, B., Mazzeo, D., Fugger, L., Viner, N., Ponsford, M., Streeter, H., Mazza, G., Wraith, D.C., and Watts, C. (2002). Destructive processing by asparagine endopeptidase limits presentation of a dominant T cell epitope in MBP. *Nat. Immunol. 3*, 169–174.

Mariathasan, S., Bachmann, M.F., Bouchard, D., Ohteki, T., and Ohashi, P.S. (1998). Degree of TCR internalization and Ca^{2+} flux correlates with thymocyte selection. *J. Immunol. 161*, 6030–6037.

Marine, J.C., Topham, D.J., McKay, C., Wang, D., Parganas, E., Stravopodis, D., Yoshimura, A., and Ihle, J.N. (1999). SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell 98*, 609–616.

Markiewicz, M.A., Brown, I., and Gajewski, T.F. (2003). Death of peripheral $CD8^+$ T cells in the absence of MHC class I is Fas-dependent and not blocked by Bcl-X_L. *Eur. J. Immunol. 33*, 2917–2926.

Mertsching, E., Burdet, C., and Ceredig, R. (1995). IL-7 transgenic mice: analysis of the role of IL-7 in the differentiation of thymocytes *in vivo* and *in vitro*. *Int. Immunol. 7*, 401–414.

Migita, K. and Ochi, A. (1993). The fate of anergic T cells *in vivo*. *J. Immunol. 150*, 763–770.

Morgan, D.J., Kreuwel, H.T.C., Fleck, S., Levitsky, H.I., Pardoll, D.M., and Sherman, L.A. (1998). Activation of low avidity CTL specific for a self epitope results in tumor rejection but not autoimmunity. *J. Immunol. 160*, 643–651.

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.

Murga, M., Fernández-Capetillo, O., Field, S.J., Moreno, B., Borlado, L., 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*, 959–970.

Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K.J., Lalioti, M.D., Mullis, P.E., Antonarakis, S.E., Kawasaki, K., Asakawa, S., Ito, F., and Shimizu, N. (1997). Positional cloning of the APECED gene. *Nat. Genet. 17*, 393–398.

Nagata, M., Santamaria, P., Kawamura, T., Utsugi, T., and Yoon, J.W. (1994). Evidence for the role of $CD8^+$ cytotoxic T cells in the destruction of pancreatic β-cells in nonobese diabetic mice. *J. Immunol. 152*, 2042–2050.

Nguyen, L.T., McKall-Faienza, K., Zakarian, A., Speiser, D.E., Mak, T.W., and Ohashi, P.S. (2000). TNF receptor 1 (TNFR1) and CD95 are not required for T cell deletion after virus infection but contribute to peptide-induced deletion under limited conditions. *Eur. J. Immunol. 30*, 683–688.

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.

Nishimura, H., Nose, M., Hiai, H., Minator, 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.

Nishizuka, Y. and Sakakura, T. (1969). Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science 166*, 753–755.

Oehen, S.U., Ohashi, P.S., Burki, K., Hengartner, H., Zinkernagel, R.M., and Aichele, P. (1994). Escape of thymocytes and mature T cells from clonal deletion due to limiting tolerogen expression levels. *Cell. Immunol. 158*, 342–352.

Ohashi, P.S. (2002). T-cell signalling and autoimmunity: molecular mechanisms of disease. *Nat. Rev. Immunol. 2*, 427–438.

Ohashi, P.S., Oehen, S., Bürki, K., Pircher, H., Ohashi, C.T., Odermatt, B., Malissen, B., Zinkernagel, R., and Hengartner, H. (1991). Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell 65*, 305–317.

Oldstone, M.B.A. (1998). Molecular mimicry and immune-mediated diseases. *FASEB J. 12*, 1255–1265.

Oldstone, M.B.A., Nerenberg, M., Southern, P., Price, J., and Lewicki, H. (1991). Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell 65*, 319–331.

Olson, J.K., Croxford, J.L., Calenoff, M.A., Dal Canto, M.C., and Miller, S.D. (2001). A virus-induced molecular mimicry model of multiple sclerosis. *J. Clin. Invest. 108*, 311–318.

Palmer, E. (2003). Negative selection – clearing out the bad apples from the T-cell repertoire. *Nat. Rev. Immunol. 3*, 383–391.

Parsons, M.J., Jones, R.G., Tsao, M.S., Odermatt, B., Ohashi, P.S., and Woodgett, J.R. (2001). Expression of active PKB in T cells perturbs both T and B cell homeostasis and promotes inflammation. *J. Immunol. 167*, 42–48.

Penhale, W.J., Farmer, A., McKenna, R.P., and Irvine, W.J. (1973). Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. *Clin. Exp. Immunol. 15*, 225–236.

Perry, W.L., Hustad, C.M., Swing, D.A., O'Sullivan, T.N., Jenkins, N.A., and Copeland, N.G. (1998). The itchy locus encodes a novel ubiquitin protein ligase that is disrupted in a^{18H} mice. *Nat. Genet. 18*, 143–146.

Pircher, H., Hoffmann Rohrer, U., Moskophidis, D., Zinkernagel, R.M., and Hengartner, H. (1991). Lower receptor avidity required for thymic clonal deletion than for effector T cell function. *Nature 351*, 482–485.

Posselt, A.M., Barker, C.F., Friedman, A.L., and Naji, A. (1992). Prevention of autoimmune diabetes in the BB rat by intrathymic islet transplantation at birth. *Science 256*, 1321–1324.

Pugliese, A., Zeller, M., Fernandez, A., Jr., Zalcberg, L.J., Bartlett, R.J., Ricordi, C., Pietropaolo, M., Eisenbarth, G.S., Bennett, S.T., and Patel, D.D. (1997). The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type I diabetes. *Nat. Genet. 15*, 293–297.

Rammensee, H.-G., Kroschewski, R., and Frangoulis, B. (1989). Clonal anergy induced in mature $V\beta6^+$ T lymphocytes on immunizing $Mls\text{-}1^b$ mice with $Mls\text{-}1^a$ expressing cells. *Nature 339*, 541–544.

Ramsdell, F. and Fowlkes, B.J. (1992). Maintenance of *in vivo* tolerance by persistence of antigen. *Science 257*, 1130–1134.

Ramsdell, F., Lantz, T., and Fowlkes, B.J. (1989). A nondeletional mechanism of thymic self tolerance. *Science 246*, 1038–1041.

Ramsey, C., Winqvist, O., Puhakka, L., Halonen, M., Moro, A., Kämpe, O., Eskelin, P., Pelto-Huikko, M., and Peltonen, L. (2002). Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum. Mol. Genet. 11*, 397–409.

Read, S., Mauze, S., Assmann, C., Coffman, R., and Powrie, F. (1998). $CD38^+$ $CD45Rb^{low}$ $CD4^+$ T cells: a T cell population with immune regulatory activities *in vitro*. *Eur. J. Immunol. 28*, 3435–3447.

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.

Reich, A., Korner, H., Sedgwick, J.D., and Pircher, H. (2000). Immune down-regulation and peripheral deletion of CD8 T cells does not require TNF receptor–ligand interactions nor CD95. *Eur. J. Immunol. 30*, 678–682.

Rellahan, B.L., Jones, L.A., Kruisbeek, A.M., Fry, A.M., and Matis, L.A. (1990). *In vivo* induction of anergy in peripheral V beta 8+ T cells by staphylococcal enterotoxin B. *J. Exp. Med. 172*, 1091–1100.

Rieux-Laucat, F., Le Deist, F., Hivroz, C., Roberts, I.A.G., Debatin, K.M., Fischer, A., and de Villartay, J.P. (1995). Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science 268*, 1347–1349.

Rocha, B., Tanchot, C., and von Boehmer, H. (1993). Clonal anergy blocks *in vivo* growth of mature T cells and can be reversed in the absence of antigen. *J. Exp. Med. 177*, 1517–1521.

Rocha, B. and von Boehmer, H. (1991). Peripheral selection of the T cell repertoire. *Science 251*, 1225–1228.

Russell, J.H., Rush, B., Weaver, C., and Wang, R. (1993). Mature T cells of autoimmune lpr/lpr mice have a defect in antigen-stimulated suicide. *Proc. Natl. Acad. Sci. USA 90*, 4409–4413.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.

Sakaguchi, S., Sakaguchi, N., Asono, M., Itoh, M., and Toda, M. (1995). Immunological self-tolerance maintained by activated T cell expressing IL-2 receptor alpha-chain [CD25]. Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155*, 1151–1164.

Scheid, M.P. and Woodgett, J.R. (2001). PKB/AKT: functional insights from genetic models. *Nat. Rev. Mol. Cell. Biol. 2*, 760–768.

Schluns, K.S., Kieper, W.C., Jameson, S.C., and Lefrançois, L. (2000). Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. *Nat. Immunol. 1*, 426–432.

Schwartz, R.H. (1990). A cell culture model for T lymphocyte clonal anergy. *Science 248*, 1349–1356.

Schwartz, R.H. (1992). Costimulation of T lymphocytes: The role of CD28, CTLA-4 and B7/BB1 in interleukin-2 production and immunotherapy. *Cell 71*, 1065–1068.

Schwartz, R.H. (2003). T cell anergy. *Annu. Rev. Immunol. 21*, 305–334.

Schwartzberg, P.L. (2001). Immunology. Tampering with the immune system. *Science 293*, 228–229.

Scott, R.S., McMahon, E.J., Pop, S.M., Reap, E.A., Caricchie, R., Cohen, P.L., Earp, N.S., and Matsushima, G.K. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature 411*, 207–211.

Sebzda, E., Kündig, T.M., Thomson, C.T., Aoki, K., Mak, S.Y., Mayer, J., Zamborelli, T.M., Nathenson, S., and Ohashi, P.S. (1996). Mature T cell reactivity altered by a peptide agonist that induces positive selection. *J. Exp. Med. 183*, 1093–1104.

Singer, G.G. and Abbas, A.K. (1994). The Fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity 1*, 365–371.

Smith, K.M., Olson, D.C., Hirose, R., and Hanahan, D. (1997). Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance. *Int. Immunol. 9*, 1355–1365.

Sotomayor, E.M., Borrello, I., Tubb, E., Rattis, F.M., Bien, H., Lu, Z., Schoenberger, S., and Levitsky, H.I. (1999). Conversion of tumor-specific $CD4^+$ T-cell tolerance to T-cell priming through *in vivo* ligation of CD40. *Nat. Med. 5*, 780–787.

Speiser, D.E., Chvatchko, Y., Zinkernagel, R.M., and MacDonald, H.R. (1990). Distinct fates of self specific T cells developing in irradiation bone marrow chimeras: Clonal deletion, clonal anergy or *in vitro* responsiveness to self Mls-1a controlled by hemopoietic cells in the thymus. *J. Exp. Med. 172*, 1305–1314.

Starr, R., Metcalf, D., Elefanty, A.G., Brysha, M., Willson, T.A., Nicola, N.A., Hilton, D.J., and Alexander, W.S. (1998). Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. *Proc. Natl. Acad. Sci. USA 95*, 14395–14399.

Starr, T.K., Jameson, S.C., and Hogquist, K.A. (2003). Positive and negative selection of T cells. *Annu. Rev. Immunol. 21*, 139–176.

Steere, A.C. and Glickstein, L. (2004). Elucidation of Lyme arthritis. *Nat. Rev. Immunol. 4*, 143–152.

Stefanova, I., Hemmer, B., Vergelli, M., Martin, R., Biddison, W.E., and Germain, R.N. (2003). TCR ligand discrimination is enforced by competing ERK positive and SHP-1 negative feedback pathways. *Nat. Immunol. 4*, 248–254.

Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. (2003). Tolerogenic dendritic cells. *Annu. Rev. Immunol. 21*, 685–711.

Steinman, R.M. and Nussenzweig, M.C. (2002). Avoiding horror autotoxicus: The importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA 99*, 351–358.

Suematsu, S., Matsuda, T., Aozasa, K., Akira, S., Nakano, N., Ohno, S., Miyazaki, J.-I., Yamamura, K.-I., Hirano, T., and Kishimoto, T. (1989). IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc. Natl. Acad. Sci. USA 86*, 7547–7551.

Sytwu, H.-K., Liblau, R.L., 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*, 17–30.

Takahashi, T., Kuniyasu, Y., Toda, M., Sakaguchi, N., Itoh, M., Iwata, M., Shimizu, J., and Sakaguchi, S. (1998). Immunologic self-tolerance maintained by $CD25^+CD4^+$ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol. 10*, 1969–1980.

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.

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.

Takeda, K., Clausen, B.E., Kaisho, T., Tsujimura, T., Terada, N., Forster, I., and Akira, S. (1999). Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity 10,* 39–49.

Takeda, K., Kaisho, T., and Akira, S. (2003). Toll-like receptors. *Annu. Rev. Immunol. 21*, 335–376.

Takeda, S., Rodewald, H.-R., Arakawa, H., Bluethmann, H., and Shimizu, T. (1996). MHC class II molecules are not required for survival of newly generated $CD4^+$ T cells, but affect their long-term life span. *Immunity 5*, 217–228.

Tan, J.T., Dudl, E., LeRoy, E., Murray, R., Sprent, J., Weinberg, K.I., and Surh, C.D. (2001). IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc. Natl. Acad. Sci. USA 98*, 8732–8737.

Tanchot, C., Lemonnier, F.A., Pérarnau, B., Freitas, A.A., and Rocha, B. (1997). Differential requirements for survival and proliferation of CD8 naïve or memory T cells. *Science 276*, 2057–2062.

Teague, T.K., Marrack, P., Kappler, J.W., and Vella, A.T. (1997). IL-6 rescues resting mouse T cells from apoptosis. *J. Immunol. 158*, 5791–5796.

Teh, H.S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Blüthmann, H., and von Boehmer, H. (1988). Thymic major histocompatibility complex antigens and the $\alpha\beta$ T cell receptor determine the CD4/CD8 phenotype of T cells. *Nature 335*, 229–233.

The Finnish-German APECED Consortium (1997). An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet. 17*, 399–403.

Thornton, A.M. and Shevach, E.M. (1998). $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J. Exp. Med. 188*, 287–296.

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.

Trollmo, C., Meyer, A.L., Steere, A.C., Hafler, D.A., and Huber, B.T. (2001). Molecular mimicry in Lyme arthritis demonstrated at the single cell level: LFA-1 alpha L is a partial agonist for outer surface protein A-reactive T cells. *J. Immunol. 166*, 5286–5291.

Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., Rainbow, D.B., Hunter, K.M., Smith, A.N., Di Genova, G., Herr, M.H., Dahlman, I., Payne, F., Smyth, D., Lowe, C., Twells, R.C., Howlett, S., Healy, B., Nutland, S., Rance, H.E., Everett, V., Smink, L.J., Lam, A.C., Cordell, H.J., Walker, N.M., Bordin, C., Hulme, J., Motzo, C., Cucca, F., Hess, J.F., Metzker, M.L., Rogers, J., Gregory, S., Allahabadia, A., Nithiyananthan, R., Tuomilehto-Wolf, E., Tuomilehto, J., Bingley, P., Gillespie, K.M., Undlien, D.E., Ronningen, K.S., Guja, C., Ionescu-Tirgoviste, C., Savage, D.A., Maxwell, A.P., Carson, D.J., Patterson, C.C., Franklyn, J.A., Clayton, D.G., Peterson, L.B., Wicker, L.S., Todd, J.A., and Gough, S.C. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423, 506–511.

Uehira, M., Matsuda, H., Hikita, I., Sakata, T., Fujiwara, H., and Nishimoto, H. (1993). The development of dermatitis infiltrated by gamma delta T cells in IL-7 transgenic mice. *Int. Immunol. 5*, 1619–1627.

Vafiadis, P., Bennett, S.T., Todd, J.A., Nadeau, J., Grabs, R., Goodyer, C.G., Wickramasinghe, S., Colle, E., and Polychronakos, C. (1997). Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat. Genet. 15*, 289–292.

van Santen, H.M., Benoist, C., and Mathis, D. (2004). Number of T reg cells that differentiate does not increase upon encounter of agonist ligand on thymic epithelial cells. *J. Exp. Med. 200*, 1221–1230.

Vasquez, N., Kaye, J., and Hedrick, S.M. (1992). *In vivo* and *in vitro* clonal deletion of double-positive thymocytes. *J. Exp. Med. 175*, 1307–1316.

Vella, A., Teague, T.K., Ihle, J., Kappler, J., and Marrack, P. (1997a). Interleukin 4 (IL-4) or IL-7 prevents the death of resting T cells: stat6 is probably not required for the effect of IL-4. *J. Exp. Med. 186*, 325–330.

Vella, A.T., Mitchell, T., Groth, B., Linsley, P.S., Green, J.M., Thompson, C.B., Kappler, J.W., and Marrack, P. (1997b). CD28 engagement and proinflammatory cytokines contribute to T cell expansion and long-term survival *in vivo*. *J. Immunol. 158*, 4714–4720.

Verdaguer, J., Schmidt, D., Amrani, A., Anderson, B., Averill, N., and Santamaria, P. (1997). Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. *J. Exp. Med. 186*, 1663–1676.

Vijayakrishnan, L., Slavik, J.M., Illes, Z., Greenwald, R.J., Rainbow, D., Greve, B., Peterson, L.B., Hafler, D.A., Freeman, G.J., Sharpe, A.H., Wicker, L.S., and Kuchroo, V.K. (2004). An autoimmune disease-associated CTLA-4 splice variant lacking the B7 binding domain signals negatively in T cells. *Immunity 20*, 563–575.

von Herrath, M.G., Dockter, J., and Oldstone, M.B.A. (1994). How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. *Immunity* 1, 231–242.

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.

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*, 985–988.

Webb, S., Morris, C., and Sprent, J. (1990). Extrathymic tolerance of mature T cells: Clonal elimination as a consequence of immunity. *Cell 63*, 1249–1256.

Welte, T., Zhang, S.S., Wang, T., Zhang, Z., Hesslein, D.G., Yin, Z., Kano, A., Iwamoto, Y., Li, E., Craft, J.E., Bothwell, A.L., Fikrig, E., Koni, P.A., Flavell, R.A., and Fu, X.Y. (2003). STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. *Proc. Natl. Acad. Sci. USA 100*, 1879–1884.

Wildin, R.S., Ramsdell, F., Peake, M., Faravelli, F., Casanova, J.-L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., Perroni, L., Bricarelli, F.D., Byrne, G., McEuen, M., Proll, S., Appleby, M., and Brunkow, M.E. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet. 27*, 18–20.

Wong, F.S., Karttunen, J., Dumont, C., Wen, L., Visintin, I., Pilip, I.M., Shastri, N., Pamer, E.G., and Janeway Jr, C.A. (1999). Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. *Nat. Med. 5*, 1026–1031.

Yagi, J. and Janeway, C.A. (1990). Ligand thresholds at different stages of T cell development. *Int. Immunol. 2*, 83–89.

Yokoi, N., Komeda, K., Wang, H.-Y., Yano, H., Kitada, K., Saitoh, Y., Seino, Y., Yasuda, K., Serikawa, T., and Seino, S. (2002). *Cblb* is a major susceptibility gene for rat type 1 diabetes mellitus. *Nat. Genet. 31*, 391–394.

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*, 878–883.

Zhao, Z., Granucci, E., Yeh, I., Schaffer, P., and Cantor, H. (1998). Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science 279*, 1344.

Zhou, T., Bluethmann, H., Zhang, J., Edwards III, C.K., and Mountz, J.D. (1992). Defective maintenance of T cell tolerance to a superantigen in MRL-*lpr/lpr* mice. *J. Exp. Med. 176*, 1063–1072.

CHAPTER

9

Regulatory T Cells

SIMON READ, KEVIN J. MALOY, AND FIONA POWRIE
Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Over the past three decades, advances in our understanding of how lymphocytes are activated at the molecular level have contributed to the discovery of several essential mechanisms involved in the maintenance of self-tolerance. These include clonal deletion of self-reactive immature lymphocytes during their development in the thymus and bone marrow (Kappler et al., 1987; von Boehmer, 1988; Cornall et al., 1995), as well as the deletion or functional inactivation (clonal anergy) of naïve lymphocytes in the periphery (Goodnow et al., 1989; Russell et al., 1991; Schwartz, 2003). While these recessive mechanisms are efficient, autoreactive T cells clearly constitute part of the normal T-cell repertoire and the occurrence of autoimmune disease is evidence of their pathogenic potential. More recently, it has become clear that dominant suppressive mechanisms also play an essential role in maintaining self-tolerance and controlling immune pathology, and that specialized populations of regulatory T cells (Tregs) are responsible for this activity (Piccirillo and Shevach, 2004; Sakaguchi, 2004). In this chapter, we describe a number of regulatory T cell types ($CD4^+CD25^+$, Tr1, Th3, NKT, and $CD8^+$) but with particular emphasis on $CD4^+CD25^+$ Treg cells.

Although the concept of T-cell-mediated suppression was first proposed over 30 years ago (Gershon and Kondo, 1970), it is only over the past decade that conclusive evidence has emerged to confirm the existence of a distinct T-cell phenotype. As will be discussed below, several complementary findings triggered this renaissance in Treg biology. Furthermore, reconstitution of immune-deficient mice with populations of normal T cells that lacked Tregs led to the development of a range of autoimmune and inflammatory pathologies (Sakaguchi et al., 1985; Powrie and Mason, 1990; Morrissey et al., 1993; Powrie et al., 1993). Second, disease development in these models was inhibited by reconstitution with Tregs from normal animals—a population that functionally resides primarily within the $CD4^+CD25^+$ T-cell fraction—the "naturally occurring" $CD4^+CD25^+$ Tregs (Read et al., 2000; Sakaguchi et al., 1995). Lastly, the identification of a transcription factor, Foxp3, which is expressed specifically in $CD4^+CD25^+$ Tregs, has been shown to play an indispensable role in their development (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). Mutations in the human Foxp3 gene were shown to be the underlying cause of an X-linked immunodeficiency syndrome, the immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome (Bennett et al., 2001; Wildin et al., 2001), a fatal inflammatory disease characterized by multiple autoimmune disorders, allergies, and inflammatory bowel disease; the same spectrum of diseases is found in mice lacking a functional Foxp3 gene and therefore $CD4^+CD25^+$ Tregs (Fontenot et al., 2003; Khattri et al., 2003). Together,

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

these studies demonstrate that expression of Foxp3 is critical for the development of the unique regulatory phenotype of $CD4^+CD25^+$ Tregs. As outlined below, these cells can suppress a variety of experimental autoimmune diseases. In fact, they have been shown to impact on almost every type of effector immune response, from the suppression of allograft rejection to impeding antitumor immunity, from inhibiting the harmful immune pathology that accompanies many antimicrobial responses to augmenting T-cell memory in others [reviewed in Maloy and Powrie (2001), Sakaguchi (2004), and Shevach (2002)].

In this chapter we focus primarily on naturally occurring $CD4^+CD25^+$ Tregs, as their nonredundant role in maintaining self-tolerance has been the most clearly established. However, it should be noted that several other populations of $CD4^+$ T cells have been described to possess regulatory activity and can be exploited therapeutically. These include natural killer T (NKT) cells that also arise naturally during development, as well as "induced" $CD4^+$ Tregs that develop following stimulation of peripheral T cells. This chapter will cover the role of these additional Treg populations in autoimmunity; however, as the relationship between $CD4^+CD25^+$ Tregs and other Treg populations is still unclear, the reader is referred to more specialized reviews for a full description of their biology (Roncarolo et al., 2003; Godfrey and Kronenberg, 2004; Mills, 2004).

NATURALLY OCCURRING REGULATORY T CELLS

Ontogeny

One important characteristic of $CD4^+CD25^+$ Tregs is that, in contrast to conventional naïve $CD4^+$ T cells, they complete their functional maturation within the thymus and are exported as bona fide Tregs (Itoh et al., 1999). The production of differentiated $CD4^+CD25^+$ Tregs, which control potentially pathogenic self-reactive T cells that have escaped negative selection, has been described as the third function of the thymus (Seddon and Mason, 2000; Apostolou et al., 2002) (see Chapter 10). A number of studies have shown that $CD4^+CD8^-CD25^+$ thymocytes possess identical phenotypic and functional characteristics to peripheral $CD4^+CD25^+$ Tregs (Papiernik et al., 1998; Itoh et al., 1999; Stephens and Mason, 2000), including high levels of expression of the transcription factor Foxp3 (Hori et al., 2003). $CD4^+CD25^+$ Tregs do not develop in Foxp3-deficient mice (Fontenot et al., 2003), and retroviral transduction of Foxp3 into $CD4^+CD25^-$ T cells converts them into functional $CD4^+CD25^+$ Tregs (Hori et al., 2003). Studies with transgenic mice have shed some light on the unique thymic ontogeny of $CD4^+CD25^+$ Tregs. For example, enhanced development of $CD4^+CD25^+$ Tregs has been observed in transgenic mice expressing both a high-affinity transgenic T-cell receptor (TCR) and the peptide ligand recognized by that TCR on thymic stromal cells (Jordan et al., 2000; Kawahata et al., 2002). $CD4^+CD25^+$ Tregs did not develop when the affinity of the TCR was reduced or the transgenic peptide was expressed at a very high level, indicating that they are subject to positive and negative selection processes. Furthermore, the development of $CD4^+CD25^+$ Tregs was dependent on the expression of major histocompatibility complex (MHC) class II and the agonist peptide by thymic cortical epithelial cells (Bensinger et al., 2001). Taken together, these findings suggest that $CD4^+CD25^+$ Tregs develop as a result of a relatively high-avidity interaction of the TCR with self-peptide–MHC II complexes expressed by thymic cortical epithelial cells.

Further evidence that $CD4^+CD25^+$ Tregs exhibit enhanced self-reactivity was recently obtained through analysis of TCR-α gene rearrangements. This revealed that the $CD4^+CD25^+$ Treg subset possesses an equally diverse, but largely distinct, TCR repertoire to that of the $CD4^+CD25^-$ T cells, and that the former contains a high frequency of TCRs with a higher avidity for self-peptide–MHC II complexes (Hsieh et al., 2004). In addition to the higher avidity of $CD4^+CD25^+$ Tregs for self-peptide–MHCII complexes, these cells are able to mediate suppression in response to a lower concentration of agonist peptide than is required for activation of conventional T cells (Takahashi et al., 1998), thus providing a potent mechanism through which dominant tolerance towards self-antigens may be maintained.

While the thymus may be the primary site of $Foxp3^+$ Treg development, there is evidence that $CD4^+CD25^+Foxp3^+$ Tregs can develop from naïve peripheral $CD4^+CD25^-$ T cells (Thorstenson and Khoruts, 2001; Apostolou et al., 2002). Such conversion has been achieved through prolonged systemic infusion of agonist peptide *in vivo* (Apostolou and von Boehmer, 2004), as well as by activation in the presence of high levels of tumor growth factor (TGF)-β *in vitro* (Chen et al., 2003). Although the physiologic significance of this pathway remains to be established, it does offer the opportunity to generate antigen-specific $Foxp3^+$ Tregs that can be used in therapeutic approaches for autoimmune and inflammatory disease.

Functional Characteristics

Markers

Naturally occurring Tregs are enriched within the 5–10% of peripheral $CD4^+$ T cells in mice and humans who express CD25. Although a useful marker of Treg activity, CD25 expression is not specific, as many T cells upregulate CD25 following activation but do not express Foxp3. Conversely, cells expressing Foxp3 mRNA can also be detected in the $CD4^+CD45RB^{low}CD25^-$ pool (Hori et al., 2003), which may

explain the described Treg activity of this population (Olivares-Villagomez et al., 2000; Stephens and Mason, 2000). Like CD25, several other activation-associated surface molecules are expressed by Tregs, such as glucocorticoid-induced TNFR family-related receptor (GITR), CTLA4, and CD103, and these have been linked with Treg function (Read et al., 2000; Takahashi et al., 2000; Lehmann et al., 2002; Shimizu et al., 2002; Banz et al., 2003; Stephens et al., 2004;). However, like CD25, expression is not restricted to Tregs and is probably more related to the activation state of the cell. Currently, Foxp3 expression is used as the definitive marker of naturally occurring Tregs; however, as its expression is confined to the nucleus, it is of limited use for the isolation of Tregs. Identification of cell-surface proteins whose expression is linked to Foxp3 may identify more specific markers of naturally arising Tregs.

Activation and Costimulation

$CD4^+CD25^+$ Tregs exhibit an anergic phenotype *in vitro*, failing to proliferate or produce interleukin (IL)-2 following polyclonal or antigen-specific stimulation, although they can mount vigorous antigen-dependent proliferative responses *in vivo* (Klein et al., 2003; Walker et al., 2003). The T-cell growth factor IL-2 appears to play a crucial role in $CD4^+CD25^+$ Treg development and peripheral activity (Furtado et al., 2002; Malek and Bayer, 2004). Mice genetically deficient in IL-2, IL-2Rα or IL-2Rβ, have reduced numbers of $CD4^+CD25^+$ Tregs in the thymus and periphery, and develop fatal autoimmune and inflammatory disease. The inflammatory syndrome in these mice can be inhibited by restoration of normal $CD4^+CD25^+$ Treg numbers (Almeida et al., 2002; Malek et al., 2002). In addition to effects on the development and maintenance of $CD4^+CD25^+$ Tregs, IL-2 is also required for Treg cell activation and clonal expansion (Thornton et al., 2004; Setoguchi et al., 2005). The latter may play a key role in Treg function *in vivo* as suppression of autoimmune and inflammatory disease involves extensive proliferation of Tregs in the secondary lymphoid organs as well as locally in the inflamed tissue (Mottet et al., 2003; Peng et al., 2004). Even in the steady-state, a proportion of $CD4^+CD25^+$ Tregs are continually proliferating (Fisson et al., 2003; Klein et al., 2003). This physiologic proliferation, presumably in response to self-antigens, may be a crucial part of $CD4^+CD25^+$ Treg-mediated dominant tolerance as treatment of normal mice with a neutralizing anti-IL-2 monoclonal antibody (mAb) prevents $CD4^+CD25^+$ Treg proliferation, leading to a reduction in their number, and development of organ-specific autoimmune disease (Setoguchi et al., 2005). IL-2 is produced primarily by activated $CD4^+CD25^-$ cells, suggesting that $CD4^+CD25^+$ Treg numbers may be linked to the level of T-cell activation, providing a feedback mechanism that ensures an appropriate balance of regulatory and effector cell responses.

A reduction in CD28 signaling, either as a result of CD28 or CD80 (B7) deficiency, also leads to reduced numbers of $CD4^+CD25^+$ Tregs both in the thymus and periphery (Salomon et al., 2000). As CD28 costimulation induces IL-2 secretion by $CD4^+CD25^-$ cells, it is possible that the effects of CD28 are mediated via IL-2. However, high levels of CD28 costimulation abrogate suppression *in vitro* (Takahashi et al., 1998; Thornton and Shevach, 1998), suggesting that the level of CD28 signalling dictates $CD4^+CD25^+$ Treg function. CTLA4, which negatively regulates TCR/CD28-mediated signaling in T cells, is also involved in $CD4^+CD25^+$ Treg function as anti-CTLA4 mAb inhibits Treg activity *in vitro* and *in vivo* (Takahashi et al., 1998; Read et al., 2000). CTLA4 is expressed by a large proportion of $CD4^+CD25^+$ Tregs, as well as by activated $CD4^+CD25^-$ cells. As such, the effects of CTLA4 blockade may be to enhance effector responses, rendering them more resistant to suppression. Alternatively, CTLA4 signaling in $CD4^+CD25^+$ Tregs may play a functional role in their activation. Recently, CTLA4 binding to CD80 (B7) on dendritic cells (DCs) has been shown to induce indoleamine 2,3-dioxygenase, leading to local immune suppression as a consequence of tryptophan depletion and production of proapoptotic metabolites (Grohmann et al., 2002; Fallarino et al., 2003; Mellor and Munn, 2004). These results raise the possibility that CTLA4 acts as a direct mediator of $CD4^+CD25^+$ Treg suppression via the induction of an local immune suppressive pathway in DCs.

Costimulatory signals may also influence the susceptibility of $CD4^+CD25^-$ cells to suppression. Anti-GITR mAb abrogates $CD4^+CD25^+$ Treg suppression *in vitro* and induces organ-specific autoimmunity when administered to young BALB/c mice (Shimizu et al., 2002). Like CTLA4, GITR is expressed by a proportion of $CD4^+CD25^+$ Tregs, as well as by activated $CD4^+CD25^-$ T cells (McHugh et al., 2002; Shimizu et al., 2002; Tone et al., 2003). More recent studies suggest that anti-GITR mAb delivers a costimulatory signal to the effector cells, rendering them resistant to suppression (Stephens et al., 2004). $CD4^+CD25^+$ Treg activity can also be controlled by activation of the Toll-like receptor (TLR) pathway, which is involved in the detection of microbial infection and innate immune activation (Takeda et al., 2003). TLR-induced IL-6 production by activated DCs renders T cells resistant to suppression, providing a mechanism by which infection can overcome dominant $CD4^+CD25^+$ Treg activity (Pasare and Medzhitov, 2003). It has also been reported that $CD4^+CD25^+$ Tregs can express TLRs, raising the possibility that they respond directly to infection (Caramalho et al., 2003).

Together the data suggest that there is a dynamic equilibrium between effector and regulatory T cells that is dictated primarily by the activation status of the local

antigen-presenting cell (APC) population. Such a mechanism ensures that under physiologic conditions Treg responses will dominate, preventing the activation of low-affinity self-reactive T cells and development of autoimmunity. However, following infection, raised levels of costimulatory signals will override Treg activity, allowing the development of protective immune responses to microbial antigens.

Mechanism of Suppression

A cardinal feature of $CD4^+CD25^+$ Tregs is their ability to suppress a range of different responses mediated by both innate and adaptive immune systems [reviewed in Maloy and Powrie (2001), Sakaguchi (2004), and Shevach (2002)]. This activity is also apparent *in vitro*, as in co-cultures $CD4^+CD25^+$ Tregs suppress the activation of both $CD4^+$ and $CD8^+$ T cells in a dose-dependent manner (Thornton and Shevach, 1998; Takahashi et al., 2000; Piccirillo and Shevach, 2001). Suppression requires direct contact between responding and regulatory T cells, and leads to a block in IL-2 synthesis by the target T cells. Induction of suppression requires TCR-mediated activation and IL-2; however, once triggered, suppression operates in an antigen nonspecific manner allowing for bystander suppression (Thornton and Shevach, 2000). The molecular mechanism of *in vitro* suppression is poorly understood; there is evidence that membrane-bound TGF-β may play a role, although this is not an absolute requirement (Nakamura et al., 2001; Piccirillo et al., 2002). Suppression of antigen-specific T cells can also be visualized *in vivo*. In adoptive transfer of mixtures of antigen-specific clonally-related $CD4^+CD25^+$ and $CD4^+CD25^-$ cells, the former were found not to suppress the initial priming of the latter but did prevent their subsequent clonal expansion (Klein et al., 2003). These results suggest $CD4^+CD25^+$ Tregs do not prevent initial T-cell activation but act to inhibit a sustained response.

In contrast to *in vitro* suppression, there is good evidence that the immune regulatory cytokines IL-10 and TGF-β play a role in $CD4^+CD25^+$ Treg-mediated suppression *in vivo*. However, the requirement for one or both of these cytokines depends on the model system (Table 9.1). Thus, prevention and cure of colitis is functionally dependent on both IL-10 and TGF-β (Read et al., 2000; Asseman et al., 2003; Mottet et al., 2003). The latter also plays a role in the prevention of diabetes (Belghith et al., 2003), but neither cytokine appears to be required for suppression of gastritis (Thornton and Shevach, 1998; Suri-Payer and Cantor, 2001; Piccirillo et al., 2002). Together the data suggest that the nature and anatomic location of the inflammatory response dictate the mechanisms of Treg-mediated control. Thus, the requirement for IL-10 in Treg-mediated control of colitis may reflect the need to suppress the innate immune response that is a prominent feature of intestinal inflammation (Maloy et al., 2003). Further evidence that control of the innate immune response by IL-10 is important in intestinal homeostasis comes from the finding that selective disruption of IL-10 signaling in myeloid cells and neutrophils is sufficient to trigger colitis (Takeda et al., 1999).

In contrast to IL-10, TGF-β plays a more general role in immune homeostasis as TGF-$\beta1^{-/-}$ mice develop multiple organ inflammatory disease early after birth (Shull et al., 1992; Kulkarni et al., 1993). TGF-β may contribute to Treg activity in several ways; in models of diabetes (Green et al., 2003) and colitis (Fahlen et al., 2005), pathogenic T cells

TABLE 9.1 Requirement for cytokines in regulatory T-cell (Treg)-mediated suppression of autoimmune disease

	IL-10	TGF-β	References
$CD4^+CD25^+$ Tregs			
Gastritis	7✓	7✓	Thornton and Shevach (1998), Suri-Payer and Cantor (2001), Piccirillo et al. (2002)
Colitis			
Prevention	3×/7✓	3×	Read et al. (2000), Annacker et al. (2003), Asseman et al. (2003)
Cure	3×	3×	Liu et al. (2003), Mottet et al. (2003)
Type 1 Diabetes		3×	Belghith et al. (2003)
Experimental autoimmune encephalomyelitis (EAE)	3×		Zhang et al. (2004)
Tr1 cells			
Colitis: prevention and cure	3×		Foussat et al. (2003)
EAE	3×		Barrat et al. (2002)
Th3 cells			
EAE		3×	Chen et al. (1994)
$Va14^+$ natural killer T cells			
Type 1 Diabetes	3× + IL-4		Sharif et al. (2001)
EAE	3× + IL-4		Singh et al. (2001)

that express a dominant negative TGF-β receptor RII (dnTGF-βRII) escape control by $CD4^+CD25^+$ T cells, indicating that TGF-β can act directly on T cells to suppress their effector function. $CD4^+CD25^+$ Tregs can express TGF-β on their surface and may focus it for presentation to effector T cells (Nakamura et al., 2001). However, there is conflicting data on whether Treg activity is dependent on TGF-β1 synthesis by $CD4^+CD25^+$ Tregs themselves (Nakamura et al., 2004; Fahlen et al., 2005). There is also emerging evidence that TGF-β can mediate positive effects on $CD4^+CD25^+$ Tregs, leading to their accumulation upon stimulation *in vitro* (Yamagiwa et al., 2001; Chen et al., 2003). This may also occur *in vivo*, as transient expression of TGF-β in the islets during diabetes inhibits disease through local expansion of Foxp3-expressing $CD4^+CD25^+$ Tregs (Peng et al., 2004).

OTHER REGULATORY T-CELL TYPES

A number of other T-cell populations have been shown to exhibit regulatory activity under some circumstances (Figure 9.1). These include other cell types that arise naturally during thymic development, like NKT cells, as well as several populations of inducible Tregs that are generated from naïve peripheral T cells.

Induced $CD4^+$ Regulatory T Cells

Naïve T cells can also be induced to differentiate into Tregs following encounter with antigen in the periphery. These induced Tregs are distinct from $CD4^+CD25^+$ Tregs in that they do not express Foxp3 and act primarily by the production of immune suppressive cytokines, such as IL-10 and TGF-β (Vieira et al., 2004). Different subsets of induced Tregs have been identified, including type 3 T-helper (Th3) and Tr1 cells, which are distinguished by their production of TGF-β and IL-10, respectively. Mucosal administration of intact proteins can induce TGF-β-secreting Th3 cells (Chen et al., 1994; Fuss et al., 2002). Tr1 cells were first generated *in vitro*; however, there is now evidence that antigen presentation by immature DCs and/or environments where high levels of immune suppressive molecules are present, such as at mucosal surfaces, can induce cells of a similar phenotype *in vivo* (Groux et al., 1997; Burkhart et al., 1999; Barrat et al., 2002; Cong et al., 2002). Induced Tr1-like cells can also develop during normal immune responses to infectious agents, especially those that cause

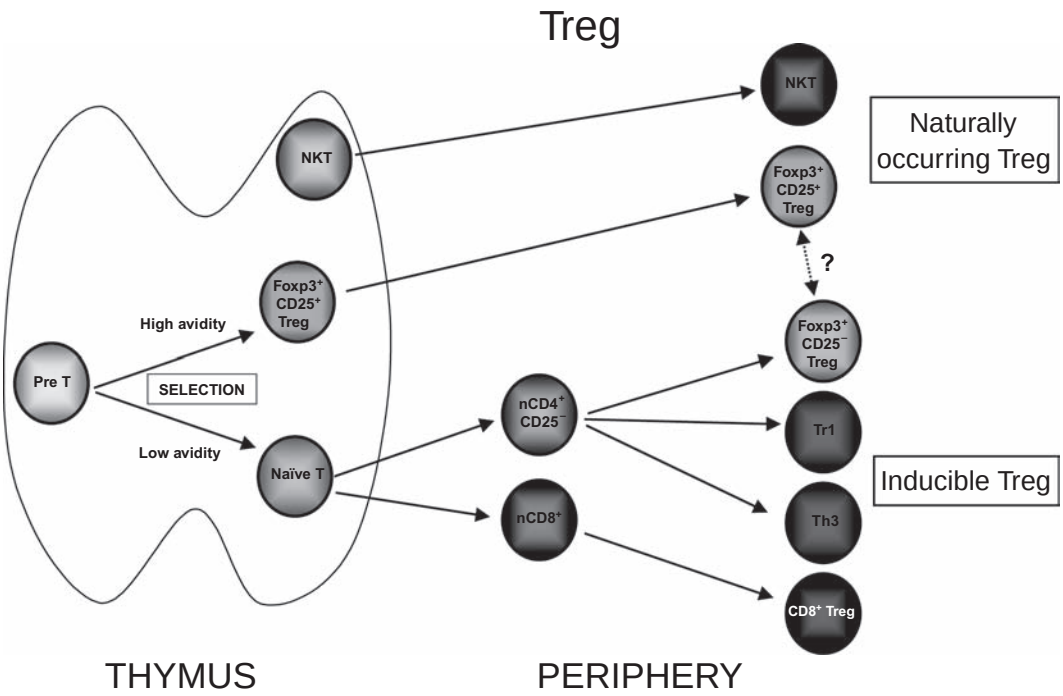


FIGURE 9.1 Major populations of regulatory T cells (Tregs). Naturally occurring Tregs ($CD4^+CD25^+$ T cells and natural killer T cells) arise during normal thymic development and do not require further differentiation in the periphery in order to mediate regulatory function. By contrast, inducible Tregs are exported from the thymus as naïve uncommitted T cells and acquire Treg function following activation and differentiation in the periphery. The precise interrelationships between the different Treg populations have not yet been fully defined.

persistent infections, and probably represent a host mechanism to limit pathology occurring as a consequence of chronic immune stimulation (Belkaid et al., 2002; Kullberg et al., 2002; Mills, 2004; Thompson and Powrie, 2004).

While the primary function of induced Tregs may not be to maintain peripheral tolerance, there is good evidence that they can be exploited therapeutically to prevent autoimmune and inflammatory diseases, where they offer the possibility of antigen-specific interventions. In autoimmune disease, Tregs responding to self-antigen have been shown to home specifically to sites where antigen is expressed and to suppress the inflammatory response via production of IL-10 and TGF-β (Barrat et al., 2002; Foussat et al., 2003). Furthermore, it may not be necessary to generate Tregs responding to all autoantigens targeted in a particular autoimmune disease. Rather it may be sufficient to generate Tregs to a single autoantigen as these cells should inhibit the response to other antigens expressed at the inflammatory site by bystander suppression.

Although the ontogeny of $CD4^+CD25^+$ Tregs and induced Tregs are different, their regulatory activity shares similar features. Indeed, recent findings suggest that the activity of these populations may be linked as $CD4^+CD25^+$ Tregs were able to induce the differentiation of cytokine-producing Tregs from $CD4^+CD25^-$ progeny *in vitro* (Dieckmann et al., 2002; Jonuleit et al., 2002; Zheng et al., 2004). While the significance of this *in vivo* has yet to be established, it would provide a mechanism to amplify the regulatory response.

$CD8^+$ T Cells

Recent evidence suggests that $CD8^+$ T cells may also mediate immune regulatory functions as several experimental systems have been described in which $CD8^+$ T cells can acquire Treg function [reviewed in Filaci and Suciu-Foca (2002) and Jiang and Chess (2000)]. These include repetitive stimulation with allogeneic APCs (Dhodapkar and Steinman, 2002; Gilliet and Liu, 2002) or presentation of self-peptides by the nonclassical MHC molecule Qa-1 (HLA-E) (Jiang et al., 1998; Sarantopoulos et al., 2004). $CD8^+$ Tregs have been implicated in the control of various experimental pathologies, including experimental autoimmune encephalomyelitis (EAE) (Jiang et al., 1992; Madakamutil et al., 2003; Hu et al., 2004), airway inflammation (Stock et al., 2004), and allograft rejection (Liu et al., 2001; Suciu-Foca et al., 2003). Although less well understood than their $CD4^+$ Treg counterparts, it appears that these induced $CD8^+$ Tregs may also utilize a variety of mechanisms to mediate suppression, including direct inhibition of autoreactive T-cell clones through cytolysis or cytokines (Jiang et al., 1998; Gilliet and Liu, 2002; Madakamutil et al., 2003; Sarantopoulos et al., 2004), as well as suppression of APC function via a cell-contact-dependent mechanism (Chang et al., 2002). Thus, it seems that induced $CD8^+$ Tregs may also contribute to immune regulation under certain circumstances, although further studies are required to precisely define their role within the regulatory cell network.

Natural Killer T Cells

NKT cells represent a distinct lineage of T cells (<1% of lymphocytes) usually identified by their expression of an invariant TCR (Vα14-Jα18 in mice, Vα24 in human), as well as characteristic NK cell markers. This invariant TCR does not recognize peptides presented in association with classical MHC molecules, but instead recognizes glycolipids presented by the nonclassical CD1d molecule (Jayawardena-Wolf and Bendelac, 2001; Godfrey and Kronenberg, 2004). The few NKT cell antigenic ligands thus far described include the synthetic glycolipid α-GalCer and the lysosomal glycosphingolipid iGb3 (Zhou et al., 2004).

NKT cells are best considered as a naturally occurring population of Tregs because, like $CD4^+CD25^+Foxp3^+$ Tregs, they are capable of immediate regulatory function following TCR stimulation. However, in contrast to $CD4^+CD25^+Foxp3^+$ Tregs, NKT cells are not always inhibitory—they may also act to exacerbate immune responses. This is due to their ability to rapidly produce both proinflammatory Th1 [interferon (IFN)-γ, tumor necrosis factor (TNF)] and/or Th2 (IL-4, IL-10) cytokines when activated. The regulatory functions of NKT cells have been most clearly demonstrated in studies of spontaneously diabetic nonobese diabetic (NOD) mice [reviewed in Godfrey and Kronenberg (2004)]. NOD mice have reduced numbers of NKT cells and increasing the size of this population by adoptive transfer (Hammond et al., 1998), by introduction of a Vα14-Jα18 transgene (Lehuen et al., 1998), or by repeated administration of α-GalCer (Hong et al., 2001; Sharif et al., 2001; Wang et al., 2001), all delay the onset of diabetes. Similarly, activation of NKT cells can prevent the development of pathology in models of EAE (Jahng et al., 2001; Singh et al., 2001; Furlan et al., 2003). However, it should be noted that some studies have reported conflicting results, with NKT-cell-derived IFN-γ exacerbating this disease (Jahng et al., 2001). Furthermore, a pathogenic role has also been attributed to IL-13–secreting NKT cells in a model of Th2-mediated colitis (Fuss et al., 2004; Heller et al., 2002). Thus, while it is clear that NKT cells can modulate autoimmune pathology, this may be either beneficial or detrimental for the host. The paradoxical functions of NKT cells mean that a greater understanding of the molecular signals that control their activation is necessary before their therapeutic potential can be fully realized (Mars et al., 2004).

Other Regulatory T-cell Populations

Additional minor subpopulations of cells with regulatory activity have been described. These include a population of $CD4^+DX5^+NK1.1^-$ T cells that were required to inhibit

diabetes in a transgenic model (Gonzalez et al., 2001), and DX5$^+$ NK cells that could suppress the expansion of autoreactive cytotoxic T lymphocytes (CTLs) (Lee et al., 2004). The spontaneous recovery from myelin basic protein (MBP) immunization-induced EAE in B10.PL mice was associated with the generation of CD4$^+$Vβ14$^+$ Tregs. These cells responded to a TCR Vβ8.2 peptide derived from the immunodominant MBP-reactive TCR, and were able to drive the deletion of activated Vβ8.2$^+$ MBP-reactive (pathogenic) T cells (Kumar and Sercarz, 1993; Kumar et al., 1996; Madakamutil et al., 2003). It is likely that under experimental conditions many cell types capable of producing suppressive cytokines or competing for survival factors may exhibit regulatory activity (Barthlott et al., 2003); however, they do not appear to be required for the maintenance of self-tolerance and prevention of autoimmunity in normal individuals.

CD4$^+$CD25$^+$ REGULATORY T CELLS IN AUTOIMMUNE DISEASE

Autoimmunity as a Consequence of a Lack of Regulatory T Cells

The roots of current thinking on naturally occurring Tregs go back to the 1960s and 1970s when two important observations were made. First, thymectomy at day 3 after birth was found to induce autoimmune oophoritis in mice (Nishizuka and Sakakura, 1969), and second, adult rats subjected to thymectomy followed by sublethal irradiation developed autoimmune thyroiditis (Penhale et al., 1973). In both cases, the autoimmune disease could be inhibited by infusion of normal lymphocytes, particularly CD4$^+$ T cells. In the 1980s attempts were made to identify the phenotype of these suppressive CD4$^+$ cells. Sakaguchi et al. (1985) found that following transfer of CD4$^+$ T cells depleted of the CD5^{high} fraction to BALB/c nude mice developed a wide spectrum of organ-specific autoimmune diseases, including gastritis, oophoritis, and thyroiditis. Similarly, transfer of CD4$^+$CD45RC^{high} cells to athymic nude rats in the absence of the CD45RC^{low} population, induced a multiorgan inflammatory disease (Powrie and Mason, 1990). Again, in both models, cotransfer of the depleted subpopulation was sufficient to prevent immune pathology, indicating that T cells capable of preventing destructive self-reactivity occur naturally in the CD4$^+$ T-cell pool in rodents. Transfer of CD4$^+$CD45RB^{high} T cells into severe combined immune deficient (SCID) mice induced an inflammatory bowel disease (IBD)-like syndrome as a consequence of immune recognition of the normal intestinal flora. Colitis was inhibited by infusion of CD4$^+$CD45RB^{low} cells, providing the first evidence that Tregs also control immune pathologic responses to environmental antigens (Morrissey et al., 1993; Powrie et al., 1993).

Pivotal studies by Sakaguchi et al. (1995) identified CD25 as a more specific marker for Tregs, as removal of the small number of CD25$^+$ cells from CD4$^+$ cells prior to their transfer to nude mice led to a higher incidence of autoimmune disease with a wider spectrum of affected organs than removal of CD5^{high} or CD45RB^{low} cells. Development of disease was prevented by restoring the CD4$^+$CD25$^+$ population. Suppression of colitis in the T-cell transfer model also enriches within the CD4$^+$CD25$^+$ pool (Read et al., 2000). However, it should be noted that Treg activity is not restricted to CD4$^+$CD25$^+$ cells as Tregs capable of inhibiting CD45RB^{high}-induced pathology in SCID mice and diabetes in ATX rats are also present in the CD45RB/C^{low}CD25$^-$ pool (Read et al., 2000; Stephens and Mason, 2000; Annacker et al., 2001; Alyanakian et al., 2003). Indeed, this may explain the observation that depletion of CD4$^+$CD25$^+$ Tregs in adult mice by administration of anti-CD25 mAb does not result in spontaneous autoimmune disease. By contrast, administration of anti-IL-2 mAb, which inhibits the functions of CD4$^+$CD25$^+$ and CD4$^+$CD25$^-$ Tregs, induces severe multiorgan autoimmune disease (Setoguchi et al., 2005).

Transfer of CD4$^+$CD25$^+$ Tregs also inhibits the development of organ-specific autoimmunity following day 3 thymectomy (d3Tx) (Asano et al., 1996). In this model, thymectomy between days 2 and 4 after birth is thought to prevent export of CD4$^+$CD25$^+$ Tregs from the thymus, allowing activation of autoreactive T cells in the periphery. However, a recent study has shown that adult d3Tx mice do retain at least some functional Foxp3$^+$ CD4$^+$CD25$^+$ Tregs (Dujardin et al., 2004), which may explain the finding that disease in these mice is considerably less severe than that in Foxp3$^{-/-}$ mice devoid of CD4$^+$CD25$^+$ Tregs.

Together these experiments suggest that a deficiency of Tregs is sufficient to allow activation of autoreactive T cells and induce autoimmune disease under conditions where there is presentation of physiologic amounts of self-antigen. However, it is likely that the presence of lymphopenia facilitates the development of autoimmunity in both T-cell transfer and d3Tx models by allowing the rapid expansion of low-affinity autoreactive T cells in the absence of clonal competition (King et al., 2004). As such, any cells that can efficiently compete with these autoreactive T cells may exhibit some suppressor function in these assays (Barthlott et al., 2003).

Recent studies on the T-cell transfer model of colitis have shown that infusion of CD4$^+$CD25$^+$ Tregs to mice with established colitis is sufficient to suppress the inflammatory response with restoration of normal intestinal architecture. The transferred Tregs homed to the site of inflammation and to the draining lymph nodes where they inhibited the proliferation of colitogenic effector T cells (Mottet et al., 2003). The ability of CD4$^+$CD25$^+$ Tregs to reverse an established inflammatory response, characterized by an accumulation of large numbers of activated effector T cells in the intestine,

strongly argues against simple clonal competition as the mechanism of suppression mediated by $CD4^+CD25^+$ Tregs. This is further supported by the fact that cure of colitis by Tregs is dependent on IL-10 and TGF-β (Liu et al., 2003; Mottet et al., 2003), arguing that $CD4^+CD25^+$ Treg-mediated suppression involves a functionally specialized response.

Furthermore, some studies suggest that protection from organ-specific autoimmunity is tissue specific, and that Tregs require continued exposure to tissue-specific antigens to mediate suppression. Thus, peripheral $CD4^+$ T cells isolated from athyroid rats were unable to suppress thyroiditis in susceptible rats, although they could prevent the development of type 1 diabetes (Seddon and Mason, 1999). By contrast, thymocytes obtained from athyroid rats could inhibit the development of thyroiditis, suggesting that maintenance of peripheral Treg activity is dependent on the presence of specific autoantigens. Similarly, in orchidectomized male mice, treatment with dihydrotestosterone induced not only the *de novo* development of a mature prostate, but also facilitated the concomitant development of $CD4^+$ T cells that inhibited prostatitis after transfer into d3Tx mice (Taguchi et al., 1994). Finally, using models of autoimmune ovarian disease, Tung et al. (2001) demonstrated that $CD4^+CD25^+$ Tregs prevented this disease in d3Tx mice and that the continuous presence of physiologically expressed autoantigen was required for the maintenance of self-tolerance in normal female mice. These experiments, together with others described below, suggest that $CD4^+CD25^+$ Tregs behave in a tissue-specific manner to prevent autoimmune disease.

Spontaneous Autoimmune Disease

Studies of the role of $CD4^+CD25^+$ Tregs in spontaneous autoimmune disease have complemented those in T-cell transfer models. In NOD mice, the destruction of insulin-producing β cells is preceded by insulitis, a nondestructive leukocytic infiltration of the islets. Strategies that reduce $CD4^+CD25^+$ Treg numbers, such as a lack of CD28, CD80, or blockade of CD80/CD86, accelerated the progression from insulitis to overt diabetes (Salomon et al., 2000). Similarly, delayed onset of diabetes induced by expression of TNF-α in the islets was found to involve enhanced recruitment of $CD4^+CD25^+$ Tregs, both to the draining lymph node and to the islets. Transfer of as few as 2000 $CD4^+CD25^+$ T cells from the pancreatic lymph node of protected mice was sufficient to prevent diabetes following adoptive transfer to young TNF-α transgenic NOD mice, providing direct evidence that $CD4^+CD25^+$ Tregs can inhibit spontaneous autoimmune disease in a lymphocyte-replete setting. $CD4^+CD25^+$ Tregs from the draining lymph node were more efficient than those from other lymph nodes, suggesting some antigen specificity in the regulatory response (Green et al., 2002). Antigen-specific $CD4^+CD25^+$ Tregs isolated from BDC2.5 TCR trangenic mice that recognize an islet antigen have also been used both to prevent and cure diabetes in NOD mice. These cells were expanded *in vitro* using either anti-CD3, anti-CD28, and IL-2, or a mimetope peptide and activated DC. Importantly, $CD4^+CD25^+$ Tregs taken from NOD mice lacked the ability to inhibit disease, again suggesting that Tregs of the appropriate specificity are required (Tang et al., 2004; Tarbell et al., 2004).

Induced Autoimmune Disease

MBP-specific TCR transgenic mice on a $RAG^{-/-}$ background develop spontaneous EAE, whereas those on a $RAG^{+/+}$ background do not (Lafaille et al., 1994). The protective effect of the RAG genes appears to involve the development of Treg populations, as $CD4^+CD25^+$ Tregs from TCR transgenic $RAG^{+/+}$ mice prevented the development of disease when transferred to TCR transgenic $RAG^{-/-}$ recipients. That Treg activity enriched within those $CD4^+CD25^+$ Tregs expressing the MBP-specific TCR provides further evidence for antigen specificity in the Treg response (Hori et al., 2002). Transfer of $CD4^+CD25^-$ T cells from TCR transgenic $RAG^{+/+}$ mice also prevented EAE in this model. In this case, suppression was found to be partially dependent on IL-10 (Olivares-Villagomez et al., 2000; Furtado et al., 2001).

A number of autoimmune diseases, including arthritis, gastritis, and encephalomyelitis can be induced by immunization with autoantigen in adjuvant. In each case, prior depletion of $CD25^+$ Tregs, although only partial, resulted in the development of significantly more severe pathology (Laurie et al., 2002; McHugh and Shevach, 2002; Morgan et al., 2003; Zhang et al., 2004). In EAE, these results suggest that $CD4^+CD25^+$ Treg activity may be involved in the spontaneous remission characteristic of this model. Consistent with this, transfer of $CD4^+CD25^+$ Tregs from normal mice into mice immunized with MBP was sufficient to ameliorate central nervous system (CNS) disease (Zhang et al., 2004). $CD4^+CD25^+$ cells isolated from $IL\text{-}10^{-/-}$ mice failed to provide any protective effect, suggesting that IL-10 production by Tregs is required to resolve CNS inflammation.

Human Autoimmune Disease

The finding that a reduction in $CD4^+CD25^+$ Treg activity is an important susceptibility factor for the development of autoimmune and inflammatory diseases in mice raises the possibility that alterations in this population, whether genetically or environmentally induced, may also contribute to polygenic autoimmune diseases in humans. In support of this there have been reports of reduced Treg activity among $CD4^+CD25^+$ cells from patients with multiple sclerosis, rheumatoid arthritis, and autoimmune polyglandular syn-

drome type II (Ehrenstein et al., 2004; Kriegel et al., 2004; Viglietta et al., 2004). Currently, it is not known whether changes in the functional activity of $CD4^+CD25^+$ Tregs are a primary cause of disease or a secondary effect. Furthermore, it cannot be excluded that the observed reduction in Treg activity is a consequence of an increase in effector cells within the activated $CD4^+CD25^+$ T-cell pool in patients with autoimmune disease as opposed to a reduction in Treg function per cell. Intriguingly, the less severe, self-remitting form of juvenile idiopathic arthritis, and in some cases remission of Goodpasture syndrome, have been associated with increases in $CD4^+CD25^+$ Treg numbers (Salama et al., 2003; de Kleer et al., 2004). Again, it is not clear whether this is the primary cause of remission or a secondary effect. However, this is an active area of interest and it is anticipated that answers to these questions will emerge with the development of better tools to track naturally occurring and other Treg population.

CONCLUDING REMARKS

During the past few years, interest in Tregs has reached unparalleled levels, culminating in the identification of the Foxp3 transcription factor that cements the link between Treg function and self-tolerance. Furthermore, there is a growing realization of the crucial physiologic role that Tregs play in maintaining the delicate equilibrium between immune responsiveness and immune pathology. Nevertheless, many important questions remain unresolved, including their precise mechanisms of action, their peptide specificities, and the interrelationships between the various subsets of Tregs. The complexities and disparities encountered in the various systems utilized to analyze Treg function are outweighed by the considerable therapeutic potential of these cells. With respect to autoimmune disease, key recent advances in mice at least include the characterization of Treg function in health and disease, the identification of more precise protocols for the generation and identification of Tregs, and the demonstration that Treg therapy can ameliorate established disease. Together, these findings strengthen the hope that continued study of Tregs will facilitate the transition from merely understanding their biology to exploiting their therapeutic potential in human disease.

References

Almeida, A.R., Legrand, N., Papiernik, M., and Freitas, A.A. (2002). Homeostasis of peripheral $CD4^+$ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls $CD4^+$ T cell numbers. *J. Immunol. 169*, 4850–4860.

Alyanakian, M.A., You, S., Damotte, D., Gouarin, C., Esling, A., Garcia, C., Havouis, S., Chatenoud, L., and Bach, J.F. (2003). Diversity of regulatory $CD4^+$ T cells controlling distinct organ-specific autoimmune diseases. *Proc. Natl. Acad. Sci. USA 100*, 15806–15811.

Annacker, O., Pimenta-Araujo, R., Burlen-Defranoux, O., Barbosa, T.C., Cumano, A., and Bandeira, A. (2001). $CD25^+CD4^+$ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. *J. Immunol. 166*, 3008–3018.

Apostolou, I., Sarukhan, A., Klein, L., and von Boehmer, H. (2002). Origin of regulatory T cells with known specificity for antigen. *Nat. Immunol. 3*, 756–763.

Apostolou, I. and von Boehmer, H. (2004). *In vivo* instruction of suppressor commitment in naive T cells. *J. Exp. Med. 199*, 1401–1408.

Asano, M., Toda, M., Sakaguchi, N., and Sakaguchi, S. (1996). Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J. Exp. Med. 184*, 387–396.

Asseman, C., Read, S., and Powrie, F. (2003). Colitogenic Th1 cells are present in the antigen-experienced T cell pool in normal mice: control by $CD4^+$ regulatory T cells and IL-10. *J. Immunol. 171*, 971–978.

Banz, A., Peixoto, A., Pontoux, C., Cordier, C., Rocha, B., and Papiernik, M. (2003). A unique subpopulation of $CD4^+$ regulatory T cells controls wasting disease, IL-10 secretion and T cell homeostasis. *Eur. J. Immunol. 33*, 2419–2428.

Barrat, F.J., Cua, D.J., Boonstra, A., Richards, D.F., Crain, C., Savelkoul, H.F., de Waal-Malefyt, R., Coffman, R.L., Hawrylowicz, C.M., and O'Garra, A. (2002). *In vitro* generation of interleukin 10-producing regulatory $CD4^+$ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med. 195*, 603–616.

Barthlott, T., Kassiotis, G., and Stockinger, B. (2003). T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med. 197*, 451–460.

Belghith, M., Bluestone, J.A., Barriot, S., Megret, J., Bach, J.F., and Chatenoud, L. (2003). TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat. Med. 9*, 1202–1208.

Belkaid, Y., Piccirillo, C.A., Mendez, S., Shevach, E.M., and Sacks, D.L. (2002). $CD4^+CD25^+$ regulatory T cells control Leishmania major persistence and immunity. *Nature 420*, 502–507.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F., and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet. 27*, 20–21.

Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J., and Laufer, T.M. (2001). Major histocompatibility complex class II-positive cortical epithelium mediates the selection of $CD4^+CD25^+$ immunoregulatory T cells. *J. Exp. Med. 194*, 427–438.

Burkhart, C., Liu, G.Y., Anderton, S.M., Metzler, B., and Wraith, D.C. (1999). Peptide-induced T cell regulation of experimental autoimmune encephalomyelitis: a role for IL-10. *Int. Immunol. 11*, 1625–1634.

Caramalho, I., Lopes-Carvalho, T., Ostler, D., Zelenay, S., Haury, M., and Demengeot, J. (2003). Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J. Exp. Med. 197*, 403–411.

Chang, C.C., Ciubotariu, R., Manavalan, J.S., Yuan, J., Colovai, A.I., Piazza, F., Lederman, S., Colonna, M., Cortesini, R., Dalla-Favera, R., and Suciu-Foca, N. (2002). Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nat. Immunol. 3*, 237–243.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med. 198*, 1875–1886.

Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., and Weiner, H.L. (1994). Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science 265*, 1237–1240.

Cong, Y., Weaver, C.T., Lazenby, A., and Elson, C.O. (2002). Bacterial-reactive T regulatory cells inhibit pathogenic immune responses to the enteric flora. *J. Immunol. 169*, 6112–6119.

Cornall, R.J., Goodnow, C.C., and Cyster, J.G. (1995). The regulation of self-reactive B cells. *Curr. Opin. Immunol. 7*, 804–811.

de Kleer, I.M., Wedderburn, L.R., Taams, L.S., Patel, A., Varsani, H., Klein, M., de Jager, W., Pugayung, G., Giannoni, F., and Rijkers, G. (2004). $CD4^+CD25$ bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis. *J. Immunol. 172*, 6435–6443.

Dhodapkar, M.V. and Steinman, R.M. (2002). Antigen-bearing immature dendritic cells induce peptide-specific $CD8^+$ regulatory T cells *in vivo* in humans. *Blood 100*, 174–177.

Dieckmann, D., Bruett, C.H., Ploettner, H., Lutz, M.B., and Schuler, G. (2002). Human $CD4^+CD25^+$ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells [corrected]. *J. Exp. Med. 196*, 247–253.

Dujardin, H.C., Burlen-Defranoux, O., Boucontet, L., Vieira, P., Cumano, A., and Bandeira, A. (2004). Regulatory potential and control of Foxp3 expression in newborn $CD4^+$ T cells. *Proc. Natl. Acad. Sci. USA 101*, 14473–14478.

Ehrenstein, M.R., Evans, J.G., Singh, A., Moore, S., Warnes, G., Isenberg, D.A., and Mauri, C. (2004). Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J. Exp. Med. 200*, 277–285.

Fahlen, L., Read, S., Gorelik, L., Hurst, S.D., Coffman, R.L., Flavell, R.A., and Powrie, F. (2005). T cells that cannot respond to TGF-beta escape control by $CD4^+CD25^+$ regulatory T cells. *J. Exp. Med. 201*, 737–746.

Fallarino, F., Grohmann, U., Hwang, K.W., Orabona, C., Vacca, C., Bianchi, R., Belladonna, M.L., Fioretti, M. C., Alegre, M.L., and Puccetti, P. (2003). Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol. 4*, 1206–1212.

Filaci, G. and Suciu-Foca, N. (2002). $CD8^+$ T suppressor cells are back to the game: are they players in autoimmunity? *Autoimmun. Rev. 1*, 279–283.

Fisson, S., Darrasse-Jeze, G., Litvinova, E., Septier, F., Klatzmann, D., Liblau, R., and Salomon, B.L. (2003). Continuous activation of autoreactive $CD4^+CD25^+$ regulatory T cells in the steady state. *J. Exp. Med. 198*, 737–746.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol. 4*, 330–336.

Foussat, A., Cottrez, F., Brun, V., Fournier, N., Breittmayer, J.P., and Groux, H. (2003). A comparative study between T regulatory type 1 and $CD4^+CD25^+$ T cells in the control of inflammation. *J. Immunol. 171*, 5018–5026.

Furlan, R., Bergami, A., Cantarella, D., Brambilla, E., Taniguchi, M., Dellabona, P., Casorati, G., and Martino, G. (2003). Activation of invariant NKT cells by alphaGalCer administration protects mice from MOG35–55-induced EAE: critical roles for administration route and IFN-gamma. *Eur. J. Immunol. 33*, 1830–1838.

Furtado, G.C., Curotto de Lafaille, M.A., Kutchukhidze, N., and Lafaille, J.J. (2002). Interleukin 2 signaling is required for $CD4^+$ regulatory T cell function. *J. Exp. Med. 196*, 851–857.

Furtado, G.C., Olivares-Villagomez, D., Curotto De Lafaille, M.A., Wensky, A.K., Latkowski, J.A., and Lafaille, J.J. (2001). Regulatory T cells in spontaneous autoimmune encephalomyelitis. *Immunol. Rev. 182*, 122–134.

Fuss, I.J., Boirivant, M., Lacy, B., and Strober, W. (2002). The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis. *J. Immunol. 168*, 900–908.

Fuss, I.J., Heller, F., Boirivant, M., Leon, F., Yoshida, M., Fichtner-Feigl, S., Yang, Z., Exley, M., Kitani, A., and Blumberg, R.S. (2004). Non-classical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J. Clin. Invest. 113*, 1490–1497.

Gershon, R.K., and Kondo, K. (1970). Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology 18*, 723–737.

Gilliet, M. and Liu, Y.J. (2002). Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J. Exp. Med. 195*, 695–704.

Godfrey, D.I., and Kronenberg, M. (2004). Going both ways: immune regulation via CD1d-dependent NKT cells. *J. Clin. Invest. 114*, 1379–1388.

Gonzalez, A., Andre-Schmutz, I., Carnaud, C., Mathis, D., and Benoist, C. (2001). Damage control, rather than unresponsiveness, effected by protective $DX5^+$ T cells in autoimmune diabetes. *Nat. Immunol. 2*, 1117–1125.

Goodnow, C.C., Crosbie, J., Jorgensen, H., Brink, R.A., and Basten, A. (1989). Induction of self-tolerance in mature peripheral B lymphocytes. *Nature 342*, 385–391.

Green, E.A., Choi, Y., and Flavell, R.A. (2002). Pancreatic lymph node-derived $CD4^+CD25^+$ Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity 16*, 183–191.

Green, E.A., Gorelik, L., McGregor, C.M., Tran, E.H., and Flavell, R.A. (2003). $CD4^+CD25^+$ T regulatory cells control anti-islet $CD8^+$ T cells through TGF-beta–TGF-beta receptor interactions in type 1 diabetes. *Proc. Natl. Acad. Sci. USA 100*, 10878–10883.

Grohmann, U., Orabona, C., Fallarino, F., Vacca, C., Calcinaro, F., Falorni, A., Candeloro, P., Belladonna, M.L., Bianchi, R., Fioretti, M.C., and Puccetti, P. (2002). CTLA-4-Ig regulates tryptophan catabolism *in vivo*. *Nat. Immunol. 3*, 1097–1101.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., De Vries, J.E., and Roncarolo, M.-G. (1997). A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature 389*, 737–742.

Hammond, K.J., Poulton, L.D., Palmisano, L.J., Silveira, P.A., Godfrey, D.I., and Baxter, A.G. (1998). Alpha/beta-T cell receptor $CD4^-CD8^-$ (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J. Exp. Med. 187*, 1047–1056.

Heller, F., Fuss, I.J., Nieuwenhuis, E.E., Blumberg, R.S., and Strober, W. (2002). Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity 17*, 629–638.

Hong, S., Wilson, M.T., Serizawa, I., Wu, L., Singh, N., Naidenko, O.V., Miura, T., Haba, T., Scherer, D. C., and Wei, J. (2001). The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat. Med. 7*, 1052–1056.

Hori, S., Haury, M., Coutinho, A., and Demengeot, J. (2002). Specificity requirements for selection and effector functions of $CD25^+CD4^+$ regulatory T cells in anti-myelin basic protein T cell receptor transgenic mice. *Proc. Natl. Acad. Sci. USA 99*, 8213–8218.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science 299*, 1057–1061.

Hsieh, C.S., Liang, Y., Tyznik, A.J., Self, S.G., Liggitt, D., and Rudensky, A.Y. (2004). Recognition of the peripheral self by naturally arising $CD25^+$ $CD4^+$ T cell receptors. *Immunity 21*, 267–277.

Hu, D., Ikizawa, K., Lu, L., Sanchirico, M.E., Shinohara, M.L., and Cantor, H. (2004). Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat. Immunol. 5*, 516–523.

Itoh, M., Takahashi, T., Sakaguchi, N., Kuniyasu, Y., Shimizu, J., Otsuka, F., and Sakaguchi, S. (1999). Thymus and autoimmunity: production of $CD25^+CD4^+$ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J. Immunol. 162*, 5317–5326.

Jahng, A.W., Maricic, I., Pedersen, B., Burdin, N., Naidenko, O., Kronenberg, M., Koezuka, Y., and Kumar, V. (2001). Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. *J. Exp. Med. 194*, 1789–1799.

Jayawardena-Wolf, J. and Bendelac, A. (2001). CD1 and lipid antigens: intracellular pathways for antigen presentation. *Curr. Opin. Immunol. 13*, 109–113.

Jiang, H. and Chess, L. (2000). The specific regulation of immune responses by $CD8^+$ T cells restricted by the MHC class Ib molecule, Qa-1. *Annu. Rev. Immunol. 18*, 185–216.

Jiang, H., Kashleva, H., Xu, L.X., Forman, J., Flaherty, L., Pernis, B., Braunstein, N.S., and Chess, L. (1998). T cell vaccination induces T cell receptor Vbeta-specific Qa-1-restricted regulatory $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA 95*, 4533–4537.

Jiang, H., Zhang, S.I., and Pernis, B. (1992). Role of $CD8^+$ T cells in murine experimental allergic encephalomyelitis. *Science 256*, 1213–1215.

Jonuleit, H., Schmitt, E., Kakirman, H., Stassen, M., Knop, J., and Enk, A.H. (2002). Infectious tolerance: human $CD25^+$ regulatory T cells convey suppressor activity to conventional $CD4^+$ T helper cells. *J. Exp. Med. 196*, 255–260.

Jordan, M.S., Riley, M.P., von Boehmer, H., and Caton, A.J. (2000). Anergy and suppression regulate $CD4^+$ T cell responses to a self peptide. *Eur. J. Immunol. 30*, 136–144.

Kappler, J.W., Roehm, N., and Marrack, P. (1987). T cell tolerance by clonal elimination in the thymus. *Cell 49*, 273–280.

Kawahata, K., Misaki, Y., Yamauchi, M., Tsunekawa, S., Setoguchi, K., Miyazaki, J., and Yamamoto, K. (2002). Generation of $CD4^+CD25^+$ regulatory T cells from autoreactive T cells simultaneously with their negative selection in the thymus and from nonautoreactive T cells by endogenous TCR expression. *J. Immunol. 168*, 4399–4405.

Khattri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003). An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. *Nat. Immunol. 4*, 337–342.

King, C., Ilic, A., Koelsch, K., and Sarvetnick, N. (2004). Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell 117*, 265–277.

Klein, L., Khazaie, K., and von Boehmer, H. (2003). *In vivo* dynamics of antigen-specific regulatory T cells not predicted from behavior *in vitro*. *Proc. Natl. Acad. Sci. USA 100*, 8886–8891.

Kriegel, M.A., Lohmann, T., Gabler, C., Blank, N., Kalden, J.R., and Lorenz, H.M. (2004). Defective suppressor function of human $CD4^+CD25^+$ regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med. 199*, 1285–1291.

Kulkarni, A.B., Huh, C.G., Becker, D., Geiser, A., Lyght, M., Flanders, K.C., Roberts, A.B., Sporn, M.B., Ward, J.M., and Karlsson, S. (1993). Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA 90*, 770–774.

Kullberg, M.C., Jankovic, D., Gorelick, P.L., Caspar, P., Letterio, J.J., Cheever, A.W., and Sher, A. (2002). Bacteria-triggered $CD4^+$ T regulatory cells suppress *Helicobacter hepaticus*-induced colitis. *J. Exp. Med. 196*, 505–515.

Kumar, V. and Sercarz, E.E. (1993). The involvement of T cell receptor peptide-specific regulatory $CD4^+$ T cells in recovery from antigen-induced autoimmune disease. *J. Exp. Med. 178*, 909–916.

Kumar, V., Stellrecht, K., and Sercarz, E. (1996). Inactivation of T cell receptor peptide-specific CD4 regulatory T cells induces chronic experimental autoimmune encephalomyelitis (EAE). *J. Exp. Med. 184*, 1609–1617.

Lafaille, J.J., Nagashima, K., Katsuki, M., and Tonegawa, S. (1994). High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell 78*, 399–408.

Laurie, K.L., Van Driel, I.R., and Gleeson, P.A. (2002). The role of $CD4^+CD25^+$ immunoregulatory T cells in the induction of autoimmune gastritis. *Immunol. Cell Biol. 80*, 567–573.

Lee, I.F., Qin, H., Trudeau, J., Dutz, J., and Tan, R. (2004). Regulation of autoimmune diabetes by complete Freund's adjuvant is mediated by NK cells. *J. Immunol. 172*, 937–942.

Lehmann, J., Huehn, J., de la Rosa, M., Maszyna, F., Kretschmer, U., Krenn, V., Brunner, M., Scheffold, A., and Hamann, A. (2002). Expression of the integrin alphaE beta 7 identifies unique subsets of $CD25^+$ as well as $CD25^-$ regulatory T cells. *Proc. Natl. Acad. Sci. USA 99*, 13031–13036.

Lehuen, A., Lantz, O., Beaudoin, L., Laloux, V., Carnaud, C., Bendelac, A., Bach, J. F., and Monteiro, R.C. (1998). Overexpression of natural killer T cells protects Valpha14- Jalpha281 transgenic nonobese diabetic mice against diabetes. *J. Exp. Med. 188*, 1831–1839.

Liu, H., Hu, B., Xu, D., and Liew, F.Y. (2003). $CD4^+CD25^+$ regulatory T cells cure murine colitis: the role of IL-10, TGF-beta, and CTLA4. *J. Immunol. 171*, 5012–5017.

Liu, Z., Yu, B., Fan, J., Cortesini, R., and Suciu-Foca, N. (2001). $CD8^+CD28^-$ T cells suppress alloresponse of $CD4^+$ T cells both in primates and rodents. *Transplant. Proc. 33*, 82–83.

Madakamutil, L.T., Maricic, I., Sercarz, E., and Kumar, V. (2003). Regulatory T cells control autoimmunity *in vivo* by inducing apoptotic depletion of activated pathogenic lymphocytes. *J. Immunol. 170*, 2985–2992.

Malek, T.R., and Bayer, A.L. (2004). Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol. 4*, 665–674.

Malek, T.R., Yu, A., Vincek, V., Scibelli, P., and Kong, L. (2002). CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. *Immunity 17*, 167–178.

Maloy, K.J. and Powrie, F. (2001). Regulatory T cells in the control of immune pathology. *Nat. Immunol. 2*, 816–822.

Maloy, K.J., Salaun, L., Cahill, R., Dougan, G., Saunders, N.J., and Powrie, F. (2003). $CD4^+CD25^+$ regulatory T cells supress innate immune pathology through cytokine-dependent mechanisms. *J. Exp. Med. 197*, 111–119.

Mars, L.T., Novak, J., Liblau, R.S., and Lehuen, A. (2004). Therapeutic manipulation of iNKT cells in autoimmunity: modes of action and potential risks. *Trends Immunol. 25*, 471–476.

McHugh, R.S., and Shevach, E.M. (2002). Cutting edge: depletion of $CD4^+CD25^+$ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J. Immunol. 168*, 5979–5983.

McHugh, R.S., Whitters, M.J., Piccirillo, C.A., Young, D.A., Shevach, E.M., Collins, M., and Byrne, M.C. (2002). $CD4^+CD25^+$ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity 16*, 311–323.

Mellor, A.L., and Munn, D.H. (2004). IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat. Rev. Immunol. 4*, 762–774.

Mills, K.H. (2004). Regulatory T cells: friend or foe in immunity to infection? *Nat. Rev. Immunol. 4*, 841–855.

Morgan, M.E., Sutmuller, R.P., Witteveen, H.J., van Duivenvoorde, L.M., Zanelli, E., Melief, C.J., Snijders, A., Offringa, R., de Vries, R.R., and Toes, R.E. (2003). $CD25^+$ cell depletion hastens the onset of severe disease in collagen-induced arthritis. *Arthritis Rheum. 48*, 1452–1460.

Morrissey, P.J., Charrier, K., Braddy, S., Liggitt, D., and Watson, J.D. (1993). $CD4^+$ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified $CD4^+$ T cells. *J. Exp. Med. 178*, 237–244.

Mottet, C., Uhlig, H.H., and Powrie, F. (2003). Cutting edge: cure of colitis by $CD4^+CD25^+$ regulatory T cells. *J. Immunol. 170*, 3939–3943.

Nakamura, K., Kitani, A., Fuss, I., Pedersen, A., Harada, N., Nawata, H., and Strober, W. (2004). TGF-beta1 plays an important role in the

mechanism of $CD4^+CD25^+$ regulatory T cell activity in both humans and mice. *J. Immunol. 172*, 834–842.

Nakamura, K., Kitani, A., and Strober, W. (2001). Cell contact-dependent immunosuppression by $CD4^+CD25^+$ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med. 194*, 629–644.

Nishizuka, Y. and Sakakura, T. (1969). Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science 166*, 753–755.

Olivares-Villagomez, D., Wensky, A.K., Wang, Y., and Lafaille, J.J. (2000). Repertoire requirements of $CD4^+$ T cells that prevent spontaneous autoimmune encephalomyelitis. *J. Immunol. 164*, 5499–5507.

Papiernik, M., de Moraes, M.L., Pontoux, C., Vasseur, F., and Penit, C. (1998). Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. *Int. Immunol. 10*, 371–378.

Pasare, C. and Medzhitov, R. (2003). Toll pathway-dependent blockade of $CD4^+CD25^+$ T cell-mediated suppression by dendritic cells. *Science 299*, 1033–1036.

Peng, Y., Laouar, Y., Li, M.O., Green, E.A., and Flavell, R.A. (2004). TGF-beta regulates *in vivo* expansion of Foxp3-expressing $CD4^+CD25^+$ regulatory T cells responsible for protection against diabetes. *Proc. Natl. Acad. Sci. USA 101*, 4572–4577.

Penhale, W.J., Farmer, A., McKenna, R.P., and Irvine, W.J. (1973). Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. *Clin. Exp. Immunol. 15*, 225–236.

Piccirillo, C.A., Letterio, J.J., Thornton, A.M., McHugh, R.S., Mamura, M., Mizuhara, H., and Shevach, E.M. (2002). $CD4^+CD25^+$ regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J. Exp. Med. 196*, 237–246.

Piccirillo, C.A. and Shevach, E.M. (2001). Cutting edge: control of $CD8^+$ T cell activation by $CD4^+CD25^+$ immunoregulatory cells. *J. Immunol. 167*, 1137–1140.

Piccirillo, C.A. and Shevach, E.M. (2004). Naturally-occurring $CD4^+CD25^+$ immunoregulatory T cells: central players in the arena of peripheral tolerance. *Semin. Immunol. 16*, 81–88.

Powrie, F., Leach, M.W., Mauze, S., Caddle, L.B., and Coffman, R.L. (1993). Phenotypically distinct subsets of $CD4^+$ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int. Immunol. 5*, 1461–1471.

Powrie, F. and Mason, D. (1990). OX-22high $CD4^+$ T cells induce wasting disease with multiple organ pathology: prevention by the OX–22low subset. *J. Exp. Med. 172*, 1701–1708.

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.

Roncarolo, M.G., Gregori, S., and Levings, M. (2003). Type 1 T regulatory cells and their relationship with $CD4^+CD25^+$ T regulatory cells. *Novartis Found. Symp. 252*, 115–127; discussion 127–131, 203–110.

Russell, D.M., Dembic, Z., Morahan, G., Miller, J.F., Burki, K., and Nemazee, D. (1991). Peripheral deletion of self-reactive B cells. *Nature 354*, 308–311.

Sakaguchi, S. (2004). Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.

Sakaguchi, S., Fukuma, K., Kuribayashi, K., and Masuda, T. (1985). Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J. Exp. Med. 161*, 72–87.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155*, 1151–1164.

Salama, A.D., Chaudhry, A.N., Holthaus, K.A., Mosley, K., Kalluri, R., Sayegh, M.H., Lechler, R.I., Pusey, C.D., and Lightstone, L. (2003). Regulation by $CD25^+$ lymphocytes of autoantigen-specific T-cell responses in Goodpasture's (anti-GBM) disease. *Kidney Int. 64*, 1685–1694.

Salomon, B., Lenschow, D.J., Rhee, L., Ashourian, N., Singh, B., Sharpe, A., and Bluestone, J.A. (2000). B7/CD28 costimulation is essential for the homeostasis of the $CD4^+CD25^+$ immunoregulatory T cells that control autoimmune diabetes. *Immunity 12*, 431–440.

Sarantopoulos, S., Lu, L., and Cantor, H. (2004). Qa-1 restriction of $CD8^+$ suppressor T cells. *J. Clin. Invest. 114*, 1218–1221.

Schwartz, R.H. (2003). T cell anergy. *Annu. Rev. Immunol. 21*, 305–334.

Seddon, B. and Mason, D. (1999). Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. *J. Exp. Med. 189*, 877–882.

Seddon, B. and Mason, D. (2000). The third function of the thymus. *Immunol. Today 21*, 95–99.

Setoguchi, R., Hori, S., Takahashi, T., and Sakaguchi, S. (2005). Homeostatic maintenance of natural Foxp3(+) $CD25^+$ $CD4^+$ regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J. Exp. Med. 201*, 723–735.

Sharif, S., Arreaza, G.A., Zucker, P., Mi, Q.S., Sondhi, J., Naidenko, O.V., Kronenberg, M., Koezuka, Y., Delovitch, T.L., and Gombert, J.M. (2001). Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat. Med. 7*, 1057–1062.

Shevach, E.M. (2002). $CD4^+$ $CD25^+$ suppressor T cells: more questions than answers. *Nat. Rev. Immunol. 2*, 389–400.

Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y., and Sakaguchi, S. (2002). Stimulation of $CD25^+CD4^+$ regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol. 3*, 135–142.

Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., and Calvin, D. (1992). Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature 359*, 693–699.

Singh, A.K., Wilson, M.T., Hong, S., Olivares-Villagomez, D., Du, C., Stanic, A.K., Joyce, S., Sriram, S., Koezuka, Y., and Van Kaer, L. (2001). Natural killer T cell activation protects mice against experimental autoimmune encephalomyelitis. *J. Exp. Med. 194*, 1801–1811.

Stephens, G.L., McHugh, R.S., Whitters, M.J., Young, D.A., Luxenberg, D., Carreno, B.M., Collins, M., and Shevach, E.M. (2004). Engagement of glucocorticoid-induced TNFR family-related receptor on effector T cells by its ligand mediates resistance to suppression by $CD4^+CD25^+$ T cells. *J. Immunol. 173*, 5008–5020.

Stephens, L.A. and Mason, D. (2000). CD25 is a marker for $CD4^+$ thymocytes that prevent autoimmune diabetes in rats, but peripheral T cells with this function are found in both $CD25^+$ and $CD25^-$ subpopulations. *J. Immunol. 165*, 3105–3110.

Stock, P., Kallinich, T., Akbari, O., Quarcoo, D., Gerhold, K., Wahn, U., Umetsu, D.T., and Hamelmann, E. (2004). $CD8^+$ T cells regulate immune responses in a murine model of allergen-induced sensitization and airway inflammation. *Eur. J. Immunol. 34*, 1817–1827.

Suciu-Foca, N., Manavalan, J.S., and Cortesini, R. (2003). Generation and function of antigen-specific suppressor and regulatory T cells. *Transpl. Immunol. 11*, 235–244.

Suri-Payer, E. and Cantor, H. (2001). Differential cytokine requirements for regulation of autoimmune gastritis and colitis by $CD4^+CD25^+$ T cells. *J. Autoimmun. 16*, 115–123.

Taguchi, O., Kontani, K., Ikeda, H., Kezuka, T., Takeuchi, M., and Takahashi, T. (1994). Tissue-specific suppressor T cells involved in

self-tolerance are activated extrathymically by self-antigens. *Immunology 82*, 365–369.

Takahashi, T., Kuniyasu, Y., Toda, M., Sakaguchi, N., Itoh, M., Iwata, M., Shimizu, J., and Sakaguchi, S. (1998). Immunologic self-tolerance maintained by $CD25^+CD4^+$ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol. 10*, 1969–1980.

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.

Takeda, K., Clausen, B.E., Kaisho, T., Tsujimura, T., Terada, N., Forster, I., and Akira, S. (1999). Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity 10*, 39–49.

Takeda, K., Kaisho, T., and Akira, S. (2003). Toll-like receptors. *Annu. Rev. Immunol. 21*, 335–376.

Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med. 199*, 1455–1465.

Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., and Steinman, R.M. (2004). $CD25^+$ $CD4^+$ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med. 199*, 1467–1477.

Thompson, C. and Powrie, F. (2004). Regulatory T cells. *Curr. Opin. Pharmacol. 4*, 408–414.

Thornton, A.E. and Shevach, E.M. (1998). $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J. Exp. Med. 188*, 287–296.

Thornton, A.M., Donovan, E.E., Piccirillo, C.A., and Shevach, E.M. (2004). Cutting edge: IL-2 is critically required for the *in vitro* activation of $CD4^+CD25^+$ T cell suppressor function. *J. Immunol. 172*, 6519–6523.

Thornton, A.M. and Shevach, E.M. (2000). Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific. *J. Immunol. 164*, 183–190.

Thorstenson, K.M. and Khoruts, A. (2001). Generation of anergic and potentially immunoregulatory $CD25^+CD4^+$ T cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. *J. Immunol. 167*, 188–195.

Tone, M., Tone, Y., Adams, E., Yates, S.F., Frewin, M.R., Cobbold, S.P., and Waldmann, H. (2003). Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *Proc. Natl. Acad. Sci. USA 100*, 15059–15064.

Tung, K.S., Agersborg, S.S., Alard, P., Garza, K.M., and Lou, Y.H. (2001). Regulatory T-cell, endogenous antigen and neonatal environment in the prevention and induction of autoimmune disease. *Immunol. Rev. 182*, 135–148.

Vieira, P.L., Christensen, J.R., Minaee, S., O'Neill, E.J., Barrat, F.J., Boonstra, A., Barthlott, T., Stockinger, B., Wraith, D.C., and O'Garra, A. (2004). IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring $CD4^+CD25^+$ regulatory T cells. *J. Immunol. 172*, 5986–5993.

Viglietta, V., Baecher-Allan, C., Weiner, H.L., and Hafler, D.A. (2004). Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med. 199*, 971–979.

von Boehmer, H. (1988). The developmental biology of T lymphocytes. *Annu. Rev. Immunol. 6*, 309–326.

Walker, L.S., Chodos, A., Eggena, M., Dooms, H., and Abbas, A.K. (2003). Antigen-dependent proliferation of $CD4^+CD25^+$ regulatory T cells *in vivo*. *J. Exp. Med. 198*, 249–258.

Wang, B., Geng, Y.B., and Wang, C.R. (2001). CD1-restricted NK T cells protect nonobese diabetic mice from developing diabetes. *J. Exp. Med. 194*, 313–320.

Wildin, R.S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J. L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., and Perroni, L. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet. 27*, 18–20.

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.

Zhang, X., Koldzic, D.N., Izikson, L., Reddy, J., Nazareno, R.F., Sakaguchi, S., Kuchroo, V.K., and Weiner, H.L. (2004). IL-10 is involved in the suppression of experimental autoimmune encephalomyelitis by $CD25^+CD4^+$ regulatory T cells. *Int. Immunol. 16*, 249–256.

Zheng, S.G., Wang, J.H., Gray, J.D., Soucier, H., and Horwitz, D.A. (2004). Natural and induced $CD4^+CD25^+$ cells educate $CD4^+CD25^-$ cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. *J. Immunol. 172*, 5213–5221.

Zhou, D., Mattner, J., Cantu, C., 3rd, Schrantz, N., Yin, N., Gao, Y., Sagiv, Y., Hudspeth, K., Wu, Y.P., and Yamashita, T. (2004). Lysosomal glycosphingolipid recognition by NKT cells. *Science 306*, 1786–1789.

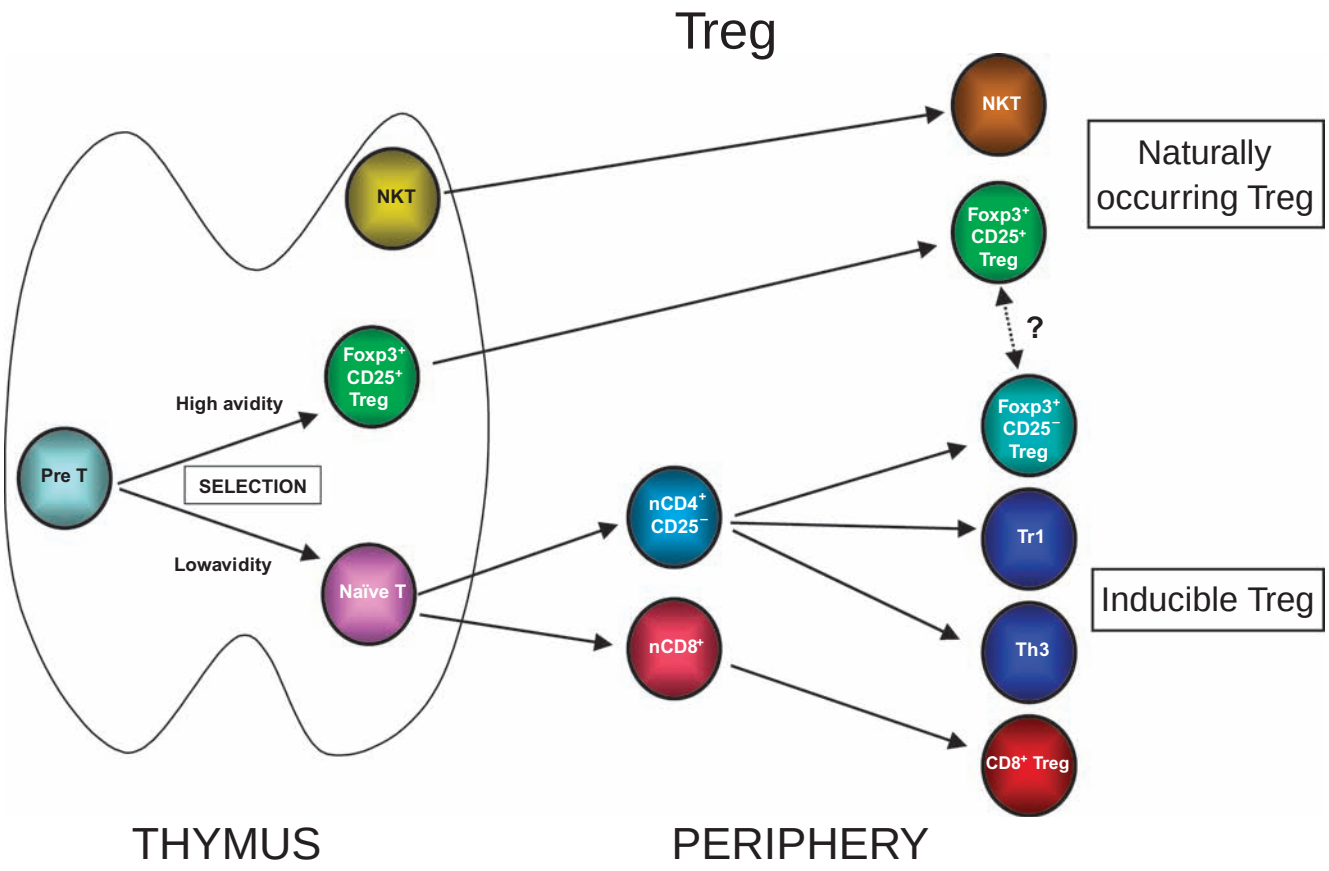


FIGURE 9.1 Major populations of regulatory T cells (Tregs). Naturally occurring Tregs ($CD4^+CD25^+$ T cells and natural killer T cells) arise during normal thymic development and do not require further differentiation in the periphery in order to mediate regulatory function. By contrast, inducible Tregs are exported from the thymus as naïve uncommitted T cells and acquire Treg function following activation and differentiation in the periphery. The precise interrelationships between the different Treg populations have not yet been fully defined.

CHAPTER

10

Peptide-Based Instruction of Suppressor Commitment in Naïve T Cells

IRINA APOSTOLOU, KARSTEN KRETSCHMER, AND HARALD VON BOEHMER

Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts, USA

Recent years have witnessed the revival of suppressor T cells that control immunity by interfering with the generation of effector T-cell function *in vivo*. A major discovery was the identification of suppressor cells that expressed the α chain of the IL-2 receptor (CD25) on their surface and the fact that $CD25^+CD4^+$ T cells were highly enriched in suppressive activity (Sakaguchi et al., 1995). This enabled the phenotypic and functional analysis of so-called natural suppressor T cells. With regard to gene expression, it was found that such cells had upregulated several activation markers and downregulated proteins characteristic of naïve T cells (Sakaguchi et al., 2001; McHugh et al., 2002; Shimuzu et al., 2002; Caramalho et al., 2003). Of great significance was the observation that these cells express high levels of the transcription factor FoxP3, which, when defective, leads to the early onset of fatal autoimmune diseases (Fontenot et al., 2003; Hori et al., 2003; Khattri et al, 2003; Walker et al., 2003a). Experiments on T-cell receptor (TCR) transgenic mice revealed that coexpression of a class II major histocompatibility complex (MHC)-restricted TCR and its ligand drove the generation of suppressor T cells (Jordan et al., 2000; 2001; Bensinger et al., 2001), and that expression on thymic stroma was an effective (Bensinger et al., 2001; Jordan et al., 2001; Apostolou et al., 2002), but not exclusive, means of generating such cells (Apostolou et al., 2002).

In this chapter we are concerned with the generation of $CD25^+$ suppressor cells by exogenous antigens in the fully mature immune system, as well as with their *in vivo* stability and function. Evidence suggests that $CD25^+$ suppressor cells represent a suitable tool to induce antigen-specific immunologic tolerance in the fully mature immune system in the absence of general immune suppression, which often has undesired side effects ranging from increased risk of infection to development of life-threatening lymphoma.

GENERATING SUPPRESSOR CELLS BY EXOGENOUS ANTIGENS

There has been a long history of attempts to induce specific *in vivo* immune suppression by delivering exogenous antigens via the oral route, intranasally, intravenously or subcutaneously (Thorstenson and Khoruts, 2001; Chai et al., 2004; Nagler-Anderson et al., 2004), and thereby induce and/or expand suppressor cells (Chen et al., 2003; Horwitz et al., 2004; Tarbell et al., 2004; Zheng et al., 2004a; 2004b). There is no doubt that some of these experiments worked, but since the readout of most was immunosuppression rather than the generation of a well-defined class of suppressor cells, no consensus emerged on what might represent the most useful approach to specifically prevent unwanted immunity. "Chronic" antigenic stimulation could result in $CD4^+$ T cells that produced mostly interleukin (IL)-10 (Bacchetta et al., 1994; Lanoue et al., 1997; Buer et al., 1998). However, in some instances, these cells only suppressed certain immune reactions and had a phenotype that differed from the later described $CD25^+$ suppressor cells (Bacchetta et al., 1994). Before the recognition of FoxP3 expression as a marker for suppressor T cells, findings were

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

reported that the subimmunogenic presentation of proteins or peptides could result in the generation of $CD25^+$ T cells that, at least in some assays, qualified as suppressor cells (Chen et al., 1994; Weiner, 1997; Thorstenson and Khoruts, 2001; Sundstedt et al., 2003). Based on our own limited and initial studies in this direction (Apostolou et al., 2002), we pursued a larger study concerned with developing suitable protocols for the extrathymic generation of $CD25^+$ suppressors, and a comparative analysis of such peripherally-generated cells versus intrathymically-generated $CD25^+$ suppressor T cells (Apostolou and von Boehmer, 2004).

Initially, we attempted to mimic a situation that may occur *in vivo* when cell-specific proteins are expressed at low levels, broken down by proteases, and the peptides presented by steady-state (mostly nonactivated) dendritic cells. To this end we continuously infused relatively small doses of peptides with the aid of mini-osmotic pumps that were transplanted subcutaneously in mice, and monitored the appearance of cells with different phenotypes over time. Studies were first conducted in TCR-HA transgenic mice on the RAG-$2^{-/-}$ background that expressed a TCR specific for the peptide 107–119 of influenza hemagglutinin presented by E^d MHC molecules in naïve T cells. To exclude any influence of the thymus, these mice were thymectomized prior to implantation of the peptide-delivering osmotic pumps. The continuous supply of peptides, even at doses as low as 10^{-3} μg/day, resulted in downregulation (endocytosis) of the TCR on many T cells, as well as the appearance of $CD25^+$ T cells with suppressive activity at day 14 of peptide infusion in the absence of immunologic priming.

These experiments were then repeated by transferring pretreated naïve TCR-HA-expressing T cells into nu/nu recipient mice and then implanting the peptide-delivering pumps. The same result was obtained, arguing that it was possible to generate $CD25^+$ cells with suppressive activity *de novo* from $CD25^-$ naïve T cells in the absence of any "tutoring" by other intrathymically-generated T cells, the latter representing a phenomenon implicit in so-called "infectious tolerance" (Waldmann et al., 2004).

Finally, a similar approach was used to generate suppressors from small numbers of naïve $CD4^+$ T cells in normal mice, emphasizing that the protocol was suitable for generating suppressors from a small number of naïve T cells in a normal environment. In transgenic as well as nontransgenic systems, the induced $CD25^+$ cells prevented the development of antigen-specific effector T cell responses in various *in vivo* readouts (Apostolou and von Boehmer, 2004). The latter setting was then used to analyze whether continuous peptide infusion provided an advantage over single-dose injection of peptides, and whether targeting of peptide to steady-state dendritic cells (DCs) was suitable for inducing such cells. This approach was used because previous experiments using the DEC-205 antibody to target peptides to DCs had led to somewhat different outcomes in different laboratories: while in some studies this mode of peptide delivery resulted in moderate cellular expansion followed by deletion and/or anergy (Hawiger et al., 2001; 2004), in at least one experimental setting it also resulted in the generation of $CD25^+$ cells with suppressive activity (Mahnke et al., 2003). Our results using this approach showed that: 1) DEC-205 delivered peptide-induced dose-dependent proliferation; 2) the cells that proliferated most extensively did not acquire constitutive CD25 expression; and 3) cells that went through only a few divisions converted most efficiently into $CD25^+$ suppressor cells during 14 days from intraperitoneally delivery of the antibody–peptide fusion protein. Thus, there was an inverse relationship between cell division and CD25 conversion, but not in the sense, as previously reported, that those cells that did not divide at all converted best (Thorstenson and Khoruts, 2001). Instead, cells that were weakly activated and proliferated to some extent showed a better conversion rate than cells that had not divided at all.

Thus, in addition to intrathymic generation of $CD25^+$ suppressor cells by "self" agonist ligands of the TCR, recent information can be exploited to generate cells that are specific for exogenous antigens in the fully mature immune system and in the absence of a functioning thymus (Apostolou and von Boehmer, 2004). This opens new avenues for inducing specific immune tolerance, an old dream of immunologists that was experimentally addressed decades ago but resulted in erratic results, most likely due to the fact that protocols aiming to induce specific immunosuppression could not be optimized because the nature of suppressor cells was poorly understood.

"LIFESTYLE" OF SUPPRESSOR T CELLS

Naturally occurring suppressor T cells have been extensively characterized *in vitro*. These cells: 1) are intrinsically anergic, i.e., do not respond to antigenic stimulation with proliferation; 2) suppress other cells through a specific T–T-cell contact, resulting in inhibition of IL-2 gene transcription; and 3) do not require the production of IL-10 for their suppressive effect, whereas the involvement of tumor growth factor (TGF)-β is debated (Piccirillo and Shevach, 2004).

These experiments could not provide information on the question of whether $CD25^+CD4^+$ FoxP3-expressing T cells represent a lineage of irreversibly committed cells or an effector T-cell population that has acquired a suppressor phenotype when antigenically stimulated. This question was addressed using the TCR-HA transgenic system by transferring $CD4^+25^+$ T cells with a TCR of known antigen specificity into an antigen-free environment and following their fate or that of induced $CD4^+25^+$ cells of 14-day peptide-

infused mice over prolonged time periods (Klein et al., 2003). It was concluded that after their induction by cognate antigen, $CD4^+25^+$ suppressor cells could survive for long periods of time in a normal lymphoid environment without any requirement for antigenic stimulation by TCR agonist ligands. The cells had an intermitotic lifespan that in murine models lasted for months (Klein et al., 2003), an observation well compatible with the notion that some $CD4^+25^+$ cells in normal mice have an intermitotic lifespan of at least 70 days (Fisson et al., 2003). Whenever tested during the observation period, the activation of these cells by cognate antigen resulted in potent suppressor activity *in vitro* and $CD4^+25^+$ cells did not lose their anergic *in vitro* phenotype when maintained for long time periods in an antigen-free environment. Thus, by these criteria, $CD4^+25^+$ suppressor T cells represent a long-lived stable lineage of T cells committed to immunosuppression only.

DYNAMICS OF *IN VIVO* SUPPRESSION

Contrary to *in vitro* readouts, the effect of suppression by $CD4^+25^+$ T cells on the immune response of $CD4^+$ or $CD8^+$ cells *in vivo* is only observed at later stages when responses begin with a relatively low frequency of suppressor T cells and other T cells responding to antigenic challenge. *In vivo* suppressor T cells are not anergic and respond to antigenic challenge with strong proliferation (Cozzo et al., 2003; Fisson et al., 2003; Klein et al, 2003; Walker et al., 2003b; Yamazaki et al., 2003) as naïve or memory T cells do. Thus, both suppressor T cells and naïve $CD4^+$ T cells will initially expand in a similar fashion in antigen-draining lymph nodes to which both suppressor T cells and $CD4^+$ T cells home with similar efficacy. Later, the proliferation of $CD4^+$ T cells is selectively diminished and their cytokine secretion is suppressed, while the activated suppressor cells produce predominantly IL-10 (Klein et al., 2003). IL-10 is, however, not always essential for the observed suppression of $CD4^+$ T cells and the effector mechanism of the *in vivo* suppression is unknown. It is also worth mentioning that under these *in vivo* conditions, the commitment of $CD4^+$ T cells to produce IL-2 or interferon-γ is not prevented, i.e., these cytokines are produced in increased amounts when the suppressed $CD4^+$ T cells are separated from the suppressors and antigenically stimulated (Martin et al., 2004).

The suppression of $CD8^+$ T cells *in vivo* does not affect initially their proliferation, such that they accumulate at very similar frequencies whether suppressors are present or not (Lin et al, 2002). However, by about day 7 of a primary CD8 immune response in the presence of suppressors, the specific suppression of cytolytic activity of the expanded $CD8^+$ T cells can be observed (Chen et al., 2005). It is of particular interest that in some instances control of CD8 responses by suppressor cells was found to be entirely dependent on TGF-β signaling by $CD8^+$ T cells, as the cytolytic activity of $CD8^+$ T cells from mice expressing a dominant negative TGF-β receptor makes them resistant to suppression by $CD4^+25^+$ suppressor cells (Green et al., 2003; Chen et al., 2005). Thus, in this particular scenario, molecular pathways are emerging of how suppressors interact with $CD8^+$ T cells, but it is not clear whether under these circumstances TGF-β must be produced by the suppressors themselves.

INTERLEUKIN-2 DEPENDENCE OF SUPPRESSOR CELLS

It has become clear that IL-2- or IL-2-receptor-deficient mice lack $CD4^+25^+$ suppressor cells in their peripheral lymphoid tissue (Papiernik et al., 1998), and are therefore highly susceptible to autoimmune disease, which in IL-2-receptor-deficient mice can be cured by giving IL-2-receptor positive $CD4^+25^+$ suppressor T cells (Malek et al., 1991). Thus, these experiments challenge earlier notions that autoimmune disease in IL-2- and IL-2-receptor-deficient mice is due to a deficiency in activation-induced cell death (Leonardo 1991; Van Parijs et al., 1997). When analyzed in more detail, it became clear that the antigen-induced proliferation of suppressors *in vivo* is not dependent on IL-2 [IL-4 can substitute for IL-2 *in vitro* (Thornton et al., 2004)], but that suppressors that are stimulated by antigen in the absence of IL-2 lose their expression of CD25 and become less potent suppressors (unpublished results), a scenario likely to cause autoimmunity in IL-2- or IL-2-receptor-deficient mice that generate $CD4^+25^+$ cells in the thymus (Malek et al., 2002).

CONCLUSION

The fact that $CD4^+25^+$ FoxP3-expressing suppressor cells have an essential role in preventing the early onset of autoimmune disease in mammals, and the fact that these cells can be artificially induced through subimmunogenic presentation of TCR agonist ligands, opens new possibilities to exploit these cells to induce specific tolerance in the fully mature immune system. This may become a powerful tool in the prevention of allergies and transplant rejection. Inducing such cells by organ-specific antigens, such as insulin or myelin basic protein peptides, may become an effective means to prevent autoimmunity in patients at risk of developing such diseases. Such attempts have had success in animal models of disease (Daniel and Wegmann, 1996; Tarbekk et al., 2004) but not in clinical trials (Diabetes Prevention Trial–Type 1 Diabetes Study Group, 2002). There is hope, however, that our acquisition of knowledge on the "lifestyle" of suppressor T cells will help to develop more

suitable procedures that may be exploited for the induction of specific immunologic tolerance in the clinic without the use of dangerous general immunosuppression.

References

Apostolou, I. and von Boehmer, H. (2004). *In vivo* instruction of suppressor commitment in naive T cells. *J. Exp. Med. 199*, 1401–1408.

Apostolou, I., Sarukhan, A., Klein, L., and von Boehmer, H. (2002). Origin of regulatory T cells with known specificity for antigen. *Nat. Immunol. 3*, 756–763.

Bacchetta, R., Bigler, M., Touraine, T.L., Parkman, R., Tovo, P.A., Abrams, J., de Waal Malfet, 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.

Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J., and Laufer, T.M. (2001). Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4(+)25(+) immunoregulatory T cells. *J. Exp. Med. 194*, 427–438.

Buer, J., Lanoue, A., Franzke, A., Garcia, C., von Boehmer, H., and Sarukhan, A. (1998). Interleukin 10 secretion and impaired effector function of major histocompatibility complex class II-restricted T cells anergized *in vivo*. *J. Exp. Med. 187*, 177–183.

Caramalho, I., Lopes-Carvalho, T., Ostler, D., Zelaney, S., Haury, M., and Demengeot, J. (2003). Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J. Exp. Med. 197*, 403–411.

Chai, J.G., James, E., Dewchand, H., Simpson, E., and Scott, D. (2004). Transplantation tolerance induced by intranasal administration of HY peptides. *Blood 103*, 3951–3959.

Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., and Weiner, H.L. (1994). Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science 265*, 1237–1240.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl S.M. (2003). Conversion of peripheral CD4+CD25− naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med. 198*, 1875–1886.

Chen, M.-L., Pittet, M., Weissleder, R., Gorelik, L., Flavell, R., von Boehmer, H., Khazaie, K. (2005). Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals *in vivo*. *Proc. Natl. Acad. Sci. USA 102*, 419–424.

Cozzo, C., Larkin, J., 3rd, and Caton, A.J. (2003). Cutting edge: self-peptides drive the peripheral expansion of CD4+CD25+ regulatory T cells. *J. Immunol. 171*, 5678–5682.

Daniel, D. and Wegmann, D.R. (1996). Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9–23). *Proc. Natl. Acad. Sci. USA 93*, 956–960.

Diabetes Prevention Trial—Type 1 Diabetes Study Group (2002). Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N. Engl. J. Med. 346*, 1685–1691.

Fisson, S., Darrasse-Jeze, G., Litvinova, E., Septier, F., Klatzmann, D., Liblau, R., and Salomon, B.L. (2003). Continuous activation of autoreactive CD4+ CD25+ regulatory T cells in the steady state. *J. Exp. Med. 198*, 737–746.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol*. *4*, 330–336 (2003).

Green, E.A., Gorelik, L., McGregor, C.M., Tran, E.H., and Flavell, R.A. (2003). CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. *Proc. Natl. Acad. Sci. USA 100*, 10878–10883.

Klein, L., Khazaie, K., and von Boehmer, H. (2003). *In vivo* dynamics of antigen-specific regulatory T cells not predicted from behavior *in vitro*. *Proc. Natl. Acad. Sci. USA 100*, 8886–8891.

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.

Hawiger, D., Masilamani, R.F., Bettelli, E., Kuchroo, V.K., and Nussenzweig, M.C. (2004). Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells *in vivo*. *Immunity 20*, 695–705.

Hori, S., Nomura, T., and Sakaguchi, S (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science 299*, 1057–1061.

Horwitz, D.A., Zheng, S.G., Gray, J.D., Wang, J.H., Ohtsuka, K., and Yamagiwa, S. (2004). Regulatory T cells generated ex vivo as an approach for the therapy of autoimmune disease. *Semin. Immunol*. *16*, 135–143.

Jordan, M.S., Riley, M.P., von Boehmer, H., and Caton, A.J. (2000). Anergy and suppression regulate CD4(+) T cell responses to a self peptide. *Eur. J. Immunol. 30*, 136–144.

Jordan, M.S., Boesteanu A., Reed, A.J., Petone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol. 2*, 301–306.

Khattri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003). An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol. 4*, 337–342.

Lanoue, A., Bona, C., von Boehmer, H., and Sarukhan, A. (1997). Conditions that induce tolerance in mature CD4+ T cells. *J. Exp. Med. 185*, 405–414.

Lenardo, M.J. (1991). Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. *Nature 353*, 858–861.

Lin, C.Y., Graca, L., Cobbold, S.P., and Waldmann, H. (2002). Dominant transplantation tolerance impairs CD8+ T cell function but not expansion. *Nat. Immunol. 3*, 1208–1213.

Mahnke, K., Qian, Y., Knop, J., and Enk, A.H. (2003). Induction of CD4+/CD25+ regulatory T cells by targeting of antigens to immature dendritic cells. *Blood 101*, 4862–4869.

Malek, T.R., Yu, A., Vincek, V., Scibelli, P., and Kong, L. (2002). CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. *Immunity 17*, 167–178.

Martin, B., Banz, A., Bienvenu, B., Cordier, C., Dautigny, N., Becourt, C., and Lucas, B. (2004). Suppression of CD4+ T lymphocyte effector functions by CD4+CD25+ cells *in vivo*. *J. Immunol. 172*, 3391–3398.

McHugh, R.S., Whitters, M.J., Piccirillo, C.A., Young, D.A., Shevach, E.M., Collins, M., and Byrne, M.C. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity 16*, 311–323.

Nagler-Anderson, C., Bhan, A.K., Podolsky, D.K., and Terhorst, C. (2004). Control freaks: immune regulatory cells. *Nat. Immunol. 5*, 119–122.

Papiernik, M., de Moraes, M.L., Pontoux, C., Vasseur, F., and Penit, C. (1998). Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. *Int. Immunol. 10*, 371–378.

Piccirillo, C.A. and Shevach, E.M. (2004). Naturally-occurring CD4+CD25+ immunoregulatory T cells: central players in the arena of peripheral tolerance. *Semin. Immunol. 16*, 81–88.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155*, 1151–1164.

Sakaguchi, S., Shimizu, J., Yamazaki, S., Sakihama, T., Itoh, M., Kuniyashu Y., Nomura, T., Toda, M., and Takahashi, T. (2001). Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev. 182*, 18–32.

Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y., and Sakaguchi, S. (2002). Stimulation of CD25+CD4+ regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol. 3*, 135–142.

Sundstedt, A., O'Neill, E.J., Nicolson, K.S., and Wraith, D.C. (2003). Role for IL-10 in suppression mediated by peptide-induced regulatory T cells *in vivo*. *J. Immunol. 170*, 1240–1248.

Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., and Steinman, R.M. (2004). CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med. 199*, 1467–1477.

Thornton, A.M., Piccirillo, C.A., and Shevach, E.M. (2004). Activation requirements for the induction of CD4+CD25+ T cell suppressor function. *Eur. J. Immunol. 34*, 366–376.

Thorstenson, K.M. and Khoruts, A. (2001). Generation of anergic and potentially immunoregulatory CD25+CD4 T cells *in vivo* after induction of peripheral tolerance with intravenous or oral antigen. *J. Immunol*. *167*, 188–195.

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*, 3738–3745.

Waldmann, H., Graca, L., Adams, E., Fairchild, P., and Cobbold, S. (2004). Regulatory T cells and organ transplantation. *Semin. Immunol. 16*, 119–126.

Walker, M.R., Kasprowicz, D.J., Gersuk, V.H., Benard, A., van Landeghen, M., Buckner, J.H., and Ziegler, S.F. (2003a). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J. Clin. Invest*. *112*, 1437–1443.

Walker, L.S., Chodos, A., Eggena, M., Dooms, H., and Abbas, A.K. (2003b). Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells *in vivo*. *J. Exp. Med. 198*, 249–258.

Weiner, H.L. (1997). Oral tolerance for the treatment of autoimmune diseases. *Annu. Rev. Med. 48*, 341–351.

Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K., and Steinman, R.M. (2003). Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J. Exp. Med. 198*, 235–247.

Zheng, S.G., Wang, J.H., Gray, J.D., Soucier, H., and Horwitz, D.A. (2004a). Natural and induced CD4+CD25+ cells educate CD4+CD25– cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. *J. Immunol. 172*, 5213–5221.

Zheng, S.G., Wang, J.H., Koss, M.N., Quismorio, F., Jr, Gray, J.D., and Horwitz, D.A. (2004b). CD4+ and CD8+ regulatory T cells generated ex vivo with IL-2 and TGF-beta suppress a stimulatory graft-versus-host disease with a lupus-like syndrome. *J. Immunol. 172*, 1531–1539.

CHAPTER

11

B Cells and Autoimmunity

STUART TANGYE*

**The Centenary Institute of Cancer Medicine and Cell Biology, Newtown, New South Wales*

FABIENNE MACKAY†

†The Garvan Institute of Medical Research, Department of Arthritis and Inflammation, Darlinghurst, New South Wales, Australia

Systemic autoimmune diseases are debilitating conditions that often require lifelong therapies. Many of these conditions, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjögren syndrome, are characterized by the production of autoantibodies. Furthermore, in recent times there has been much success in the treatment of RA by ablating B cells. Together, these findings highlight the significant contribution of B cells to the development and pathogenesis of autoimmune diseases. In this chapter, we detail recent advances in the understanding of B-cell differentiation, both in normal individuals and in those with autoimmune diseases, and the role of B-cell intrinsic and extrinsic factors to the progression of autoimmune conditions, and reveal how these findings have illuminated possible new therapies for improved treatment of such diseases.

NORMAL B CELL DEVELOPMENT AND FUNCTION

B Cell Development

B cell development occurs in two distinct stages: lymphopoiesis and immunopoiesis. Lymphopoiesis is initiated in the fetal liver and adult bone marrow (BM), and is the sequential differentiation of pluripotent hematopoietic stem cells → pro-B cells → pre-B cells → immature B cells (Figure 11.1) (Uckun, 1990; Banchereau and Rousset, 1992). Engagement of the B-cell antigen receptor (BCR) by high-affinity or multivalent self-antigen results in the deletion of potentially autoreactive B cell-clones in the BM. In contrast, exposure to monovalent or low-affinity self-antigen will result in the self-reactive B cells becoming recirculating anergic cells with a dramatically reduced half life (Goodnow et al., 1995). Immature B cells leave the BM and enter the spleen where they become transitional cells (Figure 11.1). Depending on the strength of the signals received through the BCR, as well as the receptor for BAFF [B-cell activating factor belonging to the tumor necrosis family (TNF) family; also called BLyS, zTNF4, TALL-1, THANK and TNFSF13b], these cells will undergo stimulation or deletion (Goodnow et al., 1995; Mackay et al., 2003). The end result of "productive" lymphopoiesis is a mature, naïve (i.e., antigen-inexperienced), immunocompetent B cell expressing a functional BCR (Banchereau and Rousset, 1992). Immunopoiesis continues in secondary organs and represents the antigen-driven differentiation of mature B cells into activated lymphoblasts, which further develop into antigen-specific plasma cells and memory B cells (Figure 11.2; see below) (Banchereau and Rousset, 1992; Liu and Banchereau, 1996).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

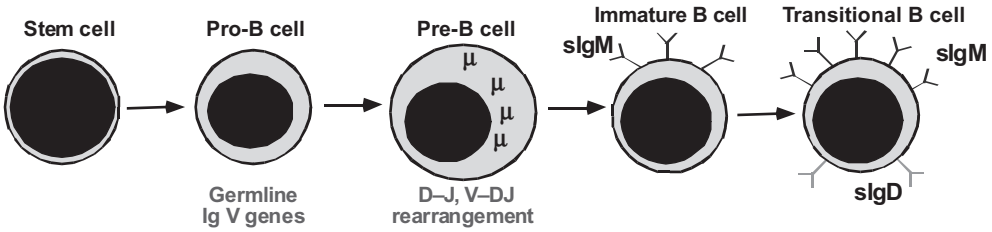


FIGURE 11.1 B-cell development. B cells arise from pluripotent stem cells present in the fetal liver and adult bone marrow. The stem cells progressively develop into pro-B cells, pre-B cells, and immature B cells, which are then exported to the periphery as transitional B cells, primarily localizing to the spleen, and undergo further maturation. Thus, the final product of this process is a mature B cell expressing a functional B-cell receptor. The different stages of B-cell development can be resolved according to the status of the immunoglobulin (Ig) genes expressed by the developing cells. Pro-B cells express unrearranged Ig genes that are in germline configuration, while D–J and V–DJ rearrangement occurs at the pre-B-cell stage. Pre-B cells also express cytoplasmic Ig μ heavy chains. Immature B cells express IgM on their surface, while transitional (and mature) B cells express both surface IgM and IgD.

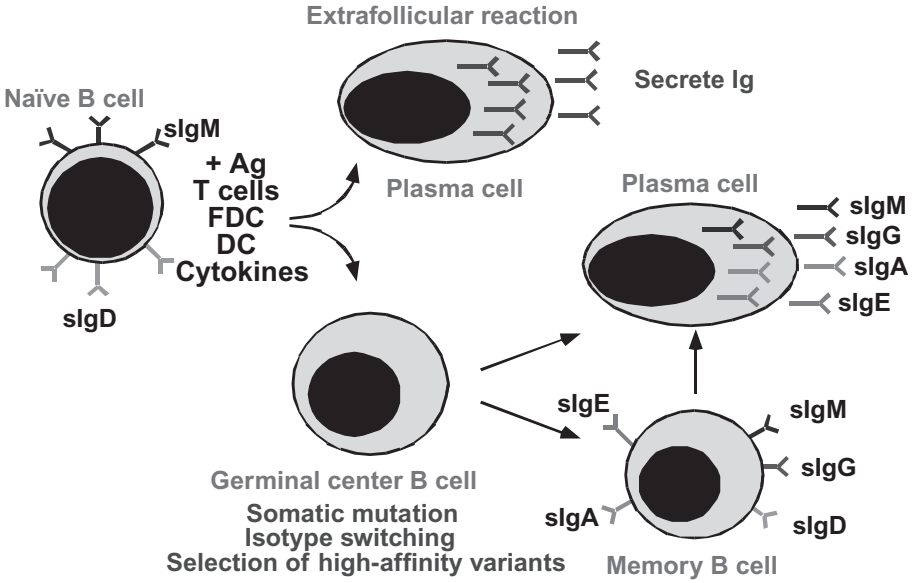


FIGURE 11.2 T-cell-dependent B-cell activation. Antigen (Ag)-specific T cells and B cells interact within secondary lymphoid tissues where, under the influence of additional signals provided by the lymphoid microenvironment in the form of dendritic cells (DCs) and follicular DCs (FDCs), B cells become activated and can enter either the extrafollicular reaction or seed a germinal center. The extrafollicular reaction yields short-lived plasma cells secreting predominantly low-affinity IgM. On the other hand, the germinal center (GC) generates long-lived cells responsible for long-term serologic humoral immunity—high-affinity memory B cells and plasma cells. On exposure to the same immunizing antigen, memory B cells can rapidly differentiate to become plasma cells. Memory B cells and plasma cells derived from a GC reaction have undergone somatic hypermutation, which results in an increase in affinity for Ag, and immunoglobulin (Ig) isotype switching, and thus express downstream Ig isotypes (IgG, A or E). However, some plasma cells and memory B cells can continue to express IgM.

T-Cell-Dependent Immune Responses: Development of Memory Cells and Plasma Cells

The differentiation of mature naïve B cells into effector memory B cells or plasma cells (PCs) in response to T-cell-dependent antigen is a complex procedure that occurs in peripheral lymphoid tissues and involves antigen, antigen-specific T cells, cytokines, and accessory cells, such as dendritic cells (DCs) and follicular dendritic cells (FDCs) (Liu and Banchereau, 1996; Liu et al., 1996b) (see Chapters 2 and 12). Interaction of naïve B cells with specific antigen in the T-cell-rich areas of lymphoid tissue induces activation and differentiation along one of two separate pathways. Antigen-specific B cells can remain in the T-cell-rich areas and rapidly differentiate to become PCs, and thus constitute the initial wave of the primary humoral immune response (the extrafollicular reaction; Figure 11.2). Alternatively,

some antigen-specific B cells become germinal center (GC) founder cells (MacLennan, 1994; Liu and Banchereau, 1996; Liu et al., 1996b).

Germinal Center Reaction

GCs are highly specialized immunologic microenvironments present within peripheral lymphoid tissues, such as lymph nodes, spleen, tonsil, and Peyer patches of the gut (see Figure 2.7). Within GCs, activated naïve B cells become centroblasts and undergo vigorous proliferation, somatic hypermutation (SHM) of the immunoglobulin V region genes, and immunoglobulin isotype switching (Liu et al., 1996a; 1996c). Histologically, proliferating GC B cells, termed centroblasts, constitute the dark zone of the GC. Centroblasts exit the dark zone and enter the FDC-rich light zone where they become nondividing B cells—centrocytes (MacLennan, 1994). It is within GCs that positive selection of antigen-specific B cells and apoptosis-mediated deletion of self-reactive or low-affinity B cells occurs. Specifically, a centrocyte expressing a mutated immunoglobulin that recognizes antigen on the surface of FDCs will undergo selection if it receives appropriate survival signals: engagement of the BCR by specific antigen, and of CD40 by CD40 ligand (CD40L), which is transiently expressed by antigen-specific T cells (Liu and Banchereau, 1996; Liu et al., 1996b). Centrocytes can then differentiate into either of two different populations of effector B cells—a memory B cell or a PC (Figure 11.2). PCs are terminally differentiated B cells that produce protective and/or neutralizing antibodies, and so maintain long-term serologic humoral immunity (Slifka and Ahmed, 1998; Manz et al., 2002), whereas memory B cells provide for an accelerated immune response upon re-exposure to the immunizing antigen (Ahmed and Gray, 1996; Rajewsky, 1996). Thus, the end product of GC reactions are populations of effector B cells that express high-affinity antigen-specific immunoglobulin and, occasionally, isotype-switched immunoglobulin heavy chain isotypes. GCs are therefore the major sites of production of memory B cells and PCs (Figure 11.2).

Phenotypic Delineation of Differentiating B Cells

The transition of naïve B cells into GC-derived effector or memory B cells has been well studied. In human tonsil, there are at least five identifiable distinct subpopulations of mature B cells (Bm1–Bm5) (Pascual et al., 1994). Bm1 cells are small resting B cells that express high levels of sIgD; Bm2 cells are activated Bm1 cells and, since both express unmutated immunoglobulin V region genes, represent naïve B-cells. Bm3 cells have a high proliferative capacity and have initiated the process of SHM, and thus represent centroblasts; Bm4 cells are centrocytes that have differentiated from Bm3 cells, and those with the greatest affinity for antigen develop into Bm5 cells, which are memory B cells (Liu et al., 1996a; Liu and Banchereau, 1996; Pascual et al., 1994). PCs can also be detected in human secondary lymphoid tissues and differ from other B cells by a $CD38^{++}CD20^{\pm}$ phenotype (Medina et al., 2002; Ellyard et al., 2004). In murine spleen, naïve B cells express sIgM, IgD, and CD38, whereas GC B cells are $CD38^{-}$ and are agglutinated by peanut agglutinin (PNA^{+}). Differentiation of naïve to memory B cells is demonstrated by loss of sIgD, expression of an antigen-specific IgG, and re-expression of CD38, whereas PCs have downregulated expression of several pan–B-cell molecules, such as B220, major histocompatibility complex (MHC) class II, and CD19, and become CD138 (Syndecan-1)$^{+}$ (Smith et al., 1996; Ridderstad and Tarlinton, 1998; Shinall et al., 2000).

Traditionally, the memory B cell has been defined as a B cell expressing a class-switched immunoglobulin isotype. However, a more reliable marker for human memory B cells is expression of CD27 (Klein et al., 1998; Tangye et al., 1998). Among human lymphoid tissues, CD27 is first expressed on B cells in the GC, and is retained by both memory B cells and PCs (Klein et al., 1998; Tangye et al., 1998; Jung et al., 2000; Ellyard et al., 2004). Thus, expression of CD27 by human B cells correlates with their involvement in a GC reaction and, when used in combination with other cell-surface markers, can accurately discriminate between multiple B-cell subsets: naïve B cells, GC B cells, memory (IgM-expressing as well as immunoglobulin isotype switched) B cells, and PCs.

Characteristics of Memory B Cells

Following the GC reaction, memory B cells acquire several characteristics that distinguish them from naïve B cells and therefore facilitate their ability to rapidly respond to antigen rechallenge (see Chapter 12).

Localization Within Lymphoid Tissue

Naïve and memory B cells differ from one another with respect to their anatomic distribution. In human tonsil, memory B cells colonize the mucosal epithelium, while naïve B cells localize to the follicular areas (Liu et al., 1995). In the spleens of humans and rodents, the white pulp is comprised of T-cell zones, B-cell follicles (the follicular zone), and marginal zones (MZs) (Liu and Banchereau, 1996). The splenic MZ surrounds the follicular zone and is adjacent to the red pulp. Much of the blood that enters the spleen traverses through the marginal sinus and MZ, and then into the red pulp, before it exits the spleen to re-enter the circulation. As a result, cells that are located in the MZ are exposed to high concentrations of blood-borne antigens (Mebius and Kraal, 2005; Pillai, 2005). Naïve B cells reside in the follicle while memory B cells colonize the MZ (Dunn-Walters et al., 1995; Liu et al., 1988; Tangye et al., 1998). The positioning of resident memory B cells within antigen-draining

sites of lymphoid tissue, especially the MZ, maximizes their exposure to circulating antigen so that specific memory B cells can rapidly respond to antigen. Similarly, circulating memory B cells disseminate immunologic memory throughout the secondary lymphoid tissues, providing a general surveillance for any sites of pathogen invasion.

Phenotype

By using the differential expression of switched immunoglobulin isotypes, and more recently CD27, the phenotype of human naïve and memory B cells has been determined. Naïve B cells present in human tonsil and spleen are morphologically small cells that express intermediate levels of IgM and CD21, high levels of IgD and CD23, and low to negligible levels of activation antigens such as CD80, CD86, and CD95. In contrast, memory B cells are larger cells, have downregulated IgD and CD23, and upregulated expression of CD21, CD40, CD80, CD86, and CD95 (Liu et al., 1995; Tangye et al., 1998; Ellyard et al., 2004). The elevated expression of the costimulatory molecules CD80 and CD86 allows memory B cells to act as efficient antigen-presenting cells (APCs) for $CD4^+$ T cells, which is of importance for autoimmunity (Liu et al., 1995). Similarly, increased expression of CD21 and CD40 on memory B cells over naïve B cells may provide them with the ability to respond to lower levels of stimulation received through immune complexes (CD21) and T-cell help (CD40). Lastly, the cytoplasmic domains of IgG and IgA significantly differ from that of IgM and are believed to facilitate a more robust response by memory B cells (Tangye and Hodgkin, 2004).

Differentiation to Plasma Cells

Naïve and memory B cells exhibit distinct responses following exposure to an activating stimulus. Memory B cells differentiate into PCs more rapidly than naïve B cells, and antigen-specific immunoglobulin was produced by human memory, but not naïve, B cells. Furthermore, this process does not require the memory B cells to enter a GC reaction, thus resulting in the rapid production of high-affinity immunoglobulin (Liu et al., 1988; 1991; Tangye and Hodgkin, 2004).

Characteristics of Plasma Cells

PCs are present in spleen, mucosal-associated lymphoid tissues, BM, and, in cases of disease, peripheral blood (Odendahl et al., 2000; Medina et al., 2002; Ellyard et al., 2004). Although similar with respect to surface phenotype, important differences have been noted between PCs isolated from different anatomic locations. For instance, the predominant immunoglobulin isotype produced is distinct in that IgM is secreted by splenic PCs, IgG by PCs in the BM, tonsils, and peripheral blood, and IgA by PCs in the gut (Merville et al., 1996; Medina et al., 2003; Ellyard et al., 2004). In contrast to GC-derived PCs, the immunoglobulin V_H genes of extrafollicular PCs typically remain in a germline configuration, having not undergone significant SHM. Furthermore, most extrafollicular PCs are short-lived, and die by apoptosis *in situ*, whereas the majority of GC-derived PCs are long-lived cells that migrate from the follicular areas of the lymphoid tissue to distant sites, or remain in the tissue of origin (Smith et al., 1996; McHeyzer-Williams and Ahmed, 1999; Calame, 2001). PCs themselves may not be intrinsically long lived, but rather depend on factors provided by particular niches to ensure their longevity (Sze et al., 2000). In other words, the local environment harboring the PCs can support only the survival of a finite number of effector cells (Sze et al., 2000). Mechanisms to support the survival and function of PCs include interactions with stromal cells within the BM or red pulp of spleen, which provides prosurvival factors such as cytokine [interleukin (IL)-6, chemokines (CXCL12), and cell-surface molecules (VLA-4)] (Manz et al., 2002; Ellyard et al., 2005).

Requirements for the Formation of Germinal Centers

Peripheral lymphoid tissues provide the appropriate environment for the formation and maintenance of a GC reaction. The microenvironment provided by DCs, FDCs, and T cells is critical for the formation of GCs, as demonstrated by the absence of GCs in mice deficient in T cells or FDCs (Liu et al., 1996b). In addition, the formation of GCs, and therefore the development of humoral immune responses to T-cell-dependent antigen, requires the expression of various cell-surface receptors, signaling molecules or transcription factors (see below).

CD40/CD154

One of the most important cellular interactions is between CD40, expressed on all B cells, and CD40L, transiently expressed by activated $CD4^+$ T cells (Van Kooten and Banchereau, 1996). GCs are absent from secondary lymphoid tissues of mice genetically deficient for CD40 or CD40L, as well as from patients with inactivating mutations in CD40L, which results in the immunodeficiency X-linked hyper-IgM syndrome (Van Kooten and Banchereau, 1996; Gulino and Notarangelo, 2003). These mice and patients fail to develop memory responses, with the lack of isotype switching to IgG (Van Kooten and Banchereau, 1996).

CD28/CD80, CD86

Another important receptor–ligand interaction for establishment of GCs is that between CD28, expressed by T cells, and CD80 or CD86, expressed by APCs. When this interaction is disrupted, mice respond poorly to specific antigen, with drastically reduced levels of specific immunoglobulin, an absence of GCs, and impaired SHM of immunoglobulin

V region genes of antigen-specific B cells (Han et al., 1995; Ferguson et al., 1996; Borriello et al., 1997).

ICOS (Inducible Costimulator)

ICOS is a CD28-like molecule expressed on activated T cells. Signaling through ICOS activates T cells and induces them to proliferate and produce IL-10, a potent growth and differentiation factor for human B cells. ICOS is expressed mainly on $CD4^+$ T cells within the light zone of GCs, while its ligand (ICOS-L) is on APCs, including B cells. Analysis of mice deficient in ICOS or ICOS-L revealed an inability to form GCs, undergo immunoglobulin isotype switching, and impaired production of IL-4 and IL-10 by $CD4^+$ T cells. ICOS-deficient humans develop common variable immunodeficiency and also fail to generate memory B cells and undergo immunoglobulin isotype switching *in vivo*. Thus, the ICOS/ICOS-L interaction, like CD28/CD80/86, is necessary for the generation of humoral immune responses (Grimbacher et al., 2003).

SAP (SLAM-Associated Protein)

SAP is encoded by the SH2D1A gene, which is mutated in X-linked lymphoproliferative disease. Affected individuals exhibit defective generation of memory B cells and hypogammaglobulinemia (Nichols et al., 2005). SAP-deficient mice exhibit a similar phenotype: they do not form GCs due to impaired $CD4^+$ T-cell help, and this results in a failure to generate a long-term humoral immune response following viral infection (Crotty et al., 2003).

Bcl-6

Bcl-6 is a transcriptional repressor expressed at the highest level in GC B cells. Mice deficient for Bcl-6 fail to form GCs (Calame et al., 2003). One function of Bcl-6 is to prevent the differentiation of activated B cells into PCs; this is achieved by the repression of the transcription factor BLIMP-1, which has been proposed as being a master regulator of PC differentiation (Calame et al., 2003).

Migration and Positioning of B-Cell Subsets

The positioning of PCs, as well as naïve and memory B cells, within different anatomic sites is regulated by the concerted actions of chemokines/chemokine receptors and adhesion molecules (see Chapter 18).

Developing and Mature B Cells

Chemokines and their cognate receptors that contribute to the localization of B cells are CXCL12-CXCR4, CXCL13-CXCR5, CCL19/CCL21-CCR7, and CXCL9/CXCL10/CXCL11-CXCR3. The responsiveness of B cells to lymphoid chemokines increases during B-cell development in both the spleen and BM, such that the greatest proportion of B cells that undergo chemotaxis towards CXCL12, CXCL13, CCL19, and CCL21 belong to the mature B-cell subset, while the least responsive cells correspond to pro- and immature B cells. This increased responsiveness correlated with the acquisition of expression of specific chemokine receptors during discrete stages of B-cell development (Bowman et al., 2000).

Plasma Cells

The majority of serum immunoglobulin is derived from PCs present in BM, to which they migrate from secondary lymphoid tissues. PCs have migratory properties distinct from mature B cells. Mature B cells migrate towards CCL19/21, CXCL12, and CXCL13, while PCs exhibit selective migration towards CXCL12 due to the downregulation of CCR7 and CXCR5, but not CXCR4. It was proposed that cells would home to particular lymphoid tissues in response to the production of a specific chemotactic factor by nonhematopoietic cells located within these tissues. Indeed, CXCL12 is produced by BM and splenic stromal cells, the latter being located in the red pulp (Ellyard et al., 2005). On the other hand, chemokines recognizing CXCR5 and CCR7 are produced by FDCs and DCs, respectively, located in lymphoid follicles. Thus, the chemotactic gradient established by the production of CXCL12 by stromal cells in the red pulp, and the inability of PCs to respond to follicular chemokines, would allow the emigration of antigen-specific PCs from the GC region to the red pulp. CXCR4 also has a pivotal role in the transit of PCs from lymphoid tissue to the BM. In the absence of CXCR4, antigen-specific PCs accumulate in the blood and are markedly reduced in number in the BM (Cyster, 2003; Kunkel and Butcher, 2003).

PCs that are located in mucosal tissues predominantly secrete IgA. This mucosal positioning appears to result from the selective expression of the chemokine receptors CCR9 and CCR10, and the mucosal homing receptor $\alpha4\beta7$ by IgA-secreting PCs, and the production of CCL25 and CCL28 (which interact with CCR9 and CCR10), and expression of MAdCAM-1, the counterstructure for $\alpha4\beta7$, by epithelial and endothelial cells located in the small intestine. Thus, expression of CCR9 and CCR10 on IgA-PCs appears to mediate the migration of these cells to specific regions in order to provide an appropriate mucosal immune response (Cyster, 2003; Kunkel and Butcher, 2003). Several inflammatory chemokines may also participate in the movement of PCs from their sites of generation to sites of inflammation. For instance, antigen-specific IgG-secreting PCs migrate towards CXCR3 ligands (CXCL9, CXCL10, and CXCL11), which are abundantly expressed by inflamed tissue resulting not only from a normal inflammatory response, but also from autoimmunity. Thus, the localization of PCs within inflamed sites may result from migration from lymphoid tissue guided by the production of such chemokines (Manz et al., 2002).

B CELLS IN AUTOIMMUNITY

Animal Models

A number of inbred mouse strains (NZB, NZW, NZM 2410, BXSB, NOD, and MRL) develop autoimmunity associated with the production of autoantibodies (Sakaguchi, 2000; see Chapter 26). Crosses of some of these mice, particularly the (NZB × NZW)F1 hybrids, are a classical model of SLE (Mohan, 2001). Understanding B-cell autoimmunity, even in these inbred models, has proven difficult due to the highly polygenic nature of the autoimmune process, since separate loci are responsible for separate aspects of the disease (Mohan, 2001). Therefore, immunologists have utilized induced and gene-specific models to understand environmental and genetic influences leading to B-cell autoimmunity.

Induced B-cell Autoimmunity

B-cell autoimmunity can be induced in mice in many ways, by hormones, environmental agents or dyregulation of various endogenous B-cell survival factors.

Hormones

Almost all B-cell-associated autoimmune disorders in humans, such as SLE, Sjögren syndrome, and RA, are more prevalent in women than men (Rider and Abdou, 2001), suggesting that sex hormones influence the susceptibility of women to autoimmunity (Castagnetta et al., 2002; see Chapter 25). Estrogen and prolactin have been shown to have pleiotropic effects on the immune system (Castagnetta et al., 2002). Clinical features observed in patients with autoimmune disorders during pregnancy also reinforced the idea of a strong effect of hormones in B-cell function and autoimmunity (McMurray, 2001). Recent work has provided some insight into the molecular basis for these effects. The functional estrogen receptors α and β are expressed on B cells and protect immature transitional B cells from BCR-mediated apoptosis (Grimaldi et al., 2002). As developing B cells normally receive an apoptotic signal when their BCR binds to self-antigen, abnormal production of estrogen at this stage may prevent this purging mechanism and allow the survival of autoreactive B cells. A role for excessive estrogen production during maturation is also suggested by the observation of an altered distribution of B-cell subsets, with a diminished transitional population and an increase in MZ B-cell numbers (Grimaldi et al., 2001). This picture is very similar to mice transgenic for BAFF (Mackay et al., 2003). BAFF transgenic mice have an altered distribution of B-cell subsets, such as an enlarged MZ B-cell compartment (Mackay et al., 2003). In addition, these mice develop severe autoimmune disorders similar to SLE and Sjögren syndrome (Mackay et al., 2003). The lactogenic hormone prolactin is also an immunomodulator involved in lymphocyte survival, activation, and proliferation (McMurray, 2001). Dysregulated prolactin production has been associated with the severity of B-cell–mediated autoimmune disorders, especially SLE (McMurray, 2001). Therefore, similar to BAFF, estrogen and prolactin may influence B-cell tolerance during maturation by altering the threshold of survival and activation of maturing B cells.

Heavy Metals

Heavy metals such as mercury are environmental pollutants which, when accidentally ingested, can lead to autoimmune disorders (see Chapter 23). These agents act on many cells, including B cells, and injection of mercuric chloride in rats and mice has been used as a model of B-cell autoimmunity leading to glomerulonephritis (Bagenstose et al., 1999; Nielsen and Hultman, 2002). However, this model only develops in susceptible rodent strains and is dependent on T-cell function (Nielsen and Hultman, 2002). The main feature of this model is the production of antinucleolar autoantibodies, with fibrillarin being the identified nucleolar reactant (Nielsen and Hultman, 2002). Modification of self-fibrillarin by mercury is thought to trigger the autoreactive response (Nielsen and Hultman, 2002).

Mineral Oils

Mineral oils are not normally toxic and are used regularly by humans. However, injection of hydrocarbon oil components such as pristane in mice can induce autoimmune disorders, notably arthritis in rats and lupus-like symptoms in mice characterized by the production of various anticytoplasmic and antinuclear autoantibodies (Holmdahl et al., 2001; Kuroda et al., 2004). Pristane has multiple effects on the immune system, including the induction of cytokines and lymphocyte activation. Pristane treatment leads to the emergence of autoreactive T cells and a reduction in regulatory T cells (Yang et al., 2003). The role of pristane on B-cell tolerance, however, is unclear. There is expansion of the MZ B-cell compartment in pristane-treated mice, which suggests a possible interference with B-cell tolerance during lymphocyte maturation, as described above (Yang et al., 2003).

Genetic Influences on B-Cell Tolerance and Autoimmunity

B-cell homeostasis results from the very fine balance between lymphocyte death and survival (Nemazee et al., 2000). This process is precise and discriminates between unwanted autoreactive cells that have to be eliminated and useful B cells that have to be positively selected for survival. Any defect of this process may either prevent the development of B cells or, conversely, allow the survival of harmful autoreactive B cells that are normally deleted. The immune

system has in place several checkpoints designed to eliminate or neutralize self-reactive B cells as they develop and emerge in the periphery (Goodnow et al., 1995; see Chapter 13). The nature of the signal delivered to B cells via their BCR is a critical factor controlling B-cell tolerance (Nemazee et al., 2000; Hardy and Hayakawa, 2001). Gene-targeting experiments have revealed numerous genes involved in B-cell tolerance. B-cell abnormalities and autoimmunity arising as the result of the absence or altered expression of B-cell-specific genes are described below.

Bcl-2 Family of Prosurvival/Proapoptosis Molecules

The Bcl-2 family of pro- and anti-apoptotic molecules plays an important role in the regulation of B-cell homeostasis (Chao and Korsmeyer, 1998; Marsden and Strasser, 2003; Strasser and Bouillet, 2003). One particular protein from this family, Bcl-2, is a central prosurvival molecule for B cells (Chao and Korsmeyer, 1998). Survival signals triggered by the BCR throughout the life of B cells are likely to involve upregulation of Bcl-2 in these cells (Strasser and Bouillet, 2003). Enforced Bcl-2 expression prolongs autoantibody responses and elicits autoimmune diseases (Strasser et al., 1991). Bim, another molecule from this family, has a proapoptotic function and has been shown to interact with Bcl-2, inactivating its survival effect in B cells (Bouillet et al., 1999). In the absence of Bim, mice have a large number of B cells and PCs (Bouillet et al., 1999). Many of these mice will die before 12 months of age from fatal immune complex-mediated glomerulonephritis (Bouillet et al., 1999). Therefore, molecules such as Bim play a critical role in preventing B-cell-mediated autoimmunity.

B-Cell Survival Factor BAFF

Expression of prosurvival molecules such as Bcl-2 in B cells can be triggered by prosurvival cytokines such as BAFF (Mackay et al., 1999), which is produced by T cells, monocytes/macrophages, neutrophils, and DCs, and is a very powerful soluble modulator of B-cell biology (Mackay and Browning, 2002; Mackay et al., 2003; Mackay and Tangye, 2004). BAFF can bind three separate receptors: BCMA (B-cell maturation antigen); BAFF receptor (BAFF-R); and TACI (transmembrane activator and CAML-interactor) (Mackay et al., 2003). BCMA is expressed at the surface of immunoglobulin-producing cells and is probably important for their survival in lymphoid tissues (Avery et al., 2003) and BM (O'Connor et al., 2004). BAFF transgenic mice have an excess of B lymphocytes in the periphery, secrete various autoantibodies, and develop an SLE-like condition marked by severe glomerulonephritis (Mackay et al., 1999; Gross et al., 2000; Khare et al., 2000), as well as a Sjögren-like syndrome characterized by the inflammation of the salivary glands, acinar cell destruction, and reduced saliva flow (Groom et al., 2002). Treatment of mice with BAFF inhibitors suppresses immune responses (Yu et al., 2000), reduces the lifespan of GCs (Rahman et al., 2003; Vora et al., 2003), prevents proteinuria and prolongs the life of mice with SLE-like disease (Gross et al., 2000). Such treatment also prevents disease in a mouse model of RA (Gross et al., 2001; Wang et al., 2001). These results suggest that excessive BAFF production may be a key factor promoting the development of autoimmunity. BAFF promotes B-cell survival *in vitro* and *in vivo* (Batten et al., 2000), suggesting a role for BAFF in peripheral B-cell homeostasis and maturation. This hypothesis was confirmed with $BAFF^{-/-}$ mice in which B-cell maturation is impaired beyond the transitional B-cell stage (Gross et al., 2001; Schiemann et al., 2001). Increased BAFF-mediated B-cell survival has also been shown to enhance humoral immune responses (Do et al., 2000). B cells in BAFF transgenic mice express higher levels of the prosurvival molecule Bcl-2 (Mackay et al., 1999). Combined, these findings suggested that abnormal BAFF production may be a key event in certain types of autoimmune diseases.

To best illustrate this, serum levels of BAFF are significantly higher in some patients with SLE or RA, and are particularly high in patients with Sjögren syndrome (Mackay and Tangye, 2004; Stohl, 2004), and levels of BAFF detected in the synovial fluid of patients with RA often exceed levels detected in the blood (Cheema et al., 2001). This suggests that BAFF is produced in inflammatory lesions *in situ* in response to inflammation and may further skew B-cell maturation towards an autoimmune-prone state by altering the threshold of B-cell survival, therefore feeding the immune system with more arthritogenic autoimmune B cells (Mackay and Mackay, 2002). The expanded MZ B-cell compartment may play some role in the autoimmune features of Sjögren syndrome because B cells with a MZ-like phenotype have been detected in inflamed salivary gland of BAFF transgenic mice (Groom et al., 2002), as well as inflamed salivary glands of patients with Sjögren syndrome (Hansen et al., 2002; Ochoa et al., 2001). The exact role of MZ B cells in autoimmunity is, however, still unclear (Lopes-Carvalho and Kearney, 2004). This population is known to include autoreactive B cells and MZ B cells are potent APCs for naïve T cells *ex vivo*, two aspects which may be important in autoimmunity in BAFF transgenic mice (Lopes-Carvalho and Kearney, 2004). Interestingly, one of the BAFF receptors, TACI, is a critical negative regulator of B-cell activation (von Bulow et al., 2001; Yan et al., 2001; Seshasayee et al., 2003). Lack of TACI leads to the expansion of the B-cell compartment, hyperreactivity, autoantibody production, and SLE-like autoimmune features, complicated by B-cell lymphomas as the mice age. Importantly, TACI is highly expressed on MZ B cells, suggesting these cells are potentially harmful and their repression via higher expression of TACI in normal animals is critical to maintain immune tolerance (Ng et al., 2004). BAFF may

promote B cell malignancies arising as a secondary problem in autoimmune disorders, and is a potent survival factor for malignant B-cells (Mackay and Tangye, 2004). Collectively, these observations suggest a possible critical role for BAFF in the pathogenesis of certain human autoimmune diseases, and BAFF inhibitors are currently being developed for the treatment of B-cell-mediated autoimmune pathologies (Mackay and Tangye, 2004; Stohl, 2004).

B-Cell Receptor Signaling and B-Cell Autoimmunity

BAFF is not the only element required for B-cell survival and development. Expression and signaling through the BCR is equally as important for B-cell survival, and engineered conditional suppression of BCR expression led to B-cell apoptosis (Lam et al., 1997; Nemazee et al., 2000; Niiro and Clark, 2002). Signaling through the BCR is very complex (see Chapter 19) and, depending on the strength of the stimuli and the stage of B-cell differentiation, the BCR can induce survival, death, anergy or activation (Nemazee et al., 2000). Signaling through the BCR is regulated by several signaling molecules and defect in these lead to B-cell autoimmunity (Yu et al., 2003).

Cell-surface BCR is formed of immunoglobulin heavy and light chains and is associated with two signaling molecules—Igα and Igβ. Following BCR ligation by antigen, the protein tyrosine kinase (PTK) Syk and the SRC-family PTK Lyn are activated [reviewed in Niiro and Clark (2002) and Chapter 19]. Lyn shares its function with other SRC kinases, Blk and Fyn. Lyn phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic tail of Igα and Igβ, which serve as docking sites for the recruitment and activation of Syk and the TEC-family PTK Btk. Syk and Btk are then central in the activation of phospholipase Cγ2 and phosphatidylinositol 3-kinase (PI3K), two crucial enzymes in the BCR signaling pathway [reviewed in (Niiro and Clark (2002)]. However, Lyn has an important inhibitory feedback function that controls signaling through the BCR to prevent B-cell autoimmunity. $Lyn^{-/-}$ B cells are hyperresponsive to BCR cross-linking, and $Lyn^{-/-}$ mice develop SLE-like symptoms characterized by splenomegaly, high levels of serum immunoglobulin, antinuclear autoantibodies, and glomerulonephritis (Hibbs et al., 1995; Nishizumi et al., 1995; Chan et al., 1997). Several molecules participate in Lyn-mediated inhibition of BCR signaling: FcγRIIb, paired immunoglobulin-like receptor B (PIR-B), CD72, and CD22, which have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic domains (Smith et al., 1998; Pan et al., 1999; Ravetch and Lanier, 2000; Niiro and Clark, 2002). ITIMs can recruit the phosphatases SH2-domain-containing protein tyrosine phosphatase 1 and 2 (SHP-1, SHP-2) and SH2-domain-containing inositol 5′ phosphatase (SHIP), which can inhibit activation initiated through the BCR (Ravetch and Lanier, 2000; Niiro and Clark, 2002).

Surprisingly, while an absence of Lyn augments BCR signaling, this defect did not prevent anergy of B cells in various transgenic models (Cornall et al., 1998). Additional experiments suggest that, depending on the developmental stage of B cells and the anatomic site, Lyn may have different and opposite effects on BCR signaling (Cornall et al., 1998). CD19 and Btk play an important role in BCR signaling, and lack of CD19 or Btk in $Lyn^{-/-}$ mice has been shown to reverse the autoimmune phenotype seen in $Lyn^{-/-}$ mice (Hasegawa et al., 2001). FcγRIIb, activated by antigen–antibody immune complexes, can also negatively regulate BCR signaling. $Fc\gamma RIIb^{-/-}$ mice develop B-cell-dependent autoimmune symptoms (Yu et al., 2003), but this effect appears to be strain specific, suggesting that additional factors contribute to the autoimmune-prone state created by the absence of FcγRII expression (Bolland and Ravetch, 2000).

CD95/CD95L Death Mechanism

CD95L is another member of the TNF family that binds to CD95 and induces apoptosis in a variety of cells, including lymphocytes (Mizuno et al., 2003; Moulian and Berrih-Aknin, 1998). CD95 appears to play a critical role in activation-induced cell death (Siegel et al., 2000). Impaired apoptosis in autoimmunity is described in detail in Chapters 15 and 70.

CD40/CD154 System and B-Cell Autoimmunity

The CD40/CD154 system is essential for B-cell activation, isotype switching, GC formation, and affinity maturation of B cells (Van Kooten and Banchereau, 1996). CD154 is transiently expressed on activated T cells but is also detectable and functional on the surface of B cells in human SLE (Banchereau et al., 2004). In transgenic mice, expression of CD154 on B cells correlated with the production of various autoantibodies and the development of nephritis (Higuchi et al., 2002). Treatment with anti-CD154-blocking antibodies is an effective way to prevent the development of disease in both mouse models of lupus (Kalled et al., 2001), and humans with SLE (Grammer et al., 2003).

Role of Complement and Toll-Like Receptors in Activating Autoreactive B Cells

Complement receptors expressed on B cells are critical for the generation of normal antibody responses, but they also help maintain B-cell tolerance (Boackle and Holers, 2003). Deficiencies of complement receptor 1 (CR1) and CR2 have been associated with SLE (Ahearn et al., 1996; Croix et al., 1996; Molina et al., 1996). Using the hen-egg lysozyme (HEL) transgenic model for B-cell tolerance, deficiency in CR1/CR2 led to impaired immune tolerance of HEL-specific transgenic B cells and the emergence of B cells responding to the self-antigen (Prodeus et al., 1998). CR1/CR2 may, therefore, participate in lowering the

threshold for negative selection of autoreactive B cells. Defects in complement receptors and complement components may also result in autoimmunity because of impaired clearance of apoptotic cells and immune complexes, as well as the inability to generate C3-coated autoantigens for CR1/CR2 (Korb and Ahearn, 1997; Boackle and Holers, 2003). Impaired expression of CR1/CR2 alone has mild effects on B-cell immune tolerance, but combined with other disease-susceptibility genes, defects in this system may contribute to severe autoimmune disorders such as SLE (Boackle and Holers, 2003).

Toll-like receptors (TLR) and DNA–autoantibody immune complexes participate in the activation of autoreactive B cells (Leadbetter et al., 2003). Low-affinity autoreactive B cells can be detected in the periphery of normal individuals, however, they are often anergic (Souroujon et al., 1988; Wardemann et al., 2003). This observation was recently explored in detail using AM14BCR transgenic mice in which B cells recognize self-IgG2a$^{j/a}$ and produce rheumatoid factors (RFs) against this particular isotype (Boackle and Holers, 2003). RF^{+} B cells develop normally despite the presence of the self-antigen and are not deleted or rendered anergic. Interestingly, these RF^{+} B cells become activated if AM14BCR transgenic mice are back-crossed onto an autoimmune-prone background, such as MRL-lpr (Wang and Shlomchik, 1999). Further studies showed that RF^{+} B cells from AM14BCR transgenic mice were in fact activated by immune complexes formed by antinucleosome IgG2a$^{j/a}$ antibodies and chromatin (Leadbetter et al., 2002). In addition, coactivation of TLR9 on B cells with the nucleic acid present in these immune complexes was required for the activation of the autoreactive RF^{+} B cells, as lack of MyD88, which is required for TLR9 signaling, in AM14BCR transgenic B cells prevented their activation by IgG2a$^{j/a}$–chromatin immune complexes (Leadbetter et al., 2002). Activation of TLR9 also induces BAFF expression by DCs, potentially contributing to further survival of autoreactive B-cells (Boule et al., 2004). This mechanism of autoreactive B-cell activation may be particularly important when clearance of chromatin from apoptotic cells is impaired, as may occur in particular autoimmune conditions (Figure 11.3; see Chapters 15 and 18).

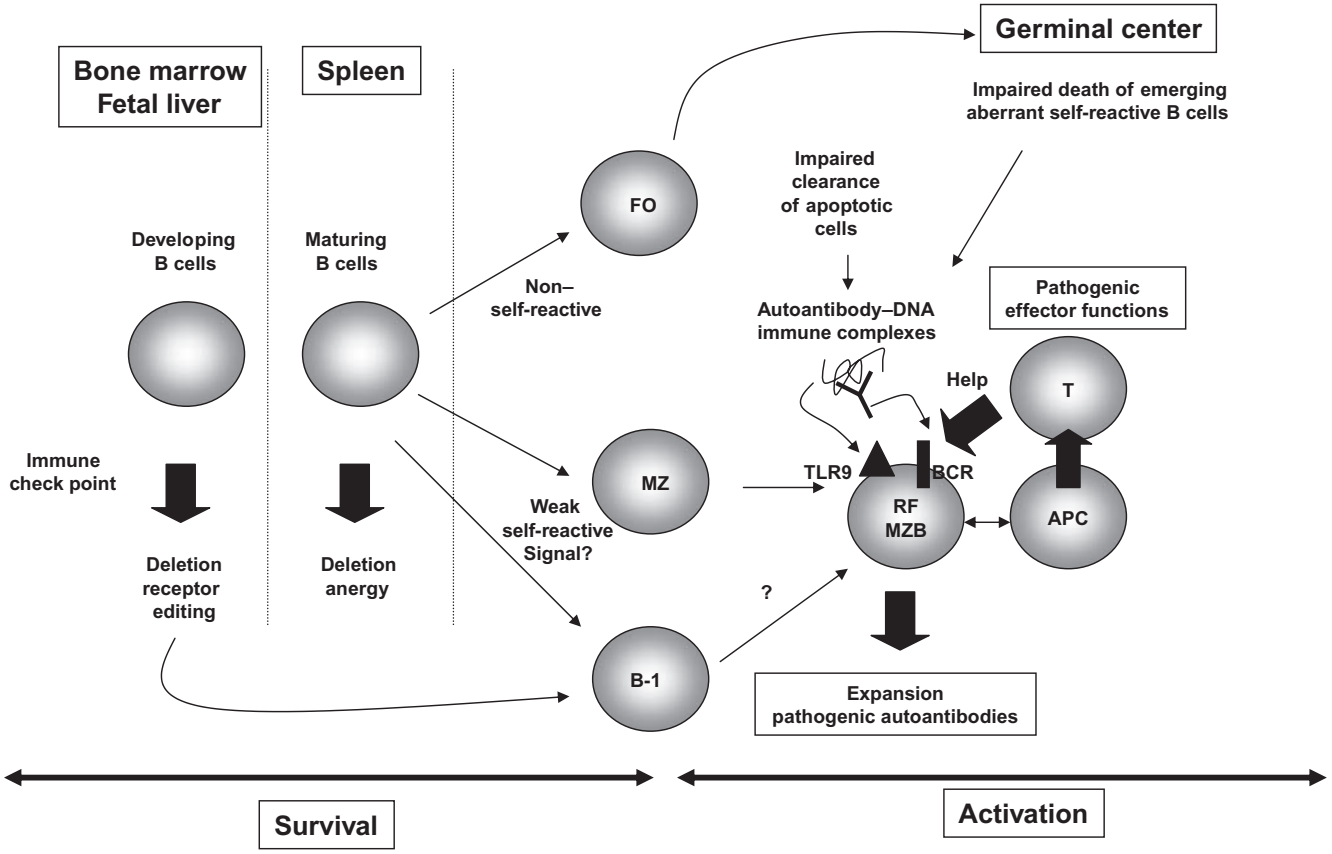


FIGURE 11.3 Mechanisms for the generation of autoreactive B cells and the development of autoimmunity. B-cells develop in the bone marrow and undergo further maturation in the spleen. Autoreactive B cells are censored at these stages either by deletion, receptor editing or anergy. If autoreactive B cells escape these checkpoints they can become activated in the periphery by aberrant signals delivered through the B-cell receptor (BCR), Toll-like receptor (TLR), or death receptor. As a result, autoreactive pathogenic antibodies will be produced. APC, antigen-presenting cell.

Pathogenicity of B cells in Autoimmunity

Over many years, scientists have questioned ways in which B cells trigger autoimmune disorders, and whether autoantibodies detected in the blood in various diseases have any pathogenic role. In human RA and SLE, levels of autoantibodies detected in the serum may correlate only weakly with disease severity and alternative pathogenic roles for B cells have been sought. Among the explanations, current diagnostic assays may not necessarily be detecting the "right" autoantibody in these patients, or truly relevant antibodies may be absorbed *in vivo* by the target tissue. However, recent studies in the mouse have shown that some autoreactive B cells do indeed secrete pathogenic autoantibodies, which alone can cause tissue destruction. This is certainly the case for arthritis in mice, illustrated by the K/BxN model developed by Mathis et al. (Kouskoff et al., 1996; Korganow et al., 1999) and to some extent in the collagen-induced arthritis model in mice. The K/BxN model resulted from mating a TCR-transgenic mouse onto a nonobese diabetic (NOD) background. The resulting progeny rapidly developed spontaneous and severe polyarthritis resembling human RA (Kouskoff et al., 1996). The autoreactive B cells in these mice secrete autoantibodies to glucose phosphate isomerase which, upon passive transfer, induce joint inflammation and destruction within 2–3 days (Korganow et al., 1999; Maccioni et al., 2002). These effects depended in part on mast cells and the C5a/C5a receptor complement system (Ji et al., 2001; Lee et al., 2002). This work shows that, in models of RA, damage to joint tissues can be caused solely by pathogenic autoantibodies. This may also be the case for SLE where immune complexes between DNA and anti-DNA autoantibodies can aberrantly activate autoreactive B cells via coactivation of the BCR and TLRs (Leadbetter et al., 2003).

However, in other diseases, including SLE, there is another role for autoreactive B cells. Here, B cells are required for the development of SLE on the MRL-lpr background (Chan et al., 1999); however, when MRL-lpr mice were engineered to produce B cells unable to secrete immunoglobulin, the mice still developed glomerulonephritis. Hence, B cells themselves, in the absence of autoantibodies, must have a pathogenic effect in this model (Chan et al., 1999). Finally, aside from producing autoantibodies, B cells can serve as APCs for T cells (Lanzavecchia, 1985; Roosnek and Lanzavecchia, 1991; Mamula et al., 1994; Liu et al., 1995; Attanavanich and Kearney, 2004). The MZ and memory B-cell subsets seem to be particularly efficient as APCs when compared to other B-cell subsets (Liu et al., 1995; Attanavanich and Kearney, 2004). As mentioned above, MZ B cells may play a role in autoimmunity and their autoimmune characteristics associated with their ability to activate T cells may be the key combination driving autoimmunity in some circumstances (Figure 11.3).

In summary, very few autoreactive B cells are present in normal mice and when they are, they are usually located away from T-cell help and are not normally activated. Increased B-cell survival may allow the emergence of autoreactive B cells and modified conditions of B-cell activation, including impaired clearance of apoptotic cells and hyperresponsive BCR signaling. Whether this participation occurs via the secretion of pathogenic autoantibodies and/or the indirect activation of self-reactive pathogenic T cells remains to be determined and may differ depending on the autoimmune condition.

Formation of Ectopic Lymphoid Tissue and Germinal Centers

In human autoimmune disease, the affected tissues are usually of nonlymphoid origin, and are thus devoid of immune cells. However, it has been known for over 40 years that the inflamed tissues of patients with diseases such as RA (synovial tissue), reactive arthritis (synovial tissue), Sjögren syndrome (salivary glands), myasthenia gravis (thymus) and Hashimoto's thyroiditis (thyroid) contain large numbers of infiltrating, and usually activated, lymphocytes (Berek and Kim, 1997). More recently, such structures have been detected in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS), as well as in animal models of this disease, and this is often accompanied by the presence of oligoclonal antibodies in the CSF (Corcione et al., 2004; Magliozzi et al., 2004; Serafini et al., 2004). In RA patients, three distinct patterns of cellular infiltrates could be resolved: 1) diffuse infiltrates containing T cells, B cells, macrophages, and DCs (approximately 50% of patients); 2) T–B-cell aggregates (approximately 25%); or 3) B cells, T cells, DCs, and FDCs, which form GC-like structures (approximately 25%) (Berek and Kim, 1997; Takemura et al., 2001a). In these latter patients, the environment of the affected tissues could support the formation of GCs. Indeed, the concentration of ectopic follicle-like structures correlated with the levels of autoantibody in RA patients, suggesting that formation of GCs in inflamed tissues directly contributes to the levels of autoantibody (Weyand and Goronzy, 2003). PCs could also be detected within the affected tissues, and these were positioned surrounding the follicle-like structure formed by the infiltrating lymphocytes, rather than within the cluster (Kim et al., 1999). This parallels the exit of PCs from GCs formed within secondary lymphoid tissues. The detection of these ectopic GCs raises the possibility that production of inflammatory chemokines may retain PCs within these tissues rather than allowing their migration to other sites. Interestingly, synoviocytes

generated from synovial tissue have been found to support the survival and immunoglobulin production by activated B cells (Dechanet et al., 1995). This led to the proposal that synoviocytes within inflamed tissues could maintain PCs present within synovial tissue, analogous to stromal cells present in spleen and BM (Ellyard et al., 2005), and thus contribute to the accumulation of PCs within this tissue (Dechanet et al., 1995).

Examination of B cells in these nonlymphoid sites revealed them to be of GC origin, i.e., memory B cells and long-lived PCs. Similarly, B cells with a phenotype corresponding to predominantly memory cells, GC cells, and PCs were detected within the CSF, but not in peripheral blood, of MS patients (Corcione et al., 2004). Clonally-related immunoglobulin V region sequences were detected within these ectopic GCs in synovial tissue and salivary glands of patients with RA and Sjögren syndrome, respectively. The patterns of mutation within the memory B cells and PCs were characteristic of antigen selection (Berek and Kim, 1997; Stott et al., 1998; Kim et al., 1999). Furthermore, clonally-related sequences encoding TCR β chain V genes were microdissected from distinct follicles within the synovial tissue of the same patient, suggesting that these clonotypic cells may recognize the same (auto) antigen (Takemura et al., 2001b). Hence, antigen-driven B-cell diversification is occurring within these ectopic GCs, and the accumulation of B cells within the tissue is not simply due to migration of such cells formed at other (lymphoid) sites. Thus, it is likely that differentiation of (auto) antigen-specific B cells into memory B cells and PCs takes place within the GCs of inflamed tissues.

A xenogeneic animal model of RA was developed by transplanting human synovial tissue containing inflammatory infiltrates into NOD.scid mouse ("RA.scid mice"). This model has facilitated examination of the role played by B cells in the development and severity of RA. These studies suggested that B cells within the inflamed tissue may not only be responsible for secreting pathogenic or arthritogenic immunoglobulin, but also for maintaining the population of activated T cells by acting as efficient APCs. It was found that when T-cell clones were transferred into RA.scid mice they elicited production of increased levels of pro-inflammatory cytokines IL-1β, TNF-α, and IFN-γ, demonstrating that these $CD4^+$ T cells are important effector cells in the inflammatory process observed in RA. Strikingly, when B-cell-depleted synovial tissue was transplanted into SCID mice, the cotransferred synovial $CD4^+$ T cells did not become activated. Similarly, when RA.scid mice were established using GC-containing synovial tissue, and treated with depleting anti-CD20 monoclonal antibody, the ability of $CD4^+$ T cells to induce generation of inflammatory cytokines was reduced by up to 80–100% (Takemura et al., 2001b). These results indicated that activation of T cells in RA is B-cell dependent, and highlights the critical role of B cells in the pathogenesis of autoimmunity.

Disturbances to B-Cell Homeostasis

Several perturbations to B-cell homeostasis have been recognized in human autoimmune diseases. Sjögren syndrome patients have reduced numbers of circulating memory B cells, perhaps resulting from the retention or accumulation of these cells in the inflamed salivary glands (Bohnhorst et al., 2001; Hansen et al., 2002). Although SLE patients are typically lymphopenic, there is a striking increase in the number of circulating plasmablasts, as well as cells with a phenotype of pre-GC cells (Odendahl et al., 2000; 2003; Arce et al., 2001; Grammer et al., 2003), and there is a correlation between the number of circulating plasmablasts and the severity and duration of SLE (Jacobi et al., 2003). Lastly, the majority of B cells in peripheral blood mononuclear cells (PBMC) of RA patients are memory B cells and plasmablasts/cells (Lindenau et al., 2003). Together, these findings suggest that the presence of increased numbers of cells that are normally restricted to lymphoid tissue suggests further dysregulated control of the GC in autoantibody-mediated autoimmunity, and this may contribute to the generation of effector B cells with pathogenic autoantigen specificity.

Altered Cytokine and Chemokine Production: Relationship to Lymphoid Neogenesis and Exacerbated B-Cell Function

Elevated levels of some cytokines have been observed in both the serum and inflamed tissue of autoimmune patients. This has been most pronounced for serum TNF-α, IL-6, and IL-10, while these cytokines, as well as lymphotoxin (LT)-α/β, IL-1β and type 1 interferons (IFNs), have been detected at elevated levels in synovial tissue of RA patients (Brennan and Feldmann, 1996; Feldmann et al., 1996) and CSF of MS patients (Corcione et al., 2004). High serum levels of CD154, as well as expression on circulating pre-GC B cells, have been observed in SLE (Banchereau et al., 2004). These cytokines could contribute to the chronic inflammation observed in, and subsequent destruction of, the tissues affected by autoimmune diseases. Indeed, in RA, TNF-α appears to be the dominant cytokine that sustains inflammation, because *in vitro* culture of synoviocytes from RA patients in the presence of neutralizing TNF-α antibody reduced the spontaneous production of IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-6, while blocking IL-1-reduced production of IL-6, but had no effect on TNF (Brennan and Feldmann, 1996; Feldmann et al., 1996; Feldmann and Maini, 2001). In addition to being

involved in the inflammatory response, some of these cytokines may contribute to the disease by facilitating and supporting the generation of ectopic lymphoid tissue, as well as by having a direct effect on the pathogenic B cells.

Lymphoid Neogenesis

TNF-α and LT are not only proinflammatory cytokines but also have fundamental roles in lymphoid neogenesis. Gene-targeting studies demonstrated that mice deficient in TNF, LT or their corresponding receptors have structural and functional defects in their secondary lymphoid organs (Fu and Chaplin, 1999). The mechanism by which TNF and LT mediate organogenesis is in part through regulating production of the lymphoid homeostatic chemokines CXCL13, CCL19, and CCL21(Ngo et al., 1999). Consistent with this, a correlation was observed between the level of expression of LT-α, LT-β, CXCL13, and CCL21, and the presence of FDCs and GCs within synovial tissue (Shi et al., 2001; Takemura et al., 2001a). Expression of these soluble mediators and the level of organization of the lymphoid cells in the synovium in RA suggested that the formation of GC was dependent on the concentrations of both CXCL13 and LT-β (Takemura et al., 2001a). CXCL13 is usually produced by FDCs in secondary lymphoid tissues. However, in RA, CXCL13-producing cells were identified as FDCs and also vascular endothelium and synoviocytes (Shi et al., 2001; Takemura et al., 2001a). Similar to normal lymphoid tissues, the predominant source of LT-β was the infiltrating B cells (Takemura et al., 2001a). Thus, within inflamed tissue, it could be that TNF-α produced by infiltrating inflammatory cells induces production of CXCL13 by vascular endothelium and synoviocytes. CXCL13 then recruits circulating B cells, and induces the expression of LT-β. LT-expressing B cells are required for the development of FDCs, which, once developed, would support the formation of GCs by acting as a repository for antigen. This model is consistent with the findings that treatment of RA patients with TNF-α antagonists resulted in reduced trafficking of leukocytes into, and reduced angiogenesis in, inflamed synovial joints (Feldmann and Maini, 2001). This model is also supported by the findings that transgenic expression of CXCL13 in murine pancreatic β cells causes infiltration of B cells, T cells, and DCs into the pancreas. Furthermore, pancreatic expression of CXCL13 resulted in the development of structures reminiscent of lymph nodes, and expression of CCL21, which probably mediates the recruitment of DCs and the majority of the T cells (Luther et al., 2000). B cells appear to be required for the establishment of pancreatic follicles, longevity of ectopic GCs, and lymphoid neogenesis because none of these features was observed in CXCL13 transgenic mice that were B-cell deficient (Luther et al., 2000). Thus, lymphoid neogenesis is secondary to the recruitment of B cells, consistent with the previous findings documenting the requirement for B cells in the development of follicles in secondary lymphoid tissues (Fu and Chaplin, 1999). The requirement for B cells in this process corresponds to the requirement for B cells in inducing activation of putative autoantigen-specific $CD4^+$ T cells in RA.scid mice, and suggests that in this animal model T-cell activation may be mediated by B-cell-dependent recruitment of T cells to the inflamed synovial tissue, or by B cells acting as potent APCs for pathogenic $CD4^+$ T cells (Takemura et al., 2001b).

It is of interest that lymphoid-like structures were demonstrable within the CNS of MS patients, and that there were high levels of CXCL12, CXCL13, LT-α, and BAFF within these structures (Corcione et al., 2004; Magliozzi et al., 2004; Serafini et al., 2004). Thus, in autoimmune disease of the CNS, LT-α and CXCL13 may contribute to the recruitment of B cells, and BAFF—produced by astrocytes (Krumbholz et al., 2005)—promotes the survival of B cells in these ectopic lymphoid tissues.

B-Cell Behavior

Cytokines such as IL-6, IL-10, IFN-α and CD40L are also elevated in the serum of patients with autoimmune diseases, and serum levels of IL-10 correlate with disease activity (Banchereau et al., 2004; Beebe et al., 2002; Llorente and Richaud-Patin, 2003). Notably, these have potent effects on the survival, growth, and differentiation of human B cells (Banchereau and Rousset, 1992; Banchereau et al., 2004).

IL-10

IL-10 may have a specific role in SLE. First, the cell types responsible for the production of IL-10 are B cells and monocytes, while T cells from SLE patients do not spontaneously produce significant levels of IL-10 (Beebe et al., 2002; Llorente and Richaud-Patin, 2003). Second, the increased levels of human IgG, as well as anti-dsDNA antibody, in the serum of SCID mice, observed following transfer of PBMC from SLE patients, was dramatically reduced in the presence of neutralizing anti-IL-10 monoclonal antibody (Llorente et al., 1995). Third, treatment of patients with anti-IL-10 monoclonal antibody resulted in a significant decrease in the SLE disease activity index for up to 6 months, as well as a reduced requirement for immunosuppression with corticosteroid drugs (Llorente et al., 2000). Similarly, using a pharmacologic approach, it was found that "lupus-like" symptoms of SCID mice reconstituted with mononuclear cells from patients with SLE and lupus-prone NZB/WF1 mice were ameliorated following treatment with the immunomodulatory drug AS101, whose effect is achieved by its ability to dramatically reduce production of IL-10 by SLE MNC (Kalechman et al., 1997). IL-10 is not only a powerful growth and differentiation factor for human

B cells, but it is also capable of protecting GCs B cells from apoptosis by increasing expression of Bcl-2 (Beebe et al., 2002). This may explain the increased number of GC-like B cells detected among PBL of SLE patients (Arce et al., 2001; Grammer et al., 2003). Thus, it is likely that IL-10 plays a complex role in SLE, rather than simply promoting the activation of autoantibody-producing B cells.

IFN-α

Another cytokine capable of inducing B-cell activation and differentiation that is overexpressed in SLE is IFN-α (Banchereau et al., 2004). Type 1 IFNs (α, β) are secreted in large quantities by plasmacytoid DCs (PDCs) activated through TLRs either by viruses or nucleic acid complexes (Banchereau et al., 2004). In SLE, these could take the form of immune complexes containing DNA or RNA. IFN-α/β can mediate the differentiation of activated human B cells into plasmablasts, which further develop into PCs in the presence of IL-6, another product of PDCs (Banchereau et al., 2004). *In vitro* studies have demonstrated that production of BAFF by DCs is increased in the presence of either exogenous IL-10 or IFN-α (Craxton et al., 2003; Litinskiy et al., 2002). It is possible that increased serum levels of IL-10 and IFN-α may act not only on the pathogenic B cell, but also on other effector cells, specifically DCs, by increasing their release of BAFF. Thus, neutralization of IL-10 and/or IFN-α may improve SLE by initially reducing circulating levels of these cytokines, and subsequently by reducing serum BAFF levels. It is presently unknown whether IL-10 induces the production of IFN-α by DCs or vice versa. Thus, it is possible that the increased production of BAFF by DCs stimulated with IL-10 or IFN-α is an indirect effect of each cytokine inducing the production the other.

DIFFERENT CYTOKINES—DIFFERENT AUTOIMMUNE DISEASES

Treatment of RA by neutralizing TNF-α has yielded great results. However, one notable side effect of this treatment has been the development of anti-DNA antibody in approximately 15% of patients (Feldmann and Maini, 2001). Interestingly, anti-TNF therapy is ineffective in SLE (and most other autoimmune diseases), suggesting that TNF has no role in the development and/or pathogenesis of such diseases (Banchereau et al., 2004). On the other hand, the immunomodulatory drug AS101 was found not only to decrease IL-10 production by mononuclear cells from patients with SLE, but also to increase production of TNF-α (Kalechman et al., 1997). These changes in serum cytokine levels of treated SCID mice were associated with reduced SLE-like pathology. Interestingly, polymorphisms in the TNF-α gene of NZW mice result in reduced production of TNF-α and increased susceptibility to the development of SLE-like disease (Jacob and McDevitt, 1988). Thus, reduced TNF-α production, or reductions in the relative amount of this cytokine due to either intrinsic or extrinsic regulatory mechanisms, may predispose individuals to autoimmune diseases such as SLE.

CONCLUDING REMARKS

Clinicians have long pondered over the association of evident B-cell overactivity and the genesis of autoimmune disease. One main reason for this is the frequently weak correlation between levels of autoantibodies and the severity of disease. However, current diagnostics may measure only a limited set of autoantibodies of which not all participate in pathologic processes. However, recent work on mice and humans has clearly identified pathogenic autoantibodies, which alone can cause tissue damage or adverse functional effects. In fact, these are probably the tip of an iceberg and tissue damage is likely triggered by several, if not different, autoantibodies, from patient to patient, with the challenge being to identify pathways by which they act. These ideas are fortified by the increasing range of autoimmune diseases alleviated by therapy with intravenous immunoglobulin for which the primary benefit depends on blocking of antibody-mediated effects (see Chapter 16). Immune cells clearly do not act alone and a mistake in the past has been to draw conclusions about autoimmune pathologies based on data from isolated subsets of lymphocytes, whether they be T cells, B cells or macrophages. Autoimmunity is a conspiracy involving the concerted misbehavior of many immune cell types. B cells play a major disruptive role in this plot as APCs and cytokine producers which, when inappropriately activated, will support the activation of harmful T cells and other cell types. The impressive efficacy of B-cell-depleting therapies in RA (e.g., anti-CD20 monoclonal antibody), which clearly spare PCs, is a strong indication that B cells as accessory cells are key elements driving autoimmunity. The challenge we face is to determine whether all B cell subsets are equal in that function and whether refined therapies targeting pathogenic B-cell subsets could be developed, sparing innocuous B cells, preventing toxicity and side effects due to prolonged and general B-cell deficiency.

References

Ahearn, J.M., Fischer, M.B., Croix, D., Goerg, S., Ma, M., Xia, J., Zhou, X., Howard, R.G., Rothstein, T.L., and Carroll, M.C. (1996). Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. *Immunity 4*, 251–262.

Ahmed, R. and Gray, D. (1996). Immunological memory and protective immunity: understanding their relation. *Science 272*, 54–60.

Arce, E., Jackson, D.G., Gill, M.A., Bennett, L.B., Banchereau, J., and Pascual, V. (2001). Increased frequency of pre-germinal center B cells

and plasma cell precursors in the blood of children with systemic lupus erythematosus. *J. Immunol. 167*, 2361–2369.

Attanavanich, K. and Kearney, J.F. (2004). Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells. *J. Immunol. 172*, 803–811.

Avery, D.T., Kalled, S.L., Ellyard, J.E., Ambrose, C., Bixler, S.A., Thien, M., Brink, R., Mackay, F., Hodgkin, P.D., and Tangye, S.G. (2003). BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J. Clin. Invest. 112*, 286–297.

Bagenstose, L.M., Salgame, P., and Monester, M. (1999). Cytokine regulation of a rodent model of mercury chloride-induced autoimmunity. *Environ. Health Perspect. 107*, 807–810.

Banchereau, J. and Rousset, F. (1992). Human B lymphocytes: phenotype, proliferation, and differentiation. *Adv. Immunol. 52*, 125–262.

Banchereau, J., Pascual, V., and Palucka, A.K. (2004). Autoimmunity through cytokine-induced dendritic cell activation. *Immunity 20*, 539–550.

Batten, M., Groom, J., Cachero, T.G., Qian, F., Schneider, P., Tschopp, J., Browning, J.L., and Mackay, F. (2000). BAFF mediates survival of peripheral immature B lymphocytes. *J. Exp. Med. 192*, 1453–1465.

Beebe, A.M., Cua, D.J., and de Waal Malefyt, R. (2002). The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). *Cytokine Growth Factor Rev. 13*, 403–412.

Berek, C. and Kim, H.J. (1997). B-cell activation and development within chronically inflamed synovium in rheumatoid and reactive arthritis. *Semin. Immunol. 9*, 261–268.

Boackle, S.A. and Holers, V.M. (2003). Role of complement in the development of autoimmunity. *Curr. Dir. Autoimmun. 6*, 154–168.

Bohnhorst, J.O., Thoen, J.E., Natvig, J.B., and Thompson, K.M. (2001). Significantly depressed percentage of CD27+ (memory) B cells among peripheral blood B cells in patients with primary Sjögren's syndrome. *Scand. J. Immunol. 54*, 421–427.

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.

Borriello, F., Sethna, M.P., Boyd, S.D., Schweitzer, A.N., Tivol, E.A., Jacoby, D., Strom, T.B., Simpson, E.M., Freeman, G.J., and Sharpe, A.H. (1997). B7-1 and B7-2 have overlapping, critical roles in immunoglobulin class switching and germinal center formation. *Immunity 6*, 303–313.

Bouillet, P., Metcalf, D., Huang, D.C.S., Tarlinton, D.M., Kay, T.W.H., Köntgen, F., Adams, J.M., and Strasser, A. (1999). Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to prelude autoimmunity. *Science 286*, 1735–1738.

Boule, M.W., Broughton, C., Mackay, F., Akira, S., Marshak-Rothstein, A., and Rifkin, I.R. (2004). Toll-like receptor 9-dependent and -independent dendritic cell activation by chromatin-immunoglobulin G complexes. *J. Exp. Med. 199*, 1631–1640.

Bowman, E.P., Campbell, J.J., Soler, D., Dong, Z., Manlongat, N., Picarella, D., Hardy, R.R., and Butcher, E.C. (2000). Developmental switches in chemokine response profiles during B cell differentiation and maturation. *J. Exp. Med. 191*, 1303–1318.

Brennan, F.M. and Feldmann, M. (1996). Cytokines in autoimmunity. *Curr. Opin. Immunol. 8*, 872–877.

Calame, K.L. (2001). Plasma cells: finding new light at the end of B cell development. *Nature Immunol. 2*, 1103–1108.

Calame, K.L., Lin, K.I., and Tunyaplin, C. (2003). Regulatory mechanisms that determine the development and function of plasma cells. *Annu. Rev. Immunol. 21*, 205–230.

Castagnetta, L., Granata, O.M., Traina, A., Cocciadiferro, L., Saetta, A., Stefano, R., Cutolo, M., and Carruba, G. (2002). A role for sex steroids in autoimmune diseases. A working hypothesis and supporting data. *Ann. N. Y. Acad. Sci. 966*, 193–203.

Chan, O.T.M., Hannum, L.G., Haberman, A.M., Madaio, M.P., and Schlomchik, M.J. (1999). A novel mouse with B cells lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. *J. Exp. Med. 189*, 1639–1647.

Chan, V.W.F., 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 downregulation. *Immunity 7*, 69–81.

Chao, D.T. and Korsmeyer, S.J. (1998). BCL-2 family: Regulators of cell death. *Annu. Rev. Immunol. 16*, 395–419.

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.

Corcione, A.S., Casazza, S., Ferretti, E., Giunti, D., Zappia, E., Pistorio, A., Gambini, C., Mancardi, G.L., Uncelli, A., and Pistoria, V. (2004). Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis. *Proc. Natl. Acad. Sci. USA 101*, 11064–11069.

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 SHIP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity 8*, 497–508.

Craxton, A., Magaletti, D., Ryan, E.J., and Clark, E.A. (2003). Macrophage and dendritic-cell dependent regulation of human B cell proliferation requires the TNF family ligand BAFF. *Blood 101*, 4464–4471.

Croix, D.A., Ahearn, J.M., Rosengard, A.M., Han, S., Kelsoe, G., Ma, M., and Carroll, M.C. (1996). Antibody response to a T-dependent antigen requires B cell expression of complement receptors. *J. Exp. Med. 183*, 1857–1864.

Crotty, S., Kersh, E.N., Cannons, J., Schwartzberg, P.L., and Ahmed, R. (2003). SAP is required for generating long-term humoral immunity. *Nature 421*, 282–287.

Cyster, J.G. (2003). Homing of antibody secreting cells. *Immunol. Rev. 194*, 48–60.

Datta, S.K. and Kalled, S.L. (1997). CD40–CD40 ligand interaction in autoimmune disease. *Arthritis Rheum. 40*, 1735–1745.

de Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C.G., and de Vries, J.E. (1991). Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med. 174*, 1209–1220.

Dechanet, J., Merville, P., Durand, I., Banchereau, J., and Miossec, P. (1995). The ability of synoviocytes to support terminal differentiation of activated B cells may explain plasma cell accumulation in rheumatoid synovium. *J. Clin. Invest. 95*, 456–463.

Do, R.K., Hatada, E., Lee, H., Tourigny, M.R., Hilbert, D., and Chen-Kiang, S. (2000). Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. *J. Exp. Med. 192*, 953–964.

Dunn-Walters, D.K., Isaacson, P.G., and Spencer, J. (1995). Analysis of mutations in immunoglobulin heavy chain variable region genes of microdissected marginal zone (MGZ) B cells suggests that the MGZ of human spleen is a reservoir of memory B cells. *J. Exp. Med. 182*, 559–566.

Ellyard, J.I., Avery, D.T., Phan, T.G., Hodgkin, P.D., and Tangye, S.G. (2004). Ag-selected Ig-secreting cells persist in human spleen as well as bone marrow. *Blood 103*, 3805–3812.

Ellyard, J.I., Avery, D.T., Mackay, C.R., and Tangye, S.G. (2005). Contribution of stromal cells to the migration, function and retention of plasma cells in human spleen: potential roles of CXCL12, IL-6 and CD54. *Eur. J. Immunol. 35*, 699–708.

Feldmann, M. and Maini, R.N. (2001). Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu. Rev. Immunol. 19*, 163–196.

Feldmann, M., Brennan, F.M., and Maini, R.N. (1996). Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol. 14*, 397–440.

Ferguson, S.E., Han, S., Kelsoe, G., and Thompson, C.B. (1996). CD28 is required for germinal center formation. *J. Immunol. 156*, 4576–4581.

Fu, Y.X. and Chaplin, D.D. (1999). Development and maturation of secondary lymphoid tissues. *Annu. Rev. Immunol. 17*, 399–433.

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.

Grammer, A.C., Slota, R., Fischer, R., Gur, H., Girschick, H., Yarboro, C., Illei, G.G., and Lipsky, P.E. (2003). Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154–CD40 interactions [see comment]. *J. Clin. Invest. 112*, 1506–1520.

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.

Grimaldi, C.M., Cleary, J., Dagtas, A.S., Moussai, D., and Diamond, B. (2002). Estrogen alters thresholds for B cell apoptosis and activation. *J. Clin. Invest. 109*, 1625–1633.

Grimbacher, B., Warnatz, K., and Peter, H.H. (2003). The immunological synapse for B-cell memory: the role of ICOS and its ligand for the longevity of humoral immunity. *Curr. Opin. Allergy Clin. Immunol. 3*, 409–419.

Groom, J., Kalled, S.L., Cutler, A.H., Olson, C., Woodcock, S.A., Schneider, P., Tschopp, J., Cachero, T.G., Batten, M., and Wheway, J. (2002). Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J. Clin. Invest. 109*, 59–68.

Gross, J.A., Johnston, J., Mudri, S., Enselman, R., Dillon, S.R., Madden, K., Xu, W., Parrish-Novak, J., Forster, D., and Lofton-Day, C. (2000). TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature 404*, 995–950.

Gross, J.A., Dillon, S.R., Mudri, S., Johnston, J., Littau, A., Roque, R., Rixon, M., Schou, O., Foley, K.P., and Haugen, H. (2001). TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLyS. *Immunity 15*, 289–302.

Gulino, A.V. and Notarangelo, L.D. (2003). Hyper IgM syndromes. *Curr. Opin. Rheumatol. 15*, 422–429.

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.

Hansen, A., Odendahl, M., Reiter, K., Jacobi, A.M., Feist, E., Scholze, J., Burmester, G.R., Lipsky, P.E., and Dorner, T. (2002). Diminished peripheral blood memory B cells and accumulation of memory B cells in the salivary glands of patients with Sjögren's syndrome. *Arthritis Rheum. 46*, 2160–2171.

Hardy, R.R. and Hayakawa, K. (2001). B cell development pathways. *Annu. Rev. Immunol. 19*, 595–621.

Hasegawa, M., Fujimoto, M., Poe, J.C., Steeber, D.A., Lowell, C.A., and Tedder, T.F. (2001). A CD19-dependent signaling pathway regulates autoimmunity in Lyn-deficient mice. *J. Immunol. 167*, 2469–2478.

Hibbs, M.L., Tarlinton, D.M., Armes, J., Grail, D., Hodgson, G., Maglitto, R., Stacket, 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.

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*, 9–12.

Holmdahl, R., Lorentzen, J.C., Lu, S., Olofsson, P., Wester, L., Holmberg, J., and Petterson, U. (2001). Arthritis induced in rats with non-immunogenic adjuvants as models for rheumatoid arthritis. *Immunol. Rev. 184*, 184–202.

Ishida, H., Hastings, R., Thompson-Snipes, L., and Howard, M. (1993). Modified immunological status of anti-IL-10 treated mice. *Cell Immunol. 148*, 371–384.

Jacob, C.O. and McDevitt, H.O. (1988). Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature 331*, 356–358.

Jacobi, A.M., Odendahl, M., Reiter, K., Bruns, A., Burmester, G.R., Radbruch, A., Valet, G., Lipsky, P.E., and Dorner, T. (2003). Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum. 48*, 1332–1342.

Ji, H., Gauguier, D., Ohmura, K., Gonzales, A., Duchatelle, V., Danoy, P., Garchon, H.-J., Degott, C., Lathrop, M., Benoist, C., and Mathis, D. (2001). Genetic influences on the end-stage effector phase of arthritis. *J. Exp. Med. 194*, 321–330.

Jung, J., Choe, J., Li, L., and Choi, Y.S. (2000). Regulation of CD27 expression in the course of germinal center B cell differentiation: the pivotal role of IL-10. *Eur. J. Immunol. 30*, 2437–2443.

Kalechman, Y., Gafter, U., Da, J.P., Albeck, M., Alarcon-Segovia, D., and Sredni, B. (1997). Delay in the onset of systemic lupus erythematosus following treatment with the immunomodulator AS101: association with IL-10 inhibition and increase in TNF-alpha levels. *J. Immunol. 159*, 2658–2667.

Kalled, S.L., Cutler, A.H., and Ferrant, J.L. (2001). Long-term anti-CD154 dosing in nephritic mice is required to maintain survival and inhibit mediators of renal fibrosis. *Lupus 10*, 9–22.

Khare, S.D., Sarosi, I., Xia, X.-Z., McCabe, S., Miner, K., Solovyev, I., Hawkins, N., Kelley, M., Chang, D., and Chang, D. (2000). Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc. Natl. Acad. Sci. USA 97*, 3370–3375.

Kim, H. J., Krenn, V., Steinhauser, G., and Berek, C. (1999). Plasma cell development in synovial germinal centers in patients with rheumatoid and reactive arthritis. *J. Immunol. 162*, 3053–3062.

Klein, U., Rajewsky, K., and Kuppers, R. (1998). Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J. Exp. Med. 188*, 1679–1689.

Krumbholz, M., Theil, D., Derfuss, T., Rosenwald, A., Schrader, F., Mororanu, C.M., Kalled, S.L., Hess, D.M., Serafini, B., Aloisi, F., Wekerle, H., Hohlfeld, R., and Meinl, E. (2005). BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma. *J. Exp. Med. 201*, 195–200.

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*, 4525–8.

Korganow, A.S., Ji, H., Mangialiao, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, G., Kikutani, H., Rajewski, K., and Pasquali, J.L. (1999). From systemic T cell reactivity to organ-specific autoimmune disease via immunoglobulin. *Immunity 10*, 451–461.

Kouskoff, V., Korganow, A.S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (1996). Organ-specific disease provoked by systemic autoimmunity. *Cell 87*, 811–822.

Kunkel, E.J. and Butcher, E.C. (2003). Plasma-cell homing. *Nat. Rev. Immunol. 3*, 822–829.

Kuroda, Y., Akaogi, J., Nacionales, D.C., Wasdo, S.C., Szabo, N.J., Reeves, W.H., and Satoh, M. (2004). Distinctive patterns of autoimmune response induced by different types of mineral oil. *Toxicolog. Sci. 78*, 222–228.

Lam, K.P., Kuhn, R., and Rajewsky, K. (1997). *In vivo* ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death [comment]. *Cell 90*, 1073–1083.

Lanzavecchia, A. (1985). Antigen-specific interaction between T and B cells. *Nature 314*, 537–539.

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors [see comment]. *Nature 416*, 603–607.

Leadbetter, E.A., Rifkin, I.R., and Marshak-Rothstein, A. (2003). Toll-like receptors and activation of autoreactive B cells. *Curr. Dir. Autoimmun. 6*, 105–122.

Lee, D.M., Friend, D.S., Gurish, M.F., Benoist, C., Mathis, D., and Brenner, M.B. (2002). Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science 297*, 1689–1692.

Lindenau, S., Scholze, S., Odendahl, M., Dorner, T., Radbruch, A., Burmester, G.R., and Berek, C. (2003). Aberrant activation of B cells in patients with rheumatoid arthritis. *Ann. N. Y. Acad. Sci. 987*, 246–248.

Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P., and Cerutti, A. (2002). DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL [see comments]. *Nat. Immunol. 3*, 822–829.

Liu, Y.J. and Banchereau, J. (1996). The paths and molecular controls of peripheral B-cell development. *Immunologist 4*, 55–66.

Liu, Y.J., Barthelemy, C., de Bouteiller, O., Arpin, C., Durand, I., and Banchereau, J. (1995). Memory B cells from human tonsils colonize mucosal epithelium and directly present antigen to T cells by rapid up-regulation of B7-1 and B7-2. *Immunity 2*, 239–248.

Liu, Y.J., Arpin, C., de Bouteiller, O., Guret, C., Banchereau, J., Martinez-Valdez, H., and Lebecque, S. (1996a). Sequential triggering of apoptosis, somatic mutation and isotype switch during germinal center development. *Semin. Immunol. 8*, 169–177.

Liu, Y.J., Grouard, G., de Bouteiller, O., and Banchereau, J. (1996b). Follicular dendritic cells and germinal centers. *Int. Rev. Cytol. 166*, 139–179.

Liu, Y.J., Malisan, F., de Bouteiller, O., Guret, C., Lebecque, S., Banchereau, J., Mills, F.C., Max, E.E., and Martinez-Valdez, H. (1996c). Within germinal centers, isotype switching of immunoglobulin genes occurs after the onset of somatic mutation. *Immunity 4*, 241–250.

Liu, Y.J., Oldfield, S., and MacLennan, I.C. (1988). Memory B cells in T cell-dependent antibody responses colonize the splenic marginal zones. *Eur. J. Immunol. 18*, 355–362.

Liu, Y.J., Zhang, J., Lane, P.J., Chan, E.Y., and MacLennan, I.C. (1991). Sites of specific B cell activation in primary and secondary responses to T cell-dependent and T cell-independent antigens. *Eur. J. Immunol. 21*, 2951–2962. [erratum appears in *Eur. J. Immunol. 22*, 615 (1992).]

Llorente, L. and Richaud-Patin, Y. (2003). The role of interleukin-10 in systemic lupus erythematosus. *J. Autoimmun. 20*, 287–289.

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.

Llorente, L., Richaud-Patin, Y., Garcia-Padilla, C., Claret, E., Jakez-Ocampo, J., Cardiel, M.H., Alcocer-Varela, J., Grangeot-Keros, L., Alarcon-Segovia, D., and Wijdenes, J. (2000). Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum. 43*, 1790–1800.

Lopes-Carvalho, T. and Kearney, J.F. (2004). Development and selection of marginal zone B cells. *Immunol. Rev. 197*, 192–205.

Luther, S.A., Lopez, T., Bai, W., Hanahan, D., and Cyster, J.G. (2000). BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis. *Immunity 12*, 471–481.

Maccioni, M., Zeder-Lutz, G., Huang, H., Ebel, C., Gerber, P., Hergueux, J., Marchal, P., Duchatelle, V., Degott, C., and van Regenmortel, M. (2002). Arthritogenic monoclonal antibodies from K/BxN mice. *J. Exp. Med. 195*, 1071–1077.

Mackay, F. and Browning, J.L. (2002). BAFF: a fundamental survival factor for B cells. *Nat. Rev. Immunol. 2*, 465–475.

Mackay, F. and Mackay, C.R. (2002). The role of BAFF in B-cell maturation, T-cell activation and autoimmunity. *Trends Immunol. 23*, 113–115.

Mackay, F. and Tangye, S.G. (2004). The role of the BAFF/APRIL system in B cell homeostasis and lymphoid cancers. *Curr. Opin. Pharmacol. 4*, 347–354.

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*, 1697–1710.

Mackay, F., Schneider, P., Rennert, P., and Browning, J.L. (2003). BAFF and APRIL: a tutorial on B cell survival. *Annu. Rev. Immunol. 21*, 231–264.

MacLennan, I.C. (1994). Germinal centers. *Annu. Rev. Immunol. 12*, 117–139.

Magliozzi, R., Columba-Cabezas, S., Serafini, B., and Aloisi, F. (2004). Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol. 148*, 11–23.

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.

Manz, R.A., Arce, S., Cassese, G., Hauser, A.E., Hiepe, F., and Radbruch, A. (2002). Humoral immunity and long-lived plasma cells. *Curr. Opin. Immunol. 14*, 517–521.

Marsden, V.S. and Strasser, A. (2003). Control of apoptosis in the immune system: Bcl-2, BH3-only proteins and more. *Annu. Rev. Immunol. 21*, 71–105.

McHeyzer-Williams, M.G. and Ahmed, R. (1999). B cell memory and the long-lived plasma cell. *Curr. Opin. Immunol. 11*, 172–179.

McMurray, R.W. (2001). Estrogen, prolactin, and autoimmunity: actions and interactions. *Int. Immunopharmacol. 1*, 995–1008.

Mebius, R.E., and Kraal, G. (2005). Structure and function of the spleen. *Nat. Rev. Immunol. 5*, 606–616.

Medina, F., Segundo, C., Campos-Caro, A., Gonzalez-Garcia, I., and Brieva, J.A. (2002). The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. *Blood 99*, 2154–2161.

Medina, F., Segundo, C., Campos-Caro, A., Salcedo, I., Garcia-Poley, A., and Brieva, J.A. (2003). Isolation, maturational level, and functional capacity of human colon lamina propria plasma cells. *Gut 52*, 383–389.

Merville, P., Dechanet, J., Desmouliere, A., Durand, I., de Bouteiller, O., Garrone, P., Banchereau, J., and Liu, Y.J. (1996). Bcl-2+ tonsillar plasma cells are rescued from apoptosis by bone marrow fibroblasts. *J. Exp. Med. 183*, 227–236.

Mizuno, T., Zhong, X., and Rothstein, T.L. (2003). Fas-induced apoptosis in B cells. *Apoptosis 8*, 451–460.

Mohan, C. (2001). Murine lupus genetics: lessons learned. *Curr. Opin. Rheumatol. 13*, 352–360.

Molina, H., Holers, V.M., Li, B., Fung, Y., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., and Chaplin, D.D. (1996). Markedly impaired humoral immune response in mice deficient in complement receptors 1 and 2. *Proc. Natl. Acad. Sci. USA 93*, 3357–3361.

Moulian, N. and Berrih-Aknin, S. (1998). Fas/APO-1/CD95 in health and autoimmune disease: thymic and peripheral aspects. *Semin. Immunol. 10*, 449–456.

Nemazee, D., Kouskoff, V., Hertz, M., Lang, J., Melamed, D., Pape, K., and Retter, M. (2000). B-cell-receptor-dependent positive and negative selection in immature B cells. *Curr. Top. Microbiol. Immunol. 245*, 57–71.

Nichols, K.E., Ma, C.S., Cannons, J., Schwartzberg, P.L., and Tangye, S.G. (2005). Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol. Rev. 203*, 180–199.

Ng, L.G., Sutherland, A., Newton, R., Qian, F., Cachero, T.G., Scott, M.L., Thompson, J.S., Wheway, J., Chtanova, T., and Groom, J. (2004). BAFF-R is the principal BAFF receptor facilitating BAFF co-stimulation of B and T cells. *J. Immunol. 173*, 807–817.

Ngo, V.N., Korner, H., Gunn, M.D., Schmidt, K.N., Riminton, D.S., Cooper, M.D., Browning, J.L., Sedgwick, J.D., and Cyster, J.G. (1999). Lymphotoxin alpha/beta and tumor necrosis factor are required for stromal cell expression of homing chemokines in B and T cell areas of the spleen. *J. Exp. Med. 189*, 403–412.

Nielsen, J.B. and Hultman, P. (2002). Mercury-induced autoimmunity in mice. *Environ. Health Perspect. 110*, 877–881.

Niiro, H. and Clark, E.A. (2002). Regulation of B-cell fate by antigen-receptor signals. *Nat. Rev. Immunol. 2*, 945–956.

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.

O'Connor, B.P., Raman, V.S., Erickson, L.D., Cook, W.J., Weaver, L.K., Ahonen, C., Lin, L., Mantchev, G.T., Bram, R.J., and Noelle, R.J. (2004). BCMA is essential for the survival of long-lived bone marrow plasma cells. *J. Exp. Med. 199*, 91–97.

Ochoa, E.R., Harris, N.L., and Pilch, B.Z. (2001). Marginal zone B-cell lymphoma of the salivary gland arising in chronic sclerosing sialadenitis (Kuttner tumor). *Am. J. Surg. Pathol. 25*, 1546–1550.

Odendahl, M., Jacobi, A., Hansen, A., Feist, E., Hiepe, F., Burmester, G.R., Lipsky, P.E., Radbruch, A., and Dorner, T. (2000). Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J. Immunol. 165*, 5970–5979.

Odendahl, M., Keitzer, R., Wahn, U., Hiepe, F., Radbruch, A., Dorner, T., and Bunikowski, R. (2003). Perturbations of peripheral B lymphocyte homoeostasis in children with systemic lupus erythematosus. *Ann. Rheum. Dis. 62*, 851–858.

Pan, C., Baumgarth, N., and Parnes, J.R. (1999). CD72-deficient mice reveal nonredundant roles of CD72 in B cell development and activation. *Immunity 11*, 495–506.

Pascual, V., Liu, Y.J., Magalski, A., de Bouteiller, O., Banchereau, J., and Capra, J.D. (1994). Analysis of somatic mutation in five B cell subsets of human tonsil. *J. Exp. Med. 180*, 329–339.

Peeva, E., Ventatesh, J., Michael, D., and Diamond, B. (2004). Prolactin as a modulator of B cell function: implications for SLE. *Biomed. Pharmacother. 58*, 310–319.

Pillai, S., Cariappa, A., and Moran, S.T. (2005). Marginal zone B cells. *Annu. Rev. Immunol. 23*, 161–196.

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*, 721–731.

Rahman, Z.S.M., Rao, S.P., Kalled, S.L., and Manser, T. (2003). Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. *J. Exp. Med. 198*, 1157–1169.

Rajewsky, K. (1996). Clonal selection and learning in the antibody system. *Nature 381*, 751–758.

Ravetch, J.V. and Lanier, L.L. (2000). Immune inhibitory receptors. *Science 290*, 84–89.

Ridderstad, A. and Tarlinton, D.M. (1998). Kinetics of establishing the memory B cell population as revealed by CD38 expression. *J. Immunol. 160*, 4688–4695.

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.

Roosnek, E. and Lanzavecchia, A. (1991). Efficient and selective presentation of antigen–antibody complexes by rheumatoid factor B cells. *J. Exp. Med. 173*, 487–489.

Sakaguchi, S. (2000). Animal models of autoimmunity and their relevance to human diseases. *Curr. Opin. Immunol. 12*, 684–690.

Schiemann, B., Gommerman, J.L., Vora, K., Cachero, T.G., Shulga-Morskaya, S., Dobles, M., Frew, E., and Scott, M.L. (2001). An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science 293*, 2111–2114.

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, L., and Aloisi, F. (2004). Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol. 14*, 164–174.

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*, 279–288.

Shi, K., Hayashida, K., Kaneko, M., Hashimoto, J., Tomita, T., Lipsky, P.E., Yoshikawa, H., and Ochi, T. (2001). Lymphoid chemokine B cell-attracting chemokine-1 (CXCL13) is expressed in germinal center of ectopic lymphoid follicles within the synovium of chronic arthritis patients. *J. Immunol. 166*, 650–655.

Shinall, S.M., Gonzalez-Fernandez, M., Noelle, R.J., and Waldschmidt, T.J. (2000). Identification of murine germinal center B cell subsets defined by the expression of surface isotypes and differentiation antigens. *J. Immunol. 164*, 5729–5738.

Siegel, R.M., Chan, F.K., Chun, H.J., and Lenardo, M.J. (2000). The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. *Nat. Immunol. 1*, 469–474.

Slifka, M.K. and Ahmed, R. (1998). Long-lived plasma cells: a mechanism for maintaining persistent antibody production. *Curr. Opin. Immunol. 10*, 252–258.

Smith, K.G., Hewitson, T.D., Nossal, G.J., and Tarlinton, D.M. (1996). The phenotype and fate of the antibody-forming cells of the splenic foci. *Eur. J. Immunol. 26*, 444–448.

Smith, K.G., 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.

Souroujon, M., White-Scharf, M.E., Andreschwartz, J., Gefter, M.L., and Schwartz, R.S. (1988). Preferential autoantibody reactivity of the preimmune B cell repertoire in normal mice. *J. Immunol. 140*, 4173–4179.

Stohl, W. (2004). Targeting B lymphocyte stimulator in systemic lupus and other autoimmune rheumatic disorders. *Expert Opin. Ther. Targets 8*, 177–189.

Stott, D.I., Hiepe, F., Hummel, M., Steinhauser, G., and Berek, C. (1998). Antigen-driven clonal proliferation of B cells within the target tissue of an autoimmune disease. The salivary glands of patients with Sjögren's syndrome. *J. Clin. Invest. 102*, 938–946.

Strasser, A. and Bouillet, P. (2003). The control of apoptosis in lymphocyte selection. *Immunol. Rev. 193*, 82–92.

Strasser, A., Whittingham, S., Vaux, D.L., Bath, M.L., Adams, J.M., Cory, S., and Harris, A.W. (1991). Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. USA 88*, 8661–8665.

Sze, D.M., Toellner, K.M., Garcia de Vinuesa, C., Taylor, D.R., and MacLennan, I.C. (2000). Intrinsic constraint on plasmablast growth and extrinsic limits of plasma cell survival. *J. Exp. Med. 192*, 813–821.

Takemura, S., Braun, A., Crowson, C., Kurtin, P.J., Cofield, R.H., O'Fallon, W.M., Goronzy, J.J., and Weyand, C.M. (2001a). Lymphoid neogenesis in rheumatoid synovitis. *J. Immunol. 167*, 1072–1080.

Takemura, S., Klimiuk, P.A., Braun, A., Goronzy, J.J., and Weyand, C.M. (2001b). T cell activation in rheumatoid synovium is B cell dependent. *J. Immunol. 167*, 4710–4718.

Tangye, S.G. and Hodgkin, P.D. (2004). Divide and conquer: the importance of cell division in regulating B-cell responses. *Immunology 112*, 509–520.

Tangye, S.G., Liu, Y.J., Aversa, G., Phillips, J.H., and de Vries, J.E. (1998). Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J. Exp. Med. 188*, 1691–1703.

Thompson, J.S., Schneider, P., Kalled, S.L., Wang, L., Lefevre, E.A., Cachero, T.G., Mackay, F., Bixler, S.A., Zafari, M., and Liu, Z.-Y. (2000). BAFF binds to the TNF receptor-like molecule BCMA and is important for maintaining the peripheral B cell population. *J. Exp. Med. 192*, 129–135.

Uckun, F.M. (1990). Regulation of human B-cell ontogeny. *Blood 76*, 1908–1923.

Van Kooten, C. and Banchereau, J. (1996). CD40-CD40 ligand: a multifunctional receptor-ligand pair. *Adv. Immunol. 61*, 1–77.

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.

Vora, K.A., Wang, L.C., Rao, S.P., Liu, Z., Majeau, G.R., Cutler, A.H., Hochman, P.S., Scott, M.L., and Kalled, S.L. (2003). Germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. *J. Immunol. 171*, 547–551.

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.

Wang, H., Marsters, S.A., Baker, T., Chan, B., Lee, W.P., Fu, L., Tumas, D., Yan, M., Dixit, V.M., Ashkenazi, A., and Grewal, I.S. (2001). TACI-ligand interactions are required for T cell activation and collagen-induced arthritis in mice. *Nat. Immunol. 2*, 632–637.

Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., and Nussenzweig, M.C. (2003). Predominant autoantibody production by early human B cell precursors. *Science 301*, 1374–1377.

Weyand, C.M. and Goronzy, J.J. (2003). Ectopic germinal center formation in rheumatoid synovitis. *Ann. N. Y. Acad. Sci. 987*, 140–149.

Yan, M., Wang, H., Chan, B., Roose-Girma, M., Erickson, S., Baker, T., Tumas, D., Grewal, I.S., and Dixit, V.M. (2001). Activation and accumulation of B cells in TACI-deficient mice. *Nat. Immunol. 2*, 638–643.

Yang, J.-Q., Singh, A.K., Wilson, M.T., Satoh, M., Stanic, A.K., Park, J.-J., Hong, S., Gadola, S.D., Mizutani, A., and Kakumanu, S.R. (2003). Immunoregulatory role of CD1d in the hydrocarbon oil-induced model of lupus. *J. Immunol. 171*, 2142–2153.

Yu, C.C.K., Mamchak, A.A., and DeFranco, A.L. (2003). Signalling mutations and autoimmunity. *Curr. Dir. Autoimmun. 6*, 61–88.

Yu, G., Boone, T., Delaney, J., Hawkins, N., Kelley, M., Ramakrishnan, M., McCabe, S., Qiu, W.-R., Kornuc, M., and Xia, X.-Z. (2000). APRIL and TALL–1 and receptors BCMA and TACI: system for regulating humoral immunity. *Nat. Immunol. 1*, 252–256.

CHAPTER

12

Memory B-Cell Development

LOUISE McHEYZER-WILLIAMS AND MICHAEL G. McHEYZER-WILLIAMS

The Scripps Research Institute, Department of Immunology, La Jolla, California, USA

Immune memory is a systemic phenomenon generated through the propagation and selective preservation of antigen-experienced memory cells in response to primary antigen exposure. Adaptive immunity to most foreign proteins requires the helper T (Th)-cell-regulated development of antigen-specific effector and memory B cells. This ordered cascade of cellular events progresses through multiple developmental checkpoints that serve to regulate immune function and limit self-reactivity. This chapter will outline what is currently understood of the temporally and spatially regulated development of antigen-specific memory B cells *in vivo*.

An adaptive immune response is a directed cellular program of development that progresses through distinguishable phases of change *in vivo*. The inflammatory context of initial antigen exposure primes the innate immune system to recruit naïve antigen-specific Th cells (phase I). Expanded effector Th cells prime antigen-experienced B cells to produce short-lived plasma cells and initiate secondary follicle formation (phase II). Secondary follicles are the microenvironmental precursors of the germinal center (GC) reaction, a cycle of somatic diversification and clonal selection that underpins affinity maturation and memory B-cell development (phase III). Multiple subsets of memory Th cells and memory B cells persist following primary antigen clearance in readiness for antigen rechallenge (phase IV). The cellular response to antigen rechallenge is substantially accelerated, resulting in the rapid and exaggerated production of memory response plasma cells and high-affinity antibody. This chapter will emphasize murine models of adaptive immunity to exemplify the emergent response to defined protein antigen. The extensive work that has been undertaken in humans is reviewed in Chapter 11.

During the primary immune response, expansion of self-reactive Th-cell or B-cell clonotypes and their differentiation into effector cells would reveal acute signs of autoimmunity. In contrast, the development and preservation of self-reactive clonotypes into the long-lived memory compartment assures a continued and self-replenishing cellular focus for chronic autoimmunity. Hence, avoiding the development of self-reactive memory is a fundamental property of the adaptive immune response to foreign antigen.

Exaggerated reactivity to antigen recall is the central defining characteristic of adaptive immunity (Burnet and Fenner, 1949). As early as 1957, Talmage (1957) and Burnet's (1957) postulates of clonal selection focused atten-

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

tion on the cellular organization of adaptive immunity. This powerful conceptual framework indicated that the cells of the immune system were the fundamental units of selection (Burnet, 1958). Early identification of cells that produced antibodies by Fragraeus (1948), and the assay of specificity with single-cell resolution by Nossal (1959), provided the initial experimental support for this model. Studies by Miller (1961) and Claman (1966) clearly indicated that mixtures of different cells were needed for efficient adaptive immunity. A series of transfer studies by Miller and Mitchell (1967) established that bone marrow produced the plasma cell precursors while the thymus-derived cells enhanced the antibody response. Subsequent studies of the hapten-carrier effect (Paul et al., 1966; Katz et al., 1970; Mitchison, 1971) began to probe the molecular basis of T–B-cell collaboration (Rajewsky et al., 1969). These classical studies also provide the experimental animal models so instrumental to our current appreciation of the cellular and molecular regulation of adaptive immunity.

PHASE I: RECRUITING SPECIFIC LYMPHOCYTES

Activating Innate Immunity

Local administration of oligovalent protein antigens without inflammatory stimuli will induce tolerance, largely due to inadequate costimulation of the adaptive system (Jenkins et al., 2001). Immune adjuvants provide the inflammatory stimulus necessary for effective protein vaccination strategies (Janeway and Medzhitov, 2002). Most adjuvants trigger cell activation through pattern-recognition receptors (PRRs), such as those of the interleukin (IL)-1R/Toll-like receptor (TLR) family (Aderem and Ulevitch, 2000). PRRs recognize conserved pathogen motifs, such as bacterial lipopolysaccharide (TLR4) or stretches of unmethylated DNA typically present in bacteria (TLR9). Dendritic cells (DCs) are uniquely efficient at protein antigen uptake and processing, and presentation of peptide major histocompatibility complexes (MHCs) *in vivo* (Banchereau and Steinman, 1998). At homeostasis, there are multiple varieties of DC resident in secondary lymphoid organs or trafficking there from regional tissue (Shortman and Liu, 2002). Upon vaccination, DCs at the site of injection produce large amounts of inflammatory chemokines, upregulate chemokine receptors and costimulatory molecules, and then relocate to the T-cell zones of regional draining lymph nodes (McHeyzer-Williams and McHeyzer-Williams, 2005) (Figure 12.1).

At sites of inflammation there will be concomitant local tissue damage. Hence, self-protein can also be processed and presented by the same activated DC population. Therefore, self-protein–MHC complexes will now be presented in the draining lymph node with adequate costimulation, providing a mechanism to activate and expand self-reactive T cells.

Immune Synapse I: Activated Antigen-Presenting Cells and Naïve Helper T Cells

In the regional lymph nodes, activated peptide–MHC II$^+$ DCs sample the naïve Th-cell compartment for T-cell receptors (TCRs) above a threshold for affinity of protein–MHC II binding (Malherbe et al., 2004). In the absence of agonist peptides, intercellular connections between antigen-presenting cells (APCs) and naïve Th cells are transient (Bromley et al., 2001). In contrast, immune synapse formation requires specific protein–MHC II recognition (Monks et al., 1998). TCR–protein–MHC II interactions, coreceptors, and intracellular signaling complexes focus centrally at the interface, surrounded by complementary adhesion molecule interactions. Exclusion of potentially negative signals, such as the receptor phosphatase CD45 or the large sialoprotein CD43, also appears to be part of the molecular rearrangement associated with immune synapsis. Longer-term immune synapsis can be visualized directly *in vivo* (Mempel et al., 2004), also requires TCR specificity (Huppa et al., 2003), and appears important for commitment to proliferation (Lee et al., 2002) and most likely differentiation of cell function.

Immune synapsis between an antigen-experienced protein–MHC II$^+$ DC and a naïve protein–MHC II-specific Th cell acts as the first critical developmental checkpoint in antiprotein immune responses (see Figure 12.1, phase I). Multiple DC subsets can be involved at this checkpoint, together with a variety of maturation states based on the quality of inflammation (Shortman and Liu, 2002; Pulendran, 2004). The route of immunization will influence the regional mix of DCs that can participate in this early phase of the response (Itano and Jenkins, 2003; Itano et al., 2003). Dose of antigen influences density of specific protein–MHC II complexes per cell, which may in turn influence Th-cell fate. We have also recently demonstrated a preexisting divide in the naïve Th-cell compartment based on differential Ly6C expression, which also divides the Th-cell repertoire and immune function (McHeyzer-Williams and McHeyzer-Williams, 2004). Hence, combinations of different cell types on either side of the immune synapse I can substantially impact Th-cell fate and function *in vivo*.

Activating Specific B Cells

To receive cognate T-cell help, naïve B cells must have first encountered a specific antigen. The epitope specificity of the two lymphocytes may differ; however, the antigen-experienced B cells must process and express the protein–MHC II complex specific for the Th cells. The B-cell receptor (BCR) can certainly recognize soluble antigen,

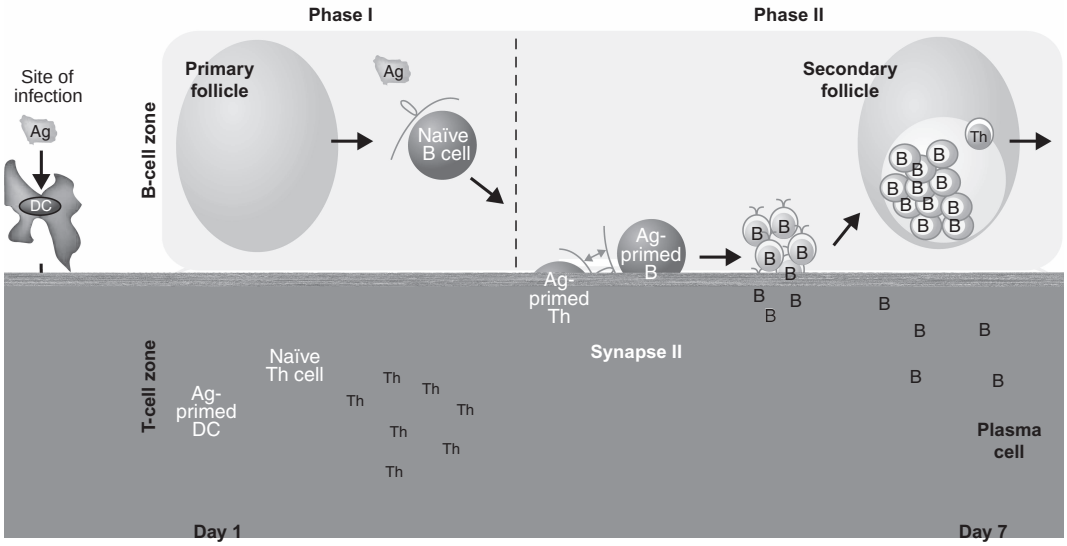


FIGURE 12.1 Initiating and regulating adaptive immunity. Phase I begins with activating antigen-presenting cells (APCs) at the site of vaccination and their migration into T-cell zones of draining lymph nodes. The formation of immune synapse I between antigen-experienced APC and naïve antigen-specific helper T (Th) cells is the first major developmental checkpoint in this pathway. Th cell clonal expansion and migration towards the T–B-cell borders initiates the search for antigen-experienced B cells. Phase II begins with immune synapse II between expanded effectors Th cells and antigen-primed B cells, the second developmental checkpoint in this pathway. A major division in B-cell development ensues with isotype switch and plasma cell differentiation or secondary follicle formation. Ag, antigen; DC, dendritic cell.

Reproduced from McHeyzer-Williams and McHeyzer-Williams (2005), with permission.

although the efficiency of B-cell responsiveness to APC-associated antigen can be 1000-fold higher (Batista et al., 2001). This cell-associated presentation has been demonstrated for Th-cell-independent B-cell responses to bacteria *in vivo* (Balazs et al., 2002), but may also be operative for protein antigens. Nevertheless, B cells must be primed with antigen prior to cognate Th-cell interactions.

PHASE II: REGULATING B-CELL FATE

Immune Synapse II: Effector Helper T Cells and Antigen-Primed B Cells

Clonal expansion precedes effector Th-cell differentiation and the delivery of cognate T-cell help to B cells (Malherbe et al., 2004). Temporal separation of these events serves to amplify low antigen-specific Th-cell precursors, increasing the probability of antigen-specific Th–B-cell contact. Upon activation and expansion, Th cells also progressively decrease CCR7 and increase CXCR5 expression as a means to relocate towards the B-cell-rich follicular regions (Ansel et al., 1999). Effector Th-cell function is delivered to B cells in a cognate manner, constituting the second major checkpoint in this emergent developmental pathway (see Figure 12.1, phase II). Immune synapse II between protein–MHC II-specific effector Th cells and antigen-primed B cells is qualitatively and quantitatively distinct from immune synapse I. The central focusing event remains the TCR–protein–MHC II interactions; however, the expression of secondary molecules capable of modifying cell fate at this juncture has substantially changed.

Diversity of effector Th-cell function in its microenvironmental context *in vivo* remains poorly resolved. Many cell-surface molecule pairs, such as CD154 (CD40L)/CD40 (Armitage et al., 1992) and ICOS/ICOSL (Dong et al., 2001; McAdam et al., 2001; Tafuri et al., 2001), play critical early roles in the delivery and reception of T-cell help to B cells. Antibody isotype switch and the GC reaction fail to develop in the absence of these key costimulator molecules. Many other members of these tumor necrosis factor (TNF)/TNF receptor (TNFR) and CD28/CD80 (B7) families have the capa-city to modify immune response outcomes (Bishop and Hostager, 2001). The production of cytokines, such as IL-4, interferon (IFN)-γ, and tumor growth factor (TGF)-β, by Th cells also have a major impact on B-cell fate by influencing isotype switch recombination (IgG1, IgG2a, and IgA, respectively) (Snapper and Paul, 1987; Cazac and Roes, 2000). Other cytokines, such as IL-5, IL-6, and IL-10, are thought to exert their influence more generally on survival and propagation of antigen-experienced B cells. Interestingly, the frequency of specific Th cells that express mRNA for any one cytokine *in vivo* is surprisingly low, with little change between primary and memory response (Panus et al., 2000). Nevertheless, the

diversity of effector Th cells serves to define a range of immune synapse II interactions that impact alternate B-cell fates *in vivo*.

The presentation of peptides from intracellular sources of self-protein in MHC II is inefficient. B cells are also poor phagocytes, thus non-BCR uptake of self-proteins is unlikely. Furthermore, if self-reactive B cells take up self-proteins and express self-protein–MHC II complexes, they still require cognate T-cell help before they can expand and differentiate into plasma cells. Low self-reactive B-cell precursor frequencies and the concomitant expansion of protein–MHC II effector Th cells for the same self-protein is another highly unlikely event. Hence, there are multiple practical barriers at the cellular, molecular, and biochemical levels that restrict exaggerated self-reactivity at this stage of the primary response.

Short-lived Plasma Cells and Secondary Follicle Formation

There is a major bifurcation in B-cell development following immune synapse II interactions during this first week after initial antigen exposure (MacLennan and Gray, 1986). Antigen-primed B cells that have received the requisite T-cell help either expand and differentiate into plasma cells or initiate secondary follicles (see Figure 12.1) (Jacob et al., 1991a). These alternate cell fates proceed separately in the T-cell zones and B-cell follicles, respectively. The early response plasma cells are characteristically short-lived with 3–5 day half lives (Ho et al., 1986) and express germline-encoded antibodies with no evidence of somatic diversification (Jacob et al., 1991b). Clonal expansion precedes plasma cell differentiation, which is considered to be the terminal event in this effector B-cell pathway. In contrast, some of the Th-cell-primed B-cell clonotypes move to the follicular area and expand rapidly. This cellular relocation, focal expansion, and regional exclusion of follicular B cells in the process establishes the secondary follicle as a dynamic new microenvironmental niche (MacLennan, 1994). These secondary follicles give rise to the GC reaction, providing the pathway to memory B-cell development.

BCR-based selection may drive this developmental decision with some evidence for the differential assortment of B-cell clonotypes into these two pathways (McHeyzer-Williams et al., 1993). Signature changes in transcription factor expression accompany differentiation to plasma cells. Transcriptional repression by Blimp-1 (Calame et al., 2003) and an XBP-1 requirement for antibody secretion (Reimold et al., 2001) are examples of these more intrinsic changes. Cognate Th-cell signals also clearly play a role in this developmental decision. Th signals that drive isotype switch in both pathways are also thought to originate at immune synapse II (Jacob et al., 1991a; McHeyzer-Williams, 2004). Combinations of Th-cell-expressed surface molecules and cytokines differentially regulate these key effector functions; however, it takes multiple cell divisions to reveal these cell fates *in vivo*. Thus, immune synapse II is the second pivotal checkpoint, controlling the quality of effector B-cell function and initiating memory B-cell development.

PHASE III: DEVELOPING B-CELL MEMORY

Germinal Center Reaction

Following initial clonal expansion, secondary follicles polarize. Continued expansion is relegated to the dark zone, always proximal to the T-cell areas, by antigen-specific GC B cells, now called centroblasts (MacLennan, 1994). The light zone at the opposite pole comprises many non-cycling GC B cells, now called centrocytes, dense processes of follicular DCs (FDCs) (non-bone marrow-derived stromal cells) (Szakal et al., 1988; Tew et al., 1990), and antigen-specific GC Th cells (Gulbranson-Judge and MacLennan, 1996; Zheng et al., 1996; Mikszta et al., 1999). This specialized microenvironment hosts a cycle of activity that underpins affinity maturation and the production of B-cell memory. As depicted in Figure 12.2, clonal expansion is accompanied by diversification of the antibody variable region genes by somatic hypermutation (SHM) (Rajewsky, 1996). The recent identification of activation-induced cytidine deaminase (AID) as an enzyme required for SHM provides a major breakthrough in understanding this multistep diversification mechanism (Honjo et al., 2002). Typically, one mutation is introduced per cell division, and cell cycle arrest then marks entry into the light zone and expression of the variant BCR (Kelsoe, 1996). Centrocytes are then selected for high-affinity BCRs and are filtered against self-reactivity (Linton et al., 1991). Although antibody production is not required for selection (Hannum et al., 2000), the FDCs and GC Th cells are thought to play a role in this critical selection event.

Immune Synapse III: Germinal Center Helper T Cells and B Cells

Positive selection in the GC cycle results in either reentry into the dark zone or exit into the long-lived memory B-cell compartment. Reiterative cycles of diversification and subsequent positive selection is the most conservative means to progressively mature the BCR affinity of the memory B-cell compartment. The precise role of the GC Th cell remains unclear; however, these cellular interactions most likely occur in a cognate manner and hence define a third and significant developmental checkpoint in the antiprotein adaptive response (Figure 12.3). Immune synapse III regulates the cellular outcome of the GC reaction

and thus controls the quality and quantity of the memory B-cell compartment.

Klinman (Linton et al., 1991) introduced the second window of tolerance to emphasize the capacity of the GC reaction to propagate self-reactivity. As SHM is random, if selection were inadvertently based on self-antigen, there would exist the potential to immortalize high-affinity neo-self-reactivity into the memory B-cell compartment. Flooding the system with soluble foreign antigen at the peak of the GC reaction interfered with normal GC development, providing some evidence that a protective mechanism was in place (Pulendran et al., 1995; Shokat and Goodnow, 1995). Importantly, the requisite self-reactive Th-cell compartment would also have to develop and be present in the GC reaction. Hence, a requirement for cognate GC Th-cell control in this process also provides a practical barrier to the development of self-reactive B-cell memory.

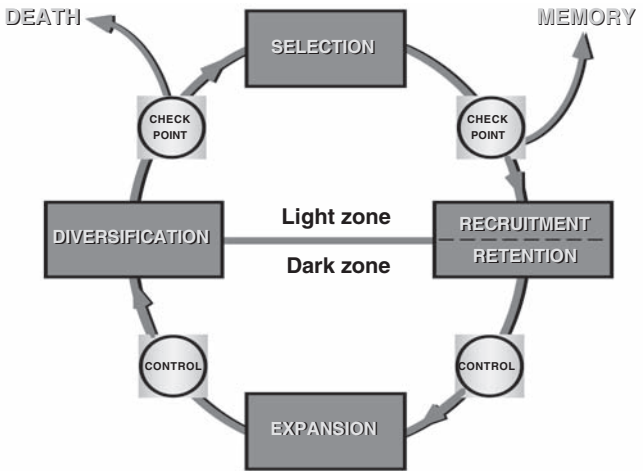


FIGURE 12.2 Germinal center (GC) cycle of activity. Antigen-specific B cells enter the follicular area and rapidly expand into secondary follicles that polarize into the light and dark zones of the GC reaction. Clonal expansion is accompanied by somatic diversification of the antibody variable region genes in the dark zone. Exit from the cell cycle and expression of variant B-cell receptors allows for antigen-specific selection of centrocytes in the light zones. Negative selection leads to apoptosis while positive selection of high-affinity variants results in GC cycle re-entry or exit from the GC into the memory B-cell compartment.

Memory B-Cell Subsets

Exit from the GC reaction is thought to signify entry into a long-lived memory B cell compartment (see Figure 12.3). Non-secreting precursors for the memory response and long-lived plasma cells are two separate subtypes of high affinity antigen-specific memory B cells that persist *in vivo* (McHeyzer-Williams et al., 2000). We have recently divided memory response precursors into two further subsets based on phenotype ***(6B2$^+$ versus 6B2$^-$ based on expression of a glycosylation variant of CD45),*** localization ***(6B2$^-$ preferentially home to BM)*** and capacity to produce antigen-specific plasma cells after adoptive transfer and antigen recall ***(6B2$^-$ have a greater propensity)*** (McHeyzer-Williams et al., 2000, Driver et al., 2001). ***We refer to these subsets as post-GC memory B cells and pre-plasma memory B cells in accordance with a proposed linear relationship in their development.*** Analysis of Blimp-1 conditional knockout animals (Shapiro-Shelef et al., 2003) indicates a novel requirement for Blimp-1 in memory B cell development. These genetic studies support a linear model of memory B cell development *in vivo* as outlined in Figure 12.3.

There is also evidence for continued antigen-based selection after the decline of the primary response GC reaction (Takahashi et al., 1998). This ongoing positive selection appears to be based on clonal competition with no further

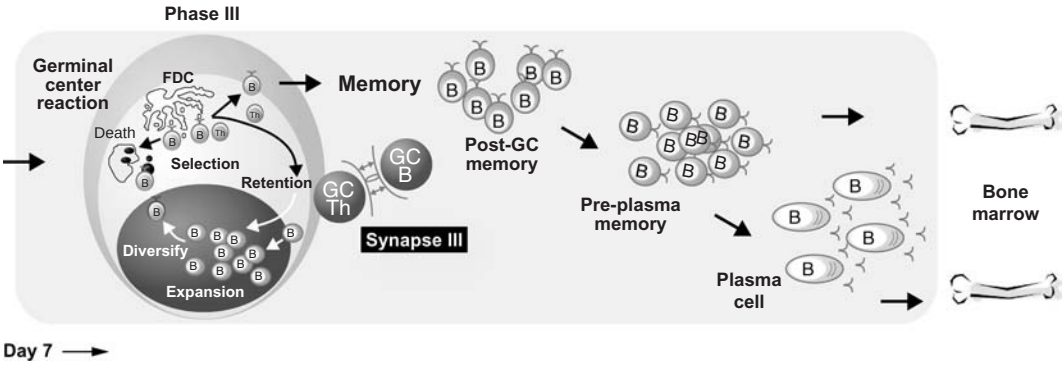


FIGURE 12.3 Memory B-cell subsets. Multiple subsets of memory B cells emerge from the germinal center (GC) reaction of the primary immune response. We propose that 6B2$^+$ post-GC memory B cells are the first cellular product to exit the GC. These cells can give rise to 6B2$^-$ preplasma memory B cells that are phenotypically and functionally distinct nonsecreting memory response precursors. The 6B2$^-$ pre-plasma memory B cells are the immediate cellular precursors of long-lived plasma cells that are a terminally differentiated antibody-secreting memory B cell compartment. Both PPM B cells and the long-lived plasma cells preferentially home to the bone marrow.

Reproduced from McHeyzer-Williams and McHeyzer-Williams (2005), with permission.

BCR diversification. Further, upon exit from the GC, memory B-cell survival is independent of BCR specificity (Maruyama et al., 2000). Individual memory B cells may not be long lived; however, the compartment persists even in the absence of the antigen-specific BCRs.

PHASE IV: RESPONDING TO ANTIGEN RECALL

Immune Synapse IV: Memory Helper T Cells and Memory B Cells

All three subsets of memory B cells persist without further antigen challenge (McHeyzer-Williams et al., 2000). The long-lived plasma cells preferentially home to the bone marrow and are the source of specific high-affinity serum antibody (McHeyzer-Williams and Ahmed, 1999). Upon antigen rechallenge, both subsets of memory response precursors expand rapidly in a Th-cell-dependent manner (McHeyzer-Williams et al., 2000). This memory response can manifest with minute quantities of soluble antigen in the absence of adjuvant. Hence, the innate system can be bypassed in the memory response. Nevertheless, effective antigen presentation and cognate Th-cell involvement are necessary for the memory response. The simplest model places the memory B-cell compartment as the APC and the elevated numbers of memory Th cells as the cognate regulators (Figure 12.4). Thus, immune synapse IV is the major developmental checkpoint for memory responses with exchanges of molecular information between memory Th cells and memory B cells.

The existence of memory Th cells and memory B cells to the same self-protein has disastrous consequences. In this scenario, inflammation would not be required to activate effector cell differentiation. There would be a potentially unlimited supply of antigen to trigger responsiveness to self. Finally, the self-replenishing characteristic of the memory compartment would broadly assure a continued supply of the precursors to damaging self-reactive effector cells. Hence, self-reactive memory is the true harbinger of unrelenting chronic autoimmunity.

Replenishment of the Memory Compartment

The number of persisting antigen-specific memory B cells of all subtypes increases following clearance of the secondary antigen challenge. The level of serum antibody specific for the antigen also increases with the secondary boost. Thus, replenishment of the memory compartment is a basic characteristic of the memory response (Traggiai et al., 2003). Self-replenishment allows continuance of

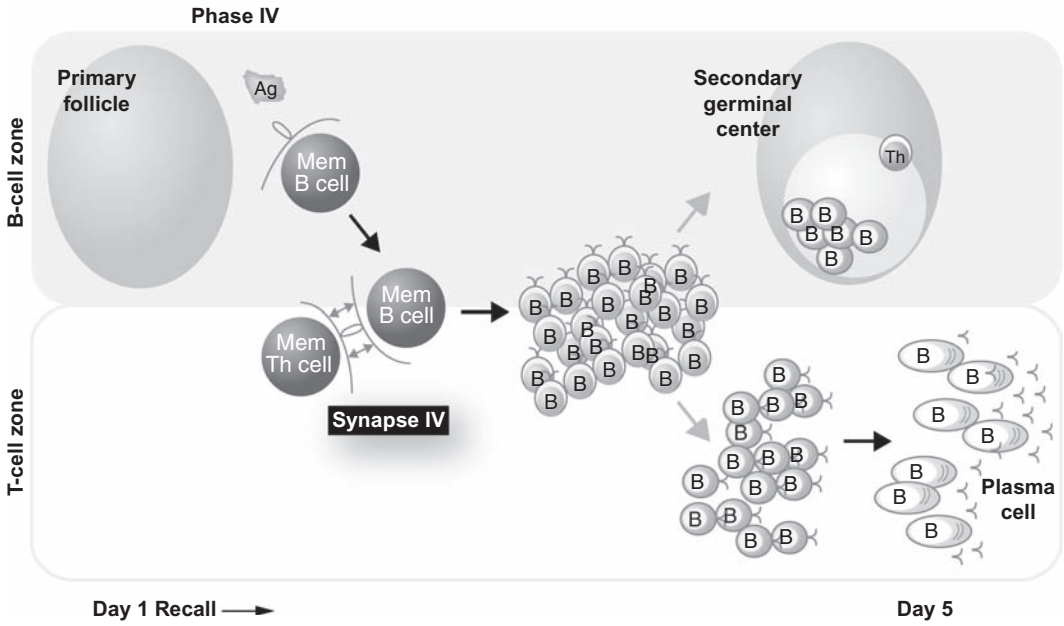


FIGURE 12.4 Response to antigen recall. Low-dose soluble antigen in the absence of adjuvant can induce a rapid and exaggerated humoral immune response. Hence, antigen-specific B cells are the most likely antigen-presenting cell (APC) for protein–MHC II complexes as immune synapse IV interactions between memory helper T (Th) cells and memory B cells are the critical developmental checkpoint for the memory response. Memory cell expansion is vigorous and produces large numbers of high-affinity memory response plasma cells as the dominant cellular outcome. Secondary germinal center (GC) reactions are also part of the memory response, although more a minor outcome than the primary response.

particular reactivity, but also heightens the basic capacity of the memory compartment to respond in the future.

Isotype-Specific Memory Cells

The spectrum of antibody isotype is the most clearly identifiable and differential functional characteristic of B-cell immunity. There is some information on the effector Th-cell control of isotype switch during the initial priming response, which has been discussed above. However, very little is known of the differential regulation of isotype-specific memory B cells into the memory response to antigen rechallenge. While IL-4 is a switch factor for some IgG1 and most or all IgE in B-cell responses (Snapper et al., 1988), it is not clear what $IgG1^+$ or IgE^+ memory B cells require for re-entry into the memory response. The distinct behavior of memory B cells expressing different isotypes *in vivo* has not been carefully studied. It seems likely that IgA memory B cells relocate to mucosal surfaces; however, it seems unlikely that TGF-β is required for their continued survival or re-entry into a memory response (Cazac and Roes, 2000). Hence, the developmental experience, resultant gene expression pattern, and intrinsic program of cellular behavior define memory B cells of different isotypes.

We have proposed the existence of functionally distinct memory Th-cell subsets that support the reactivation of isotype-specific memory B cells as one reasonable model of memory Th-cell organization. Unfortunately, the development of Th-cell memory itself remains poorly understood. How, where or even when Th-cell memory is developed during the primary responses is not clear. Direct experimental access to antigen-specific Th cells *in vivo* will help to resolve these issues (Altman et al., 1996); however, frequencies of responders remain quite low, even at the peak of the primary response, making functional assessment of effector and memory Th cells technically challenging. This is not the only organization proposed for Th-cell memory (see Chapter 6). Nevertheless, understanding functional diversity and developmental control of antigen-specific Th cells will be necessary to fully understand the control of antigen-specific B-cell memory.

CONCLUSION

The development and propagation of self-specific B-cell memory underpins many pathologic elements of autoimmune disease. The murine model provides access to the emergent properties of a developing adaptive response in secondary lymphoid organs that have been difficult to monitor in humans. The strategies that have been discussed in this chapter offer the means to analyze more directly the extent of antigen-specific immunity in humans. These strategies can potentially use peripheral blood to access memory lymphocytes and to quantify frequencies and qualify cellular diversity therein. Isolation and characterization of self-specific memory B cells and their memory Th-cell counterparts promise unique targets for immunotherapeutics at both the cellular and molecular levels.

References

Aderem, A. and Ulevitch, R.J. (2000). Toll-like receptors in the induction of the innate immune response. *Nature 406*, 782–787.

Altman, J.D., Moss, P.A., Goulder, P.J., Barouch, D.H., McHeyzer-Williams, M.G., Bell, J.I., McMichael, A.J., and Davis, M.M. (1996). Phenotypic analysis of antigen-specific T lymphocytes. *Science 274*, 94–96.

Ansel, K.M., McHeyzer-Williams, L.J., Ngo, V.N., McHeyzer-Williams, M.G., and Cyster, J.G. (1999). *In vivo*-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. *J. Exp. Med. 190*, 1123–1134.

Armitage, R.J., Fanslow, W.C., Strockbine, L., Sato, T.A., Clifford, K.N., Macduff, B.M., Anderson, D.M., Gimpel, S.D., Davis-Smith, T., and Maliszewski, C.R. (1992). Molecular and biological characterization of a murine ligand for CD40. *Nature 357*, 80–82.

Balazs, M., Martin, F., Zhou, T., and Kearney, J. (2002). Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses. *Immunity 17*, 341–352.

Banchereau, J. and Steinman, R.M. (1998). Dendritic cells and the control of immunity. *Nature 392*, 245–252.

Batista, F.D., Iber, D., and Neuberger, M.S. (2001). B cells acquire antigen from target cells after synapse formation. *Nature 411*, 489–494.

Bishop, G.A. and Hostager, B.S. (2001). B lymphocyte activation by contact-mediated interactions with T lymphocytes. *Curr. Opin. Immunol. 13*, 278–285.

Bromley, S.K., Burack, W.R., Johnson, K.G., Somersalo, K., Sims, T.N., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., and Dustin, M.L. (2001). The immunological synapse. *Annu. Rev. Immunol. 19*, 375–396.

Burnet, F.M. (1957). A modification of Jerne's theory of antibody production using the concept of clonal selection. *Australas. J. Sci. 20*, 67–69.

Burnet, F.M. (1958). *Clonal Selection Theory of Acquired Immunity*. Cambridge: Cambridge University Press.

Burnet, F.M. and Fenner, F. (1949). *The Production of Antibodies*. Melbourne: MacMillan.

Calame, K.L., Lin, K.I., and Tunyaplin, C. (2003). Regulatory mechanisms that determine the development and function of plasma cells. *Annu. Rev. Immunol. 21*, 205–230.

Cazac, B.B. and Roes, J. (2000). TGF-beta receptor controls B cell responsiveness and induction of IgA *in vivo*. *Immunity 13*, 443–451.

Claman, H.N., Chaperon, E.A., and Triplett, R.F. (1966) Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. Exp. Biol. Med. 122*, 1167–1171.

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 409*, 97–101.

Driver, D.J., McHeyzer-Williams, L.J., Cool, M., Stetson, D.B. and McHeyzer-Williams, M.G. (2001). Development and maintenance of a B220-memory B cell compartment. *J. Immunol. 167*, 1393–1405.

Fagraeus, A. (1948). The plasma cell reaction and its relation to the formation of antibodies *in vitro*. *J. Immunol. 58*, 1–3.

Gulbranson-Judge, A. and MacLennan, I. (1996). Sequential antigen-specific growth of T cells in the T zones and follicles in response to pigeon cytochrome c. *Eur. J. Immunol. 26*, 1830–1837.

Hannum, L.G., Haberman, A.M., Anderson, S.M., and Shlomchik, M.J. (2000). Germinal center initiation, variable gene region hypermutation,

and mutant B cell selection without detectable immune complexes on follicular dendritic cells. *J. Exp. Med. 192*, 931–942.

Ho, F., Lortan, J.E., MacLennan, I.C.M., and Khan, M. (1986). Distinct short-lived and long-lived antibody-producing cell populations. *Eur. J. Immunol. 16*, 1297–1301.

Honjo, T., Kinoshita, K., and Muramatsu, M. (2002). Molecular mechanism of class switch recombination: linkage with somatic hypermutation. *Annu. Rev. Immunol. 20*, 165–196.

Huppa, J.B., Gleimer, M., Sumen, C., and Davis, M.M. (2003). Continuous T cell receptor signaling required for synapse maintenance and full effector potential. *Nat. Immunol. 4*, 749–755.

Itano, A.A. and Jenkins, M.K. (2003). Antigen presentation to naive CD4 T cells in the lymph node. *Nat. Immunol. 4*, 733–739.

Itano, A.A., McSorley, S.J., Reinhardt, R.L., Ehst, B.D., Ingulli, E., Rudensky, A.Y., and Jenkins, M.K. (2003). Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity 19*, 47–57.

Jacob, J., Kassir, R., and Kelsoe, G. (1991a). *In situ* studies of the primary immune response to (4-hydroxy–3-nitrophenyl)acetyl. I. The architecture and dynamics of responding cell populations. *J. Exp. Med. 173*, 1165–1175.

Jacob, J., Kelsoe, G., Rajewsky, K., and Weiss, U. (1991b). Intraclonal generation of antibody mutants in germinal centres. *Nature 354*, 389–392.

Janeway, C. A. Jr. and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol. 20*, 197–216.

Jenkins, M.K., Khoruts, A., Ingulli, E., Mueller, D.L., McSorley, S.J., Reinhardt, R.L., Itano, A., and Pape, K.A. (2001). *In vivo* activation of antigen-specific CD4 T cells. *Annu. Rev. Immunol. 19*, 23–45.

Katz, D.H., Paul, W.E., Goidl, E.A., and Benacerraf, B. (1970). Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J. Exp. Med. 132*, 261–282.

Kelsoe, G. (1996). The germinal center: a crucible for lymphocyte selection. *Semin. Immunol. 8*, 179–184.

Lee, K.H., Holdorf, A.D., Dustin, M.L., Chan, A.C., Allen, P.M., and Shaw, A.S. (2002). T cell receptor signaling precedes immunological synapse formation. *Science 295*, 1539–1542.

Linton, P.J., Rudie, A., and Klinman, N.R. (1991). Tolerance susceptibility of newly generating memory B cells. *J. Immunol. 146*, 4099–4104.

MacLennan, I. C. (1994). Germinal centers. *Annu. Rev. Immunol. 12*, 117–139.

MacLennan, I.C.M. and Gray, D. (1986). Antigen-driven selection of virgin and memory B cells. *Immunol. Rev. 91*, 61–85.

Malherbe, L., Hausl, C., Teyton, L., and McHeyzer-Williams, M.G. (2004). Clonal selection of helper T cells is determined by an affinity threshold with no further skewing of TCR binding properties. *Immunity 21*, 669–679.

Maruyama, M., Lam, K.P., and Rajewsky, K. (2000). Memory B-cell persistence is independent of persisting immunizing antigen. *Nature 407*, 636–642.

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 409*, 102–105.

McHeyzer-Williams, M.G. (2004). *B Cell Signaling and Activation*. Philadelphia: Lippincott Williams & Wilkins.

McHeyzer-Williams, M.G. and Ahmed, R. (1999). B cell memory and the long-lived plasma cell. *Curr. Opin. Immunol. 11*, 172–179.

McHeyzer-Williams, L.J. and McHeyzer-Williams, M. (2005). Antigen-specific memory B cell development. *Annu. Rev. Immunol. 23*, 487–513.

McHeyzer-Williams, L.J. and McHeyzer-Williams, M.G. (2004). Developmentally distinct Th cells control plasma cell production *in vivo*. *Immunity 20*, 231–242.

McHeyzer-Williams, M.G., McLean, M.J., Lalor, P.A., and Nossal, G.J.V. (1993). Antigen-driven B cell differentiation *in vivo*. *J. Exp. Med. 178*, 295–307.

McHeyzer-Williams, L.J., Cool, M., and McHeyzer-Williams, M.G. (2000). Antigen-specific B cell memory: expression and replenishment of a novel b220(−) memory B cell compartment. *J. Exp. Med. 191*, 1149–1166.

Mempel, T.R., Henrickson, S.E., and Von Andrian, U.H. (2004). T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature 427*, 154–159.

Mikszta, J.A., McHeyzer-Williams, L.J., and McHeyzer-Williams, M.G. (1999). Antigen-driven selection of TCR *in vivo*: related TCR alpha-chains pair with diverse TCR beta-chains. *J. Immunol. 163*, 5978–5988.

Miller, J.F. (1961). Immunological function of the thymus. *Lancet 2*, 748–749.

Miller, J.F. and Mitchell, G.F. (1967). The thymus and the precursors of antigen reactive cells. *Nature 216*, 659–663.

Mitchison, N.A. (1971). The carrier effect in the secondary response to hapten-protein conjugates. I. Measurement of the effect with transferred cells and objections to the local environment hypothesis. *Eur. J. Immunol. 1*, 10–17.

Monks, C.R., Freiberg, B.A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature 395*, 82–86.

Nossal, G.J. (1959). Antibody production by single cells. III. The histology of antibody production. *Br. J. Exp. Pathol. 40*, 301–311.

Panus, J.F., McHeyzer-Williams, L.J., and McHeyzer-Williams, M.G. (2000). Antigen-specific T helper cell function: differential cytokine expression in primary and memory responses. *J. Exp. Med. 192*, 1301–1316.

Paul, W.E., Siskind, G.W., and Benacerraf, B. (1966). Studies on the effect of the carrier molecule on antihapten antibody synthesis. II. Carrier specificity of anti-2,4-dinitrophenyl-poly-l-lysine antibodies. *J. Exp. Med. 123*, 689–705.

Pulendran, B. (2004). Modulating vaccine responses with dendritic cells and Toll-like receptors. *Immunol. Rev. 199*, 227–250.

Pulendran, B., Kannourakis, G., Nouri, S., Smith, K.G., and Nossal, G.J. (1995). Soluble antigen can cause enhanced apoptosis of germinal-centre B cells. *Nature 375*, 331–334.

Rajewsky, K. (1996). Clonal selection and learning in the antibody system. *Nature 381*, 751–758.

Rajewsky, K., Schirrmacher, V., Nase, S., and Jerne, N.K. (1969). The requirement of more than one antigenic determinant for immunogenicity. *J. Exp. Med. 129*, 1131–1143.

Reimold, A.M., Iwakoshi, N.N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravallese, E.M., Friend, D., Grusby, M.J., Alt, F., and Glimcher, L.H. (2001). Plasma cell differentiation requires the transcription factor XBP-1. *Nature 412*, 300–307.

Shapiro-Shelef, M., Lin, K.I., McHeyzer-Williams, L.J., Liao, J., McHeyzer-Williams, M.G., and Calame, K. (2003). Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity 19*, 607–620.

Shokat, K.M. and Goodnow, C.C. (1995). Antigen-induced B-cell death and elimination during germinal-centre immune responses. *Nature 375*, 334–338.

Shortman, K. and Liu, Y.J. (2002). Mouse and human dendritic cell subtypes. *Nat. Rev. Immunol. 2*, 151–161.

Snapper, C.M. and Paul, W.E. (1987). Interferon-gamma and B cell stimulatory factor–1 reciprocally regulate Ig isotype production. *Science 236*, 944–947.

Snapper, C.M., Finkelman, F.D., and Paul, W.E. (1988). Regulation of IgG1 and IgE production by interleukin 4. *Immunol. Rev. 102*, 51–75.

Szakal, A.K., Kosco, M.H., and Tew, J.G. (1988). A novel *in vivo* follicular dendritic cell-dependent iccosome-mediated mechanism for delivery of antigen to antigen-processing cells. *J. Immunol. 140*, 341–353.

Tafuri, A., Shahinian, A., Bladt, F., Yoshinaga, S.K., Jordana, M., Wakeham, A., Boucher, L.M., Bouchard, D., Chan, V.S., Duncan, G., Odermatt, B., Ho, A., Itie, A., Horan, T., Whoriskey, J.S., Pawson, T., Penninger, J.M., Ohashi, P.S., and Mak, T.W. (2001). ICOS is essential for effective T-helper-cell responses. *Nature 409*, 105–109.

Takahashi, Y., Dutta, P.R., Cerasoli, D.M., and Kelsoe, G. (1998). *In situ* studies of the primary immune response to (4-hydroxy–3-nitrophenyl)acetyl. V. Affinity maturation develops in two stages of clonal selection. *J. Exp. Med. 187*, 885–895.

Talmage, D.W. (1957). Allergy and immunology. *Annu. Rev. Med. 8*, 239–256.

Tew, J.G., Kosco, M.H., Burton, G.F. and Szakal, A.K. (1990). Follicular dendritic cells as accessory cells. *Immunol. Rev. 117*, 185–211.

Traggiai, E., Puzone, R., and Lanzavecchia, A. (2003). Antigen dependent and independent mechanisms that sustain serum antibody levels. *Vaccine 21* (Suppl 2), S35–37.

Zheng, B., Han, S., Zhu, Q., Goldsby, R., and Kelsoe, G. (1996). Alternative pathways for the selection of antigen-specific peripheral T cells. *Nature 384*, 263–266.

CHAPTER

13

Tolerance and Autoimmunity: B Cells

ANTONY BASTEN AND ROBERT BRINK

Centenary Institute of Cancer Medicine and Cell Biology, University of Sydney, Sydney, NSW Australia

GROUND RULES FOR B-CELL SELF-TOLERANCE

Tolerance and memory constitute the two pillars on which the adaptive (specific) immune system is based. For self-antigens, tolerance or immunologic unresponsiveness is the appropriate outcome, whereas for foreign antigens, immunity and long-term memory are required.

The concept of self-tolerance can be traced back to the early 20th century when Ehrlich and Morgenroth [cited in Ehrlich and Morgenroth (1957)] described the production of hemolytic antibodies in goats following immunization with erythrocytes obtained from other goats, whereas autologous red cells failed to elicit a response. This and other findings on alloantisera led them to conclude that autoimmune responses "constitute a danger threatening the organism more frequently and more severely than all exogenous injuries." The fact that collectively autoimmune diseases are relatively uncommon is an indication of how effective the immune system normally is in distinguishing between self and nonself, or as Matzinger (1994) put it in her "danger" model, between harmless and dangerous entities.

Tolerance, like immunity, was shown early on to be somatically acquired as a result of exposure to antigen rather than being genetically pre-programmed into the germline [reviewed in Schwartz (1993)]. The simplest way of explaining how these two phenomena might be distinguished was on the basis of differences in the structure and properties of self- and foreign antigens. This proposition, however, was shown to be false by Traub (1938), Owen (1945), and Billingham et al. (1953) in their classic experiments demonstrating induction of tolerance to normally foreign antigens when introduced antenatally. Thus, the timing of antigen exposure appeared to be more important than the origin of the antigen per se. Based on these observations, Burnet (1959) encapsulated the concept of immunity and tolerance in his clonal selection theory, which states that: "self-not-self recognition means simply that all those clones which would recognize (that is produce antibody against) a self component have been eliminated in embryonic life. All the rest are retained." Thus every individual is born with a repertoire of "immunocytes," as Burnet called them, purged of self-reactive cells but still capable of mounting responses to foreign antigens. Clonal selection therefore paved the way for the concepts of positive and negative selection of cells recognizing nonself and self, respectively, although this thinking came to be applied more to the thymus than bone marrow.

Shortly afterwards, Lederberg (1959), while working in Burnet's laboratory, came to appreciate that tolerance could be imposed on developing lymphocytes, not just antenatally but throughout life, as they differentiate from precursor cells into mature immunocompetent cells. This line of reasoning

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

served to focus attention on primary lymphoid tissues (thymus and bone marrow) as the major sites for clonal elimination (deletion). In the event of a failure in self-tolerance, it was envisaged that a "forbidden clone" could emerge from these sites, resulting in onset of autoimmunity.

Cell–Cell Interactions and the Two-Signal Hypothesis

During the 20 years following promulgation of the clonal selection theory by Burnet, the immunologic function of the thymus was discovered (Miller, 1961), the lymphocyte was identified as the antigen-specific immunocyte (Gowans and Knight, 1964), the two-cell system of T and B lymphocytes was defined (Mitchell and Miller, 1968), and the phenomenon of major histocompatibility complex (MHC) restriction was described (Zinkernagel and Doherty, 1974). The picture of the immune system to emerge from these findings was one of an intricate set of cell–cell interactions initiated by exposure to antigen and regulated by multiple positive and negative signals derived from lymphocytes, antigen-presenting cells (APCs), and stromal cells located in primary and secondary lymphoid tissue. Thus, tolerance and immunity were no longer considered to be totally discrete entities, but rather the poles of a continuum of immune responsiveness, the former appropriate to self- and the latter to foreign antigens.

The ground rules for analyzing tolerance and immunity within this framework were first articulated by Bretscher and Cohn (1970) in their two-signal hypothesis. As originally applied to a generic antigen-responsive cell, the hypothesis envisaged that an immune response required associative recognition of two determinants (signal 1 and signal 2) on a given antigen, by two different cells. On the other hand, if the first recognition event (signal 1) occurred alone, then tolerance would ensue. For self-antigens, to which the immune system is exposed early in life, tolerance would be the most likely outcome since the frequency of cells with specificity for such antigens would be very low indeed and their chances of meeting remote. For foreign antigens, which are not continually present, precursors specific for multiple epitopes could accumulate prior to contact with antigen, thus allowing a cooperative immune response to proceed. Subsequently the two-signal paradigm was modified to accommodate collaborative interactions between T cells, B cells, and APCs (Lafferty and Cunningham, 1975; Schwartz, 1993).

For B-cell responses, the current thinking may be summarized as follows: signal 1 is provided by antigen binding to the B-cell receptor (BCR) (Figure 13.1). In the case of T-dependent antibody responses, B cells present antigenic peptides to adjacent activated helper T (Th) cells. These cells express cell-surface CD40L and release B-cell stimulatory cytokines like interleukin (IL)-4, and the binding of these ligands to their specific receptors collectively delivers signal 2 to the B cell (Hodgkin and Basten, 1995) (Figure 13.1). The efficacy of the interaction is enhanced by pairs of costimulatory molecules, like CD28/CD86 and ICOS/ICOSL, present on the surface of T and B cells, respectively. In the case of T-independent antibody responses, direct interaction of antigen with B cells can provide signal 2 as well as signal 1. For example, some antigens (signal 1) can form complexes with DNA-derived CpG motifs, thereby leading to cross-linking of BCR with TLR9 (signal 2) and a productive antibody response (Leadbetter et al., 2002). Also, certain multivalent antigens, such as polymerized flagellin (Basten and Miller, 1974), or complement-fixing polysaccharides, such as Ficoll and dextran (Thyphronitis et al., 1991), can not only bind to the BCR (signal 1) but costimulate the B cell through interaction with the complement receptor, CD21 (signal 2). A corollary to the two-signal hypothesis is that the immunogenicity of antigen, i.e., whether it is "strong" or "weak," influences the decision between tolerance and immunity; weak antigens are those that cross-link the BCR poorly and/or fail to generate a second signal, e.g., deaggregated immunoglobulin or anti-Fab, and tend to be tolerogenic. Strong antigens, on the other hand, cross-link the BCR and recruit second signals efficiently. Examples include viruses and bacteria that elicit T-dependent responses as well as the multivalent T-independent antigens mentioned above.

In addition to the nature of the antigen, the decision between tolerance and immunity may be influenced by the duration of antigen exposure and its timing during B-cell development. Immature (including transitional) B cells as opposed to mature B cells are thought to be hardwired to undergo tolerance (negative selection) on exposure to antigen, possibly due to differences in the balance between pro- and anti-apopotic molecules of the Bcl-2 family (Marsden and Strasser, 2002). Such a scenario can explain why congenital rubella is associated with a failure by the fetus to eliminate the virus. On the other hand, intrauterine infections other than rubella can elicit a positive immune response (Nossal, 1957; Buchmeier et al., 1980) and tolerance can be readily imposed on mature B cells (see below). The key factor in some instances may therefore be the duration of antigen exposure rather than its timing. Thus, persistent BCR stimulation by low levels of antigen appears to favor tolerance, whereas immunity would be the outcome of sudden BCR engagement, as proposed in the danger hypothesis (Matzinger, 1994; Jun and Goodnow, 2003). Thus, the latter rather than the former scenario is more likely to be associated with rapid delivery of signal 2.

Requirement for Tolerance in B-Cell and T-Cell Repertoires

The B-cell repertoire is generated in two waves by distinct mechanisms that shape and modify the genes encoding the antigen-binding site of immunoglobulin molecules. The

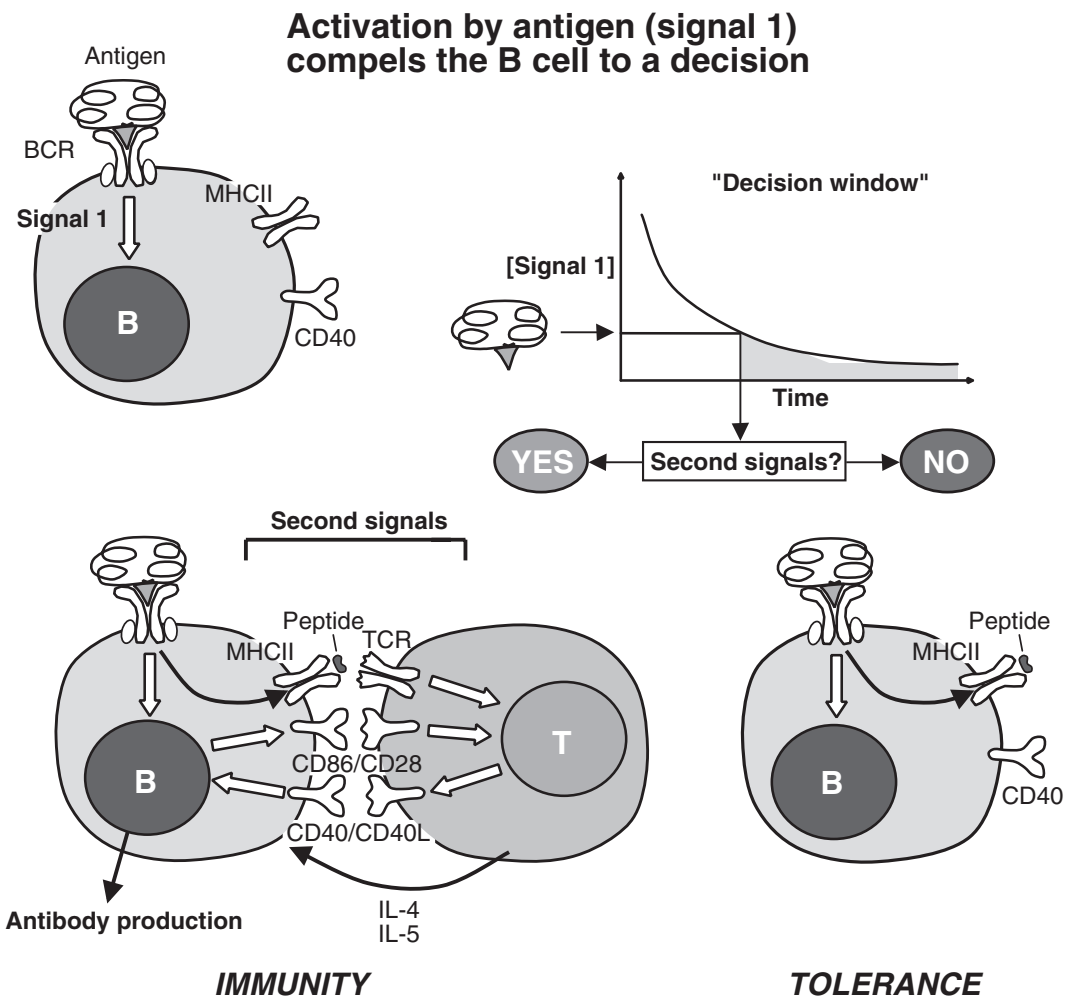


FIGURE 13.1 Two-signal hypothesis as it applies to B cells. Binding of antigen to the B-cell receptor (BCR) (signal 1) leads to primary activation. The B cell then has a finite amount of time to find a source of a second (costimulatory) signal. If it does, a productive response ensues. If it fails to do so, the cell will become tolerant and die. The time available for receipt of signal 2 is inversely proportional to the strength of signal 1. The case of a T-dependent B-cell response is shown here, where signal 2 is delivered collectively by multiple molecules expressed by activated T helper (Th) cells, such as cell-surface CD40L and the soluble cytokines IL-4 and IL-5. Effective collaboration with Th cells requires the B cell to both present peptide fragments of the antigen on class II MHC and to upregulate the costimulatory molecule CD86. Anergic B cells cannot upregulate CD86 in response to signal 1, which prevents them from receiving signal 2 from Th cells.

first is the process of VDJ gene recombination, which occurs in B-cell precursors within fetal liver and adult bone marrow. This process combines germline V, D, and J gene segments to form rearranged immunoglobulin heavy (VDJ) and light chain (VJ) genes. The products of these rearranged genes then combine to produce the nascent BCRs expressed on immature B cells and thus shape the primary B-cell repertoire. The second mechanism by which the B-cell repertoire is shaped is somatic hypermutation (SHM) of immunoglobulin variable region genes. This occurs largely in germinal centers (GCs) following B-cell activation and collaboration with Th cells, and generates what is referred to as the secondary B-cell repertoire. While the principal aim of both VDJ recombination and SHM is to diversify the B-cell repertoire in order to best deal with invading foreign antigens, the essentially random nature of both processes inevitably generates self-reactive B cells with the potential to produce pathogenic autoantibodies. The mechanisms whereby such clones are normally excluded from productive antibody responses represent the central issue in B-cell self-tolerance.

Following the discovery of the two-cell system and the demonstration of a helper function for T cells in antibody production by B cells in the late 1960s, it became apparent

that tolerance could be imposed on both T cells and B cells, although the threshold for its induction was lower in T cells (Chiller et al., 1970). This was accompanied by the notion of "low" and "high" zone tolerance in which small amounts of antigen led to T-cell unresponsiveness, whereas larger doses tolerized B cells as well as T cells. Moreover, the difference in the threshold of tolerance induction between T cells and B cells led Miller (1971) to suggest: "It may in fact turn out that tolerance to self components is a property confined exclusively to the T-cell population, tolerance in B cells being merely a laboratory artifice."

However, there are several reasons why an unpurged B-cell repertoire could pose a threat to the host. First, residual self-reactive B cells can be triggered by T-independent stimuli, such as the polyclonal activator CpG (type I T-independent antigen) or highly repetitive complement-fixing polysaccharides like those present on many capsulated bacteria (type II T-independent antigens). Moreover, self-reactive B cells play diverse roles in autoimmune responses; thus, the minority B1-cell subset is a major source of IgM autoantibodies with specificity for intracellular self-antigens (Casali et al., 1994), while class-switching B2 cells contribute to T-dependent autoimmune responses, either by secreting pathogenic IgG autoantibodies or by presenting self-antigens to activated $CD4^+$ T cells, which can then induce damage in target tissues. In nonobese diabetic (NOD) mice, for example, that spontaneously develop autoimmune (type 1) diabetes, B cells appear to play a direct role in disease pathogenesis, predominantly through the latter rather than the former mechanism (Silveira et al., 2004). There is also some evidence from animal models of rheumatoid arthritis that B cells may be directly pathogenic in joints. This may explain the efficacy of anti-CD20 monoclonal antibodies in treating this disease (Edwards et al., 2004).

Another reason why tolerance needs to be imposed on the B-cell as well as the T-cell repertoire is the situation where a self-reactive B cell may also recognize (cross-react with) a foreign antigen. Even if it is argued that T-cell self-tolerance alone would prevent self-reactive B cells from responding to endogenous antigens, cross-reactivity of such B cells with an epitope on an invading foreign antigen would effectively bypass T-cell self-tolerance by facilitating collaboration with nontolerant antiforeign T cells. A similar situation can arise when non-self-reactive B cells recognize a foreign antigen, collaborate with antiforeign T cells and undergo SHM of their immunoglobulin variable region genes in the GC. Because the nature of SHM is essentially random, B cells with new antiself specificities or increased affinity for self-antigens can appear in the GC and, if they still recognize the original immunogen, continue to receive T-cell help. The absence of self-tolerance mechanisms within either the primary or secondary B-cell repertoires would therefore significantly increase the potential for pathogenic autoantibody responses driven by antiforeign T cells. For all these reasons it is desirable for self-tolerance to be imposed on the B-cell as well as the T-cell repertoire.

MECHANISMS OF B-CELL SELF-TOLERANCE

Classification

Deletion as initially described by Burnet (1959) is not the only mechanism responsible for preventing autoimmunity. Rather self-tolerance has come to be regarded as a syndrome in which multiple mechanisms operate to polarize the immune response towards a negative outcome (Nossal, 1983). In addition to the landmarks in the evolution of the phenomenon of tolerance already mentioned, the past 30 years have seen the introduction of the idiotype regulation concept (Jerne, 1971), receptor editing (Gay et al., 1993; Tiegs et al., 1993), and the use of B-cell transgenic models for analyzing underlying mechanisms (see below). For convenience these mechanisms can be classified into *de novo* and regulatory (Tables 13.1 and 13.2). The former includes deletion and anergy, which operate throughout B-cell differentiation and contribute to the shaping of the repertoire, whereas regulatory mechanisms control the level of responsiveness in the mature B-cell pool without exerting a major effect on repertoire selection.

De Novo Mechanisms

The low frequency of antigen-specific B cells in the normal repertoire (approximately 10^{-3}–10^{-5}) made it difficult in the past to determine the relative importance of the various primary mechanisms of self-tolerance in the intact host. Although *in vitro* use of the surrogate antigen anti-immunoglobulin provided some clues about the normal fate of self-reactive B cells [reviewed in Nossal (1983; 1992)], definitive answers had to await the advent of transgenesis in the 1980s. By introducing transgene-encoded prearranged immunoglobulin heavy and light chain genes, mice were generated in which the majority of B cells expressed BCRs with a single defined specificity. These cells could therefore be tracked *in vivo* by flow cytometry using antiallotypic or anti-idiotype antibodies recognizing the transgenic BCR. Antigen-related as well as cellular variables in self-tolerance could therefore be studied by creating double transgenic mice consisting of a BCR transgenic line crossed with a second line expressing the corresponding self- or neo–self-antigen endogenously.

The overall conclusion from experiments in many such models is that B-cell self-tolerance is indeed mediated by a range of different mechanisms (Table 13.1). Among the most flexible and widely used double transgenic system is

TABLE 13.1 Primary mechanisms of B-cell self-tolerance

Mechanism	Site		Antigenic properties	Mode of action
	Central	Peripheral		
Deletion*	+	+	Multivalent high affinity	Activation of proapoptotic Bcl-2 family molecules
Receptor Editing*	+	–	Mulitivalent	Revised B-cell receptors (BCRs) with nonself specificities
Anergy*	+	+	Paucivalent soluble	BCR desensitization Lack of access to BAFF
Ignorance†	+	+	Low affinity Low concentration Poor access	No BCR recognition

*Result in exclusion of self-reactive B cells from mature B-cell pool.
†Self-reactive B cells present in mature B-cell pool.

TABLE 13.2 Secondary mechanisms of B-cell self-tolerance*

Mechanism	Examples	Mode of action
B-cell intrinsic	ITIM-containing motifs, e.g., FcγRIIb, CD22 Signaling molecules, e.g., Lyn, CD19	Increase threshold of B-cell receptor (BCR) activation and reduce B-cell expansion
B-cell extrinsic	Idiotype network	Neutralization of autoantibodies
	Classical complement pathway	Removal of apoptosing cells, guiding B cells to niches of negative selection
	Regulatory T cells	Reduce T-cell help for self-reactive B cells

*Operate mainly in peripheral lymphoid tissues.
ITIM, immunoreceptor tyrosine-based inhibition motif.

the hen-egg lysozyme (HEL)/anti-HEL BCR model originally developed by Goodnow et al. (1988) and recently modified such that the B cells can undergo switching from IgM to downstream isotypes (Phan et al., 2003). This, and other models where relevant, will be used to illustrate the various primary mechanisms of B-cell self-tolerance.

Deletion

Self-reactive B cells undergo negative selection on exposure to antigen at both immature and mature stages in their development within the bone marrow and peripheral lymphoid tissue, including the outer periarteriolar lymphatic sheath (PALS) of the T-cell zone and GCs (Russell et al., 1991, reviewed in Goodnow et al. 1995). Self-antigen in multivalent form favors deletion in the absence of signal 2, provided that a critical threshold of avidity is attained (Nemazee and Bürki, 1989; Hartley et al., 1991) (Figures 13.1 and 13.2). B1 cells as well as B2 cells are susceptible to deletion (Murakami et al., 1992). The molecular basis of B-cell deletion has yet to be fully worked out. However, the balance between pro- and anti-apoptotic members of the Bcl-2 family of molecules belonging to the classical mitochondrial pathway appears to be important for negative selection of B cells in the bone marrow and deletion of mature antigen-activated B cells in the periphery (Marsden and Strasser, 2002).

Receptor Editing

Self-reactive B-cell-expressing BCRs of medium to high affinity can also have their self-reactivity abolished by the process of receptor editing (Gay et al., 1993; Tiegs et al., 1993). Following engagement of BCR on immature B cells within the bone marrow, recombinase-activating genes (RAG 1 and 2) can be re-expressed, resulting in new VJ rearrangements at the immunoglobulin light chain loci. If a new light chain is successfully produced and the revised BCR loses self-reactivity, the B cell can then migrate to join the mature lymphocyte pool (Figure 13.2). During the past decade it has become apparent that receptor editing plays a more significant role in shaping the immature B-cell repertoire than deletion per se. Thus, in a recent study of B cells taken from patients with SLE, the relative contributions of the two mechanisms were found to be approximately 4 : 1, respectively (Wardemann et al., 2003).

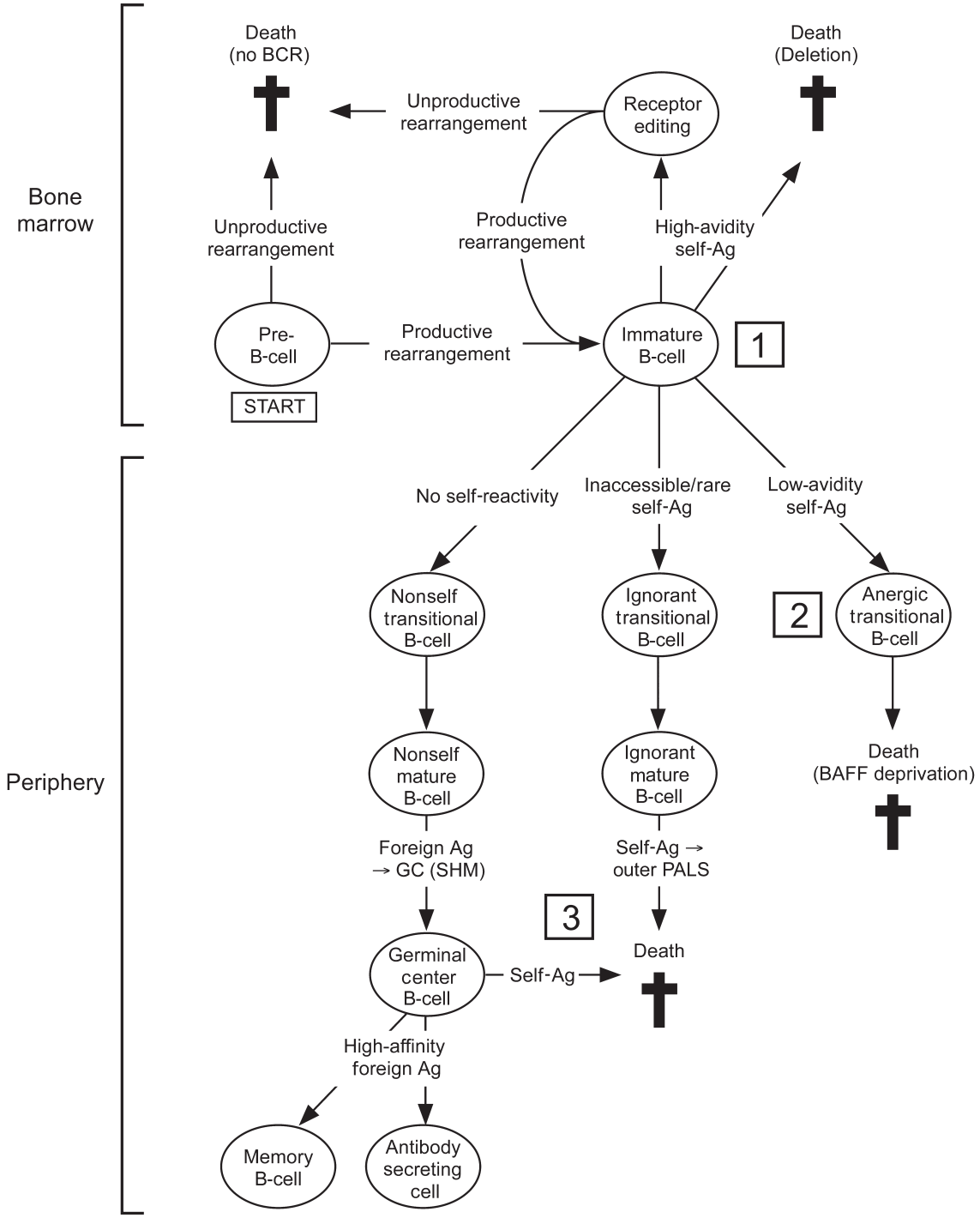


FIGURE 13.2 B-cell self-tolerance is enforced at multiple checkpoints during bone marrow and peripheral B-cell development. Self-reactive B cells are removed at three critical checkpoints during B-cell development: 1) following B-cell receptor (BCR) expression in the bone marrow (deletion or receptor editing); 2) at the immature transitional stage in the periphery (death of anergic B cells); and 3) following contact of mature B cells with antigen in the periphery (death of previously ignorant B cells and B cells that acquire self-reactivity in the germinal center (GC). See text for details. Ag, antigen; PALS, periarteriolar lymphatic sheath; SHM, somatic hypermutation.

Anergy

The term anergy was originally coined by Nossal (Nossal and Pike, 1980) to describe B cells which had *not been deleted but were functionally silenced* following *in vitro* cross-linking of BCR in the absence of second signals. Subsequently, anergy was clearly demonstrated *in vivo* in the HEL double transgenic model when transgenic B cells were exposed to soluble rather than multivalent neo–self-antigen (Goodnow et al., 1988). These anergic cells migrated from bone marrow to the splenic follicle to join the mature B-cell pool (Figure 13.2).

In the absence of competition from non-self-reactive B cells, anergic B cells survive in the periphery for several weeks. However, when normal levels of competition are present, the lifespan of anergic B cells drops to 2–3 days, effectively purging them from the mature B-cell pool. The reason for this effect is thought to be that anergic B cells do not compete efficiently for factor(s) required for B-cell survival and maturation. Recent studies using the HEL double transgenic model indicate that the factor in question is the B-cell survival factor, BAFF. Thus, anergic B cells respond poorly to BAFF and can be rescued from their normal early death when BAFF is overexpressed *in vivo* (Lesley et al., 2004; Thien et al., 2004). Increased expression of BAFF, as reported in patients with Sjögren syndrome and SLE, may therefore contribute to the pathogenesis of these autoimmune diseases by subverting the normal mechanisms of B-cell self-tolerance [reviewed in Mackay and Tangye (2004)] (See Chapter 11).

The principal biochemical lesion in anergic B cells is inactivation of signaling through the BCR (Cooke et al., 1994). In these cells there is a failure to activate the JNK and NF-κB pathways [reviewed in Jun and Goodnow (2003)] and to upregulate the costimulatory molecule CD86 required for antigen presentation to activated T cells, meaning that signal 1 is effectively inactivated. When this molecular lesion is taken in conjunction with the exclusion of anergic B cells from the long-lived B-cell pool, the outcome is a dramatic reduction in the risk of activation of such self-reactive clones by antigen-dependent or polyclonal stimuli (Phan et al., 2003).

Ignorance

Ignorance is not an active mechanism of self-tolerance; rather it refers to self-reactive B cells which are not anergic but persist in a potentially active state (Figure 13.2). This situation occurs if the binding of self-antigen to BCR is below a critical threshold (25–50% receptor occupancy) (Goodnow et al., 1988) or if self-antigen is expressed in a tissue not normally accessed by developing B cells (Akkaraju et al., 1997). The persistence of such cells poses a relevant threat to the maintenance of self-tolerance. For example, B cells reactive against vitreous-associated ocular antigens can produce autoantibodies if these normally inaccessible autoantigens leak into the circulation (Grisanti et al., 1994).

Regulatory Mechanisms

Superimposed on the *de novo* mechanisms of B-cell self-tolerance are a number of cellular and biochemical failsafe stratagems designed to control the level of B-cell responsiveness in peripheral lymphoid tissue once an autoimmune response has been initiated (Table 13.2). They can be divided into B-cell *intrinsic* and *extrinsic* mechanisms. Among the former are immunoreceptor tyrosine-based inhibitory motif (ITIM) surface molecules, such as FcγRIIb and CD22, which can reduce B-cell proliferation and/or raise the threshold of BCR signaling (Doody et al., 1996; Bolland and Ravetch, 2000). B-cell extrinsic factors comprise antibodies, complement, and its receptors, regulatory T cells, and an absence of T-cell help leading to "death by neglect."

In addition to feedback inhibition via FcγRIIb, antibodies may regulate ongoing B-cell responses through the *idiotypic network*, as originally proposed by Jerne (1971). The effect of this network was originally thought to be directed largely against B1 cells that produce polyreactive "natural" autoantibodies against a range of self-antigens, including nucleic acids, phospholipids, and cytoskeletal proteins (Casali et al., 1994). Recent work, however, has revealed significant levels of IgG anti-idiotypic specificities in normal human serum directed against pathologic as well as natural autoantibodies. Moreover, there is growing support for the concept that one of the modes of action of intravenous immunoglobulin (IVIG) in controlling autoimmune diseases like autoimmune thrombocytopenic purpura is related to the presence of anti-idiotypic antibodies capable of neutralizing the effects of antiplatelet autoantibodies (Kazatchkine and Kaveri, 2001).

The *classical complement pathway* appears to play an intriguing role in regulating autoimmunity by one of three mechanisms. The first and best known is related to the capacity of complement-binding immune complexes to be removed from the circulation. Depending on their size and charge, these complexes may either be rendered harmless, e.g., in the liver, or be deposited in the tissues, thereby causing damage. Secondly, complement fragments have been shown to enhance uptake and clearance, not just of soluble immune complexes but of apoptotic cells by phagocytes. The importance of this mechanism is related to the fact that blebs on apoptosing cells are highly immunogenic by virtue of their content of CpG-containing nuclear antigens. Thirdly, complement-containing self-antigen complexes may contribute to B-cell self-tolerance by guiding $CD21/35^{+}$ B cells to their stromal cell niches in bone marrow and spleen where negative selection normally takes place.

This mechanism may explain the enhanced susceptibility of C1q- and C4-deficient mice and humans to lupus, and of transitional B cells to tolerance, since the CD21/35 receptor complex is first expressed at this stage in B-cell differentiation and influences the threshold of BCR signaling [reviewed in Carroll (2004)].

Finally, T cells play an important role in the maintenance of B-cell self-tolerance in one of three ways. First, B2 cells, including those with antiself reactivity, have a finite lifespan in the absence of T-cell help and "die by neglect" following antigen encounter (Figure 13.1). Secondly, anergic self-reactive B cells are eliminated by a Fas-dependent mechanism should they be exposed to self-antigen and activated Th cells (Rathmell et al., 1995). Thirdly, and probably of greatest biologic significance for controlling antiself B cells as well as T cells, is the mechanism of *T-dependent regulation (suppression)*. After 20 years in the wilderness, regulatory T cells have re-emerged as important cells in preventing autoimmunity. Interestingly, B cells as well as T cells were originally thought to be the direct target of these cells (Adelstein et al., 1990). The current view, however, is that regulatory T cells appear to operate on B cells predominantly by interacting with other T cells, including $CD4^+$ Th cells, and are involved in the phenomenon of immune deviation or "split tolerance" (Parish, 1972). Like the other mechanisms in this category, regulatory T cells operate to control both antiforeign and antiself T-dependent responses.

Multiple Checkpoints of B-Cell Self-Tolerance

The diversification of the B-cell repertoire in two stages and at diverse anatomic sites highlights the necessity to have multiple "checkpoints" or filters for controlling self-reactivity in the B-cell lineage (Goodnow et al., 1995). This concept is illustrated in Figure 13.2. In brief, developing B-lineage cells that successfully express a BCR following VDJ recombination are subject to deletion or receptor editing in the bone marrow if they recognize a strongly cross-linking (e.g., cell surface) self-antigen (checkpoint 1). In conjunction with unsuccessful VDJ recombination, this results in the loss every day of approximately 75% of the 5×10^7 B-lineage cells generated by the mouse (Osmond, 1991). Of the remaining 25%, a further 15% die in the periphery at the sensitive transitional B-cell stage, including those recognizing more weakly cross-linking (e.g., soluble) self-antigens (checkpoint 2). The 10% of B cells that survive to become mature B cells again become susceptible to negative selection following exposure to antigens in the periphery (checkpoint 3). This occurs either at the outer PALS for previously "ignorant" self-reactive B cells due to lack of T-cell help, or in GCs where antiforeign B cells undergo SHM and potentially acquire self-reactivity for the first time. Superimposed on these deletional checkpoints, regulatory mechanisms operate to reinforce negative selection and to control the responsiveness of any residual B cells expressing antiself BCR.

AUTOIMMUNITY: A BREAKDOWN IN B-CELL SELF-TOLERANCE

A pool of self-reactive B cells comprising potentially responsive and anergic cells exists in peripheral lymphoid tissue (see above) with the capacity to generate pathogenic autoimmune responses in the event of a failure in self-tolerance. Broadly speaking, there are five situations that can lead to activation of this pool of self-reactive B cells, which are summarized here. Additional examples of autoimmunity are provided in Chapter 11.

In the first, T-cell self-tolerance is intact, but foreign antigen-specific T cells may interact with residual nontolerant self-reactive B cells. In other words, self-reactive (ignorant) B cells may present cross-reactive epitopes present on a foreign antigen (*molecular mimicry*) or a self-antigen modified by exposure to drugs or viruses to such antiforeign T cells [reviewed by Goodnow et al. (1990) and Hodgkin and Basten (1995)]. Examples of the former situation include rheumatic fever following Group A streptococcal infection and cold agglutinin disease following mycoplasma infection, while α-methyldopa-induced autoimmune hemolytic anemia and rubella virus-induced autoimmune thrombocytopenic purpura may represent instances of the latter [reviewed in Ebringer et al. (2003)].

Secondly, residual self-reactive B cells, even if they are anergic, can be stimulated directly by T-independent ligands such as lipopolysaccharide and DNA CpG motifs, which interact with TLR 4 and 9 on the B-cell surface, respectively [reviewed in Vinuesa and Goodnow (2002)]. Normally, such interactions lead to production of low-affinity IgM autoantibodies of little pathogenic significance. However, autoimmune-prone mice expressing large numbers of IgG2a self-reactive B cells, for example, contain IgG2a complexed with self-DNA or chromatin, which cross-link BCR with TLR9, resulting in high levels of circulating rheumatoid factor (Leadbetter et al., 2002). A similar mechanism may explain the occurrence of rheumatoid factors as well as contribute to "epitope spreading" in patients with systemic autoimmune diseases like rheumatoid arthritis, Sjögren syndrome, and mixed connective tissue disease.

In the third situation, T-cell tolerance is defective as it occurs, for example, in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, which is due to mutations in the AIRE gene (Peterson et al., 2004). Since this gene regulates expression of self-peptides in the thymus, self-reactive T cells escape to the periphery where they can interact with nontolerant

(ignorant) self-reactive B cells, leading to secretion of multiple autoantibodies associated with a range of organ-specific diseases.

Fourthly, a primary defect may occur in self-reactive B cells per se, resulting in a breakdown in self-tolerance. For example, receptor editing is impaired in autoimmune-prone MRL mice and in some patients with lupus (Wardemann et al., 2003). Moreover, defective negative selection at the transitional stage of B-cell differentiation can also be a feature of lupus, while a P227A polymorphism in CD40 has been detected on B cells from members of several lupus families (Grammer and Lipsky, 2003). Superimposed on these B-cell-selective abnormalities are genetic defects in ITIMs, signaling pathways, and proapoptotic proteins, which further predispose to a breakdown in self-tolerance, often in T cells as well as B cells. The mouse models of autoimmune disorders associated with dysregulation of CD22 or FcγRIIb, lyn or CD19, and Bim, respectively, are all examples of this group of mechanisms (Cornall et al., 1998; see Chapter 11), as are the various types of human autoimmune lymphoproliferative syndrome (ALPS) characterized by mutations in Fas (or FasL) (Rieux–Laucat et al., 2003) (see Chapters 11 and 19). Of particular interest here is the recent finding that partial restoration of FcγRIIB levels on B cells from lupus-prone mice restores the tolerant state (McGaha et al., 2005) (See Chapter 70).

Finally, the defect may lie not in the primary but in the secondary mechanisms of B-cell self-tolerance related to complement-signaling pathways and regulatory T cells that control the level of responsiveness in peripheral lymphoid tissue (Table 13.2; see above). The association between autoimmunity and primary or acquired immunodeficiency states is one cogent piece of evidence supporting this possibility. Thus, in addition to the well-recognized linkage of SLE with primary complement deficiencies (Carroll, 2004) and of autoantibody-mediated hemolytic anemia with B-cell lymphomas, complicated by hypogammaglobulinemia, an increasing number of clinical situations as well as experimental models are being reported in which defective immune regulation predisposes to autoimmunity involving the B-cell lineage. Perhaps the most striking example is the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. In this primary immune deficiency, loss of function mutations in the FoxP3 gene expressed by natural $CD4^+CD25^+$ regulatory T cells can lead to certain organ-specific autoimmune diseases, inflammatory bowel disease, and allergy [reviewed in Nieves et al. (2004); see Chapter 9]. Moreover, about one-third of multiple sclerosis patients given the lymphocyte-depleting antibody, CAMPATH-IH, develop autoimmune thyroid disease (Coles et al., 1999). Collectively these examples lend strong support to the notion that immune dysregulation is of major importance in the development of autoimmunity.

References

Adelstein, S., Pritchard-Briscoe, H., Loblay, R.H., and Basten, A. (1990). Suppressor T cell memory. *Curr. Top. Microbiol. Immunol. 159*, 123–138.

Akkaraju, S., Canann, K., and Goodnow, C.C. (1997). Self-reactive B cells are eliminated or inactivated by autoantigen expressed on thyroid epithelial cells. *J. Exp. Med. 15*, 2005–2012.

Basten, A. and Goodnow, C.C. (1990). Self tolerance in T cells and B cells. In M. Kendall and M. Ritter (eds), *Thymus Update 3* (pp. 113–126). London: Harwood Academic.

Basten, A. and Miller, J.F.A.P. (1974). Cellular interactions in the immune response. In R.P. Cox (ed.), *Cell Communication* (pp. 187–216). Chichester: John Wiley & Sons.

Billingham, R.E., Brent, L., and Medawar, P.B. (1953). Actively acquired tolerance of foreign cells. *Nature 172*, 603–606.

Bolland, S. and Ravetch, J.V. (2000). Spontaneous autoimmune disease in FcγRIIb-deficient mice results from strain-specific epistasis. *Immunity 13*, 277–285.

Bretscher, P., and Cohn, M. (1970). A theory of self-nonself discrimination. *Science 169*, 1042–1049.

Buchmeier, M.J., Welsh, R.M., Dutko, F.J., and Oldstone, M.B. (1980). The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol. 30*, 275–331.

Burnet, F.M. (1959). *The Clonal Selection Theory of Acquired Immunity*. Nashville: Vanderbilt University Press.

Carroll, M.C. (2004). Complement system in B cell regulation. *Mol. Immunol. 41*, 141–146.

Casali, P., Kasaian, M.T., and Haughton, G. (1994). C-1 (CD5 B) cells. In A. Coutino and M.D. Kazatchkine (eds), *Autoimmunity–Physiology and Disease* (pp. 57–88). New York: Wiley-Liss.

Chiller, J.M., Habicht, G.S., and Weigle, W.O. (1970). Cellular sites of immunologic unresponsiveness. *Proc. Natl. Acad. Sci. USA 65*, 551–556.

Coles, A.J., Wing, M., Smith, S., Coraddu, F., Greer, S., Taylor, C., Weetmann, A., Hale, G., Chatterjee, V.K., Waldmann, W., and Compston, A. (1999). Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet 354*, 1691–1695.

Cooke, M.P., Heath, A.W., Shokat, K.M., Zeng, Y., Finkelman, F.D., Linsley, P.S., Howard, M., and Goodnow, C.C. (1994). Immunoglobulin signal transduction guides the specificity of B cells T cell interactions and is blocked in tolerant self-reactive B cells. *J. Exp. Med. 179*, 425–438.

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 signalling and selection. *Immunity 8*, 497–508.

Doody, G.M., Dempsey, P.W., and Fearon, D.T. (1996). Activation of B lymphocytes: Integrating signals from CD19, CD22 and FcγRIIb. *Curr. Opin. Immunol. 8*, 378–382.

Ebringer, A., Rashid, T., and Wilson, C. (2003). In M Zouali (ed.), Molecular mimicry as the basis of a new theory of autoimmunity. *Frontiers in Autoimmunity* (pp. 79–99). Okmsha: IOS Press.

Edwards, J.C.W., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Emery, P, Close, D.R., Stevens, R.M., and Shaw, T. (2004). Efficacy of B cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N. Engl. J. Med. 350*, 2572–2581.

Ehrlich, P. and Morgenroth, J. (1957). On haemolysins: Third and fifth communications. Reprinted in *The Collected Papers on Paul Ehrlich,* vol. 2 (pp. 246–255). London: Pergamon.

Gay, D., Saunders, T., Camper, S., and Weigert, M. (1993). Receptor editing: An approach by autoreactive B cells to escape tolerance. *J. Exp. Med. 177*, 999–1008.

Goodnow, C.C., Crosbie, J., Adelstein, S., Lavoie, T.B., Smith-Gill, S.J., Brink, R.A., Pritchard-Briscoe, H., Wotherspoon, J.S., Loblay, R.H., Raphael, K., Trent, R.J., and Basten, A. (1988). Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature 334*, 676–682.

Goodnow, C.C., Adelstein, S., and Basten, A. (1990). The need for central and peripheral tolerance in the B cell repertoire. *Science 248*, 1373–1379.

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.

Gowans, J.L. and Knight, E.J. (1964). The route of re-circulation of lymphocytes in the rat. *Proc. R. Soc. London Ser. B 159*, 257.

Grisanti, S., Heimann, K., and Wiedemann, P. (1994) Immune response to specific molecules of the retina in proliferative vitreoretinal disorders. *Graefes Arch. Clin. Exp. Ophthalmol. 232*, 302–307.

Hartley, S.B., Crosbie, J., Brink, R., Kantor, A.B., Basten A., and Goodnow, C.C. (1991). Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognising membrane-bound antigens. *Nature 353*, 765–769.

Hodgkin, P.D. and Basten, A. (1995). B cell activation, tolerance and antigen presenting function. *Curr. Opin. Immunol. 7*, 121–129.

Jerne, N.K. (1971). Towards a network theory of the immune system. *Ann. Int. Pasteur (Paris) 125C*, 373–389.

Jun, J.E. and Goodnow, C.C. (2003). Scaffolding of antigen receptors for immunogenic versus tolerogenic signalling. *Nat. Immunol. 4*, 1057–1064.

Kazatchkine, M.D. and Kaveri, S.V. (2001) Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N. Engl. J. Med.* 345, 747–755.

Lafferty, K.J. and Cunningham, A.J. (1975). A new analysis of allogeneic interactions. *Australas. J. Exp. Biol. Med. Sci. 53*, 27–42

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature 416*, 603–607.

Lederberg, J. (1959). Genes and antibodies: Do antigens bear instructions for antibody specificity or do they select cell lines that arise by mutation? *Science 129*, 1649–1653.

Lesley, R., Zu, Y., Kalled, S.L., Hess, D.M., Schwab, S.R., Shu, H.B. and Cyster, J.G. (2004). Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. *Immunity 20*, 441–453.

Li, H., Jiang, Y., Prak, L.L., Radic, M., and Weigert, M. (2001). Editors and editing of anti-DNA receptors. *Immunity 15*, 947–957.

Mackay, F. and Tangye S.G. (2004). The role of the BAFF/APRIL system in B cell homeostasis and lymphoid cancers. *Curr. Opin. Pharmacol. 4*, 347–354.

Marsden, V.S. and Strasser, A. (2002). Control of apoptosis in the immune system: Bcl-2, BH3-Only proteins and more. *Annu. Rev. Immunol. 21*, 71–105.

Matzinger, P. (1994). Tolerance, danger and the extended family. *Annu. Rev. Immunol. 12*, 991–1045.

McGaha, T.L., Sorrentino, B., and Ravetch, J.V. (2005). Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science 307*, 590–593.

Miller, J.F.A.P. (1961). Immunological function of the thymus. *Lancet 2*, 748–749.

Miller, J.F.A.P. (1971). The thymus in immunity. *Rec. Austral. Acad. Sci. 2*, 82–98.

Mitchell, G.F. and Miller, J.F.A.P. (1968). Cell to cell interaction in the immune response II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J. Exp. Med. 128*, 821–837.

Murakami, M., Tsubata, T., Okamoto, M., Shimizu, A., Kumagai, S., Imura, H., and Honjo, T. (1992). Antigen induced apoptotic death of Ly-1B cells responsible for autoimmune disease in transgenic mice. *Nature 357*, 77–80.

Nemazee, D.A. and Bürki, K. (1989). Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature 337*, 562–566.

Nieves, D.S., Phipps, R.P., Pollock, S.J., Ochs, H.D., Zhu, Q.L., Scott, G.A., Ryan, C.K., Kobayashi, I., Rossi, T.M., and Goldsmith, L.A. (2004). Dermatologic and immunologic findings in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *Arch. Dermatol. 140*, 466–472.

Nossal, G.J.V. (1957). The immunological response of foetal mice to influenza virus. *Austral. J. Exp. Biol. 35*, 549.

Nossal, G.J.V. (1983). Cellular mechanisms of immunologic tolerance. *Annu. Rev. Immunol. 1*, 33–62.

Nossal, G.J.V. (1992). Cellular and molecular mechanisms of B lymphocyte tolerance. *Adv. Immunol. 52*, 283–331.

Nossal, G.J.V. and Pike, B.L. (1980). Clonal anergy: Persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proc. Natl. Acad. Sci. USA 70*, 1602–1606.

Osmond, D.G. (1991). Proliferation kinetics and the life span of B cells in central and peripheral lymphoid organs. *Curr. Opin. Immunol. 3*, 179–185.

Owen, R.D. (1945). Immunogentic consequences of vascular anastomoses between bovine twins. *Science 102*, 400–401.

Parish, C.R. (1972). The relationship between humoral and cell mediated immunity. *Trans. Rev. 13*, 35–66.

Peterson, P., Pitkanen, J., Sillanpaa, N., and Krohn, K. (2004). Autoimmune polyendocrinopathy candidiases ectodermal dystrophy (APECED): a model disease to study molecular aspects of endocrine autoimmunity. *Clin. Exp. Immunol. 135*, 348–357.

Phan, T.G., Amesbury, M., Gardam, S., Crosbie, J., Hasbold, J., Hodgkin, P.D., Basten, A., and Brink, R.A. (2003). B cell receptor-independent stimuli trigger immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. *J. Exp. Med. 197*, 845–860.

Rathmell, J.C., Cooke, M.P., Ho, W.Y., Grein, J., Townsend, S.E., Davis, M.M, and Goodnow, C.C. (1995). CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with $CD4^+$ T cells. *Nature 376*, 181–184.

Rieux-Laucat, F., Le Deist, F., and Fischer, A. (2003). Autoimmune lymphoproliferative syndromes: genetic defects of apoptosis pathways. *Cell Death Diff. 10*, 124–133.

Russell, D.M., Dembic, Z., Morahan, G., Miller, J.F.A.P., Bürki, K., and Nemazee, D. (1991). Peripheral deletion of self-reactive B cells. *Nature 354*, 308–311.

Schwartz, R.H. (1993). Immunological tolerance. In W.E. Paul (ed.), *Fundamental Immunology*, 3rd ed. (pp. 677–732). New York: Raven.

Silveira, P.A., Dombrowsky, J., Johnson, E., Chapman, H.D., Nemazee, D., and Serreze, D.V. (2004). B cell selection defects underlie the development of diabetogenic APCs in nonobese diabetic mice. *J. Immunol. 172*, 5086–5094.

Thien, M., Phan, T.G., Gardam, S., Amesbury, M., Basten, A., Mackay, F., and Brink, R. (2004). Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity 20*, 785–798.

Thyphronitis, G., Kinoshita, T., Inoue, K., Schweinle, J.E., Tsokos, G.C., Metcalf, E.S., Finkelman, F.D., and Balow, J.E. (1991). Modulation of mouse complement receptor 1 and receptor 2 suppresses antibody responses *in vivo*. *J. Immunol. 147*, 224–230.

Tiegs, S.L., Russell, D.M., and Namazee, D.A. (1993). Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med. 177*, 1009–1020.

Traub, E. (1938). Factors influencing the persistence of choriomeningitis virus in the blood of mice after clinical recovery. *J. Exp. Med. 68*, 229–250.

Vinuesa, C.G. and Goodnow, C.C. (2002). Immunology: DNA drives autoimmunity. *Nature 416*, 595–598.

Wardemann, H., Yurasov, S., Schaefer, A., Young, J.W., Meffre, E., and Nussenzweig, M.C. (2003). Predominant autoantibody production by early human B cell precursors. *Science 301*, 1374–1377.

Zinkernagel, R.M. and Doherty, P.C. (1974). Restriction of *in vitro* T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature (London) 248*, 701–702.

CHAPTER

14

Generation of T-cell Antigenic Determinants in Autoimmunity and Their Recognition

EMANUAL MAVERAKIS*
*Department of Dermatology, University of California Davis, Sacramento, California

KAMAL D. MOUDGIL†
†Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland

ELI E. SERCARZ‡
‡Division of Immune Regulation, Torrey Pines Institute for Molecular Studies, San Diego, California, USA

This chapter focuses on the cellular strategy inherent in the creation of the self-determinants that become involved in autoreactivity. For an excellent and concise review of the biochemistry of T-cell receptor (TCR)–major histocompatibility complex (MHC)–antigen interaction, the reader is referred to Krogsgaard and Davis (2005).

NATURE OF AUTOANTIGENIC DETERMINANTS

Immunodominant, Subdominant, and Cryptic Determinants

Two decades ago, when T-cell determinant structures of model antigens were first characterized, a surprising observation was made: only a few regions of the intact pro-tein could elicit immune responses (Katz et al., 1982; Shimonkevitz et al., 1984). An initial theory to explain the unresponsiveness to other regions was simply that the T-cell repertoire was restricted and lacked receptors capable of responding to these other regions (i.e., holes existed in the T-cell repertoire). While examples of holes in the repertoire can be cited (Nanda et al., 1991; Moudgil et al., 1996), we favored the idea that a primary consideration for immunodominance was the competitive nature of the processing events within the antigen-presenting cell (APC).

The concept of dominance and crypticity was established with the use of T-cell proliferation assays. Dominant determinants were defined as regions of the protein molecule that could recall a strong *in vitro* proliferative response after the animal was primed with a whole-protein antigen. Cryptic determinants were defined as regions unable to recall such a response after the animal was primed with whole-protein antigen, but potentially excellent inducers of a proliferative response if used as the initial priming immunogen. Thus, outside their molecular context they were perfectly capable of initiating an immune response, but in the context of the intact protein antigen they were "invisible" to the immune system. It was hypothesized that dominant determinants were regions of the protein molecule readily processed by

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

the APC (Sercarz et al., 1993). In contrast, cryptic determinants were hypothesized to be poorly displayed after processing. However, certain cryptic determinants ("latent" or "facultative" cryptics) can be efficiently displayed under conditions of upregulated antigen processing and presentation (Lehmann et al., 1992; Moudgil and Sercarz, 1994; Opdenakker and Van Damme, 1994). Finally, there are determinants that fit between these extremes, "subdominant" determinants, which induce a weak-to-moderate level of T-cell response in comparison to the dominant determinants.

Thus, a determinant hierarchy exists on the surface of an APC. Determinant display hierarchies have now been confirmed by direct elution experiments. Unanue and colleagues (Viner et al., 1995) endogenously expressed hen-egg lysozyme (HEL) and then eluted the processed peptides from the surface of APCs, showing that relatively few regions of the intact molecule are presented by the APC. Since our original description of dominance and crypticity, other reasons for crypticity have been described (Grewal et al., 1995; Viner et al., 1995; Moudgil et al., 1996; 1998), and, although usually the case, there is not always a direct correlation between determinant display and T-cell proliferation.

Immunogenic, Dominant Self-Determinants, and the Self-Directed T-Cell Repertoire

Initially, it was thought that central and peripheral tolerance could effectively purge all self-reactive T cells. Although certain strains of mice were known to be susceptible to autoimmunity, self-reactive T cells were thought to be unique to these strains. It is now known, however, that all individuals harbor self-directed T cells capable of causing autoimmunity (Shaw et al., 1992; Abromson-Leeman et al., 1993; 1995). How do residual self-reactive responses remain if central and peripheral tolerances are so effective? Experimentally supported theories have arisen to explain how a potentially autoreactive T cell avoids tolerance induction. Most are centered on the principle that if presentation of a self-determinant in the thymus is too low (Gammon and Sercarz, 1989; Liu et al., 1995), negative selection will not occur and potentially autoreactive T cells will escape to the periphery (see Chapters 6 and 8).

Two types of sequestration can deny a determinant reasonable access to an MHC groove. In *geographic sequestration*, a self-determinant can be expressed in an immune-privileged site within the body (e.g., the eye) with no expression in the thymus (Griffith et al., 1995; Klein et al., 2000). However, several "geographically sequestered antigens" have now been shown to be expressed in the thymus. For example, the retinal antigen IRBP is expressed in the thymus at a low level, but consonant with inducing functional tolerance (Avichezer et al., 2003).

In fact, many other neuroproteins that had been thought of as sequestered can be found in the thymus (Pribyl et al., 1993; Feng et al., 2000; Klein et al., 2000). Interestingly, SJL mice mount a vigorous T-cell response to the self-peptide proteolipid protein (PLP) 139–151 due to a high precursor frequency of 139–151-specific T cells. This intact repertoire of self-reactive T cells exists because a splice variant (DM20) of PLP, which lacks the 139–151 portion, is the form of PLP expressed in the thymus. Thus, tolerance is achieved to all potential determinants on PLP with the exception of this region, leaving a 139–151 repertoire particularly poised to cause autoimmunity (Klein et al., 2000).

A similar situation exists with respect to a principal human HLA-DR2-restricted epitope, amino acids 85–99 of myelin basic protein (MBP), the display of which is inversely related to the level of a cysteine protease, asparaginyl endopeptidase (AEP). Since AEP is well expressed in the thymus, the presentation of 85–99 is low, allowing 85–99-specific T cells to escape tolerance induction (Anderton et al., 2002; Manoury et al., 2002). Thymic expression of self-antigens is also dependent in part on the AIRE (autoimmune regulatory element) protein, which is a transcription factor that promiscuously promotes expression of genes that are otherwise unique to other tissues (pancreatic antigens, thyroid antigens, myelin antigens). Lack of this factor results in multisystem autoimmunity (see Chapter 8). Nevertheless, as emphasized above, although many self-antigens are displayed, only some determinants on each antigen will become visible to ambient T cells.

In *molecular sequestration,* weakly displayed determinants: 1) are poorly processed from the native molecule; 2) have a poor binding affinity for the MHC molecules; 3) are out-competed for binding to the MHC class II molecules by a flanking determinant(s) (Maverakis et al., 2000b); or 4) are degraded by exopeptidases as a result of residing too close to another determinant that was successfully "captured" by a different MHC molecule.

The theory of molecular sequestration is based on the existence of hierarchies of determinant display for each self-protein, established by the processing and presentation mechanisms of the APCs' MHC class II pathways (Sercarz and Maverakis, 2003). It is essentially based on the affinity of the MHC molecule for different determinants within the protein and the location of these determinants with respect to antigen-processing sites within the protein (Schneider et al., 2000), and is subject to various secondary effects, such as hindrance by flanking residues (Grewal et al., 1995; Moudgil et al., 1996), competition by flanking determinants (Maverakis et al., 2003; Seamons et al., 2003; Sercarz and Maverakis, 2003), and effects contributed by the chaperone H-2M and H-20 proteins (Nanda and Sant, 2000).

Self-reactive T cells specific to well-displayed self-determinants are subject to the strongest tolerogenic pressures. With regard to well-displayed self-determinants,

tolerance generally eliminates the highest-avidity self-directed T cells, although with medium-to-poor determinant display, as is the case with subdominant and cryptic determinants, respectively, much of the available repertoire may remain intact. Thus, the self-directed T-cell repertoire will be deficient in high-affinity cells capable of responding to well-expressed determinants (Cibotti et al., 1994; Maverakis et al., 2000a), but will maintain a high-affinity repertoire to the cryptic self because the presentation of these determinants is insufficient to induce strong tolerance. The subdominant self-directed repertoire will be comprised of cells of intermediate affinity but should be deprived of most cells of high affinity for MHC–antigen.

The relationship between determinant hierarchies and tolerance induction has been supported experimentally. In studies with the model antigen, hen egg-white lysozyme, it was shown that B10.A (H-2^a) mice, when immunized with the intact protein, responded to certain codominant determinants, including HEL peptide 46–61. When animals were tolerized to whole HEL, responsiveness to the dominant peptide disappeared, but peptide responses to subdominant and cryptic determinants could still be elicited (Gammon and Sercarz, 1989). More interestingly, the degree of responsiveness to these subdominant/cryptic determinants was proportional to the amount of antigen used in the tolerance induction (Gammon and Sercarz, 1989). Likewise, when BALB/c (H-2^d) mice were made transgenic for HEL, making HEL a neo-self-antigen, the T-cell repertoire directed against the dominant I-E^d determinant, HEL:106–116, was functionally neutralized (Cibotti et al., 1992). By comparing the HEL-specific immune response of the HEL-transgenic mice to that of the nontransgenic H-2^d animals, it was discovered that T cells directed toward well-displayed "dominant" self-determinants are preferentially tolerized. Interestingly, the degree of tolerance induction was again found to be directly related to the amount of HEL expressed in the HEL-transgenic animal (Cibotti et al., 1992). Thus, although there was some residual responsiveness to HEL determinants in the transgenic animals, the HEL-specific T-cell repertoires of transgenic and wild-type animals were quite different. Likewise, the MBP-specific T-cell responses in MBP-deficient mice have been described as functionally different from those seen in MBP-expressing animals (Harrington et al., 1998; Targoni and Lehmann, 1998; Yoshizawa et al., 1998), as are self (mouse) lysozyme (ML) – specific T cell responses in the wild type versus ML knock-out mice (Sinha et al., 2004).

PROCESSING CREATES THE SELF

Antigen Processing

Antigen-processing events, both in the thymus and the periphery, influence the spectrum of determinants available to T cells, through influencing the display of dominant determinants, which either positively or negatively select the available repertoire. In the class II MHC processing program, both long and short peptides can bind to the open-ended groove. Whole molecules can bind to class II molecules, but some determinant(s) must be rendered available for binding, either by endopeptidases and/or disulfide bond reduction (Sercarz and Maverakis, 2003). The first endopeptidic cut provides mobility and availability to the stretches of peptide flanking the cut, and therefore, a determinant in this region is likely to be the first to bind to the MHC, and if the binding is of relatively high affinity, to become a dominant determinant on the antigen (Sercarz and Maverakis, 2003).

When a long peptide binds at a single site, the flanking ends will presumably become radically shortened, owing to exopeptidase action as well as some additional endopeptidase cuts, until peptide fragments of 13–22 amino acids are the majority remaining, with ends protruding from the MHC class II groove. Intriguing evidence for the binding of large fragments of HEL (Castellino et al., 1998) showed that many larger peptides with molecular weights between 3000 and 7000 Da could be eluted from either I-A or I-E molecules. Furthermore, complexes of 120 kDa could be found in the endocytic pathway, with a single HEL polypeptide chain of about 70 amino acids bound to two different MHC class II isotypes, I-A and I-E. This shows that binding of MHC class II molecules can occur before excessive antigen processing takes place.

Another set of experiments with HEL (Schneider et al., 2000) tested the proposition that a cryptic determinant could be rendered into a dominant one, if a processing site susceptible to an endopeptidase from the family of proprotein convertases (Seidah and Chretien, 1997) could be created adjacent to the determinant in question. This was accomplished by mutating a single residue on HEL to create a dibasic target site. The mutant HEL, with the extra processing site, induced a dominant response from a previously cryptic determinant. The importance of reductases was shown with the enzyme, interferon-γ-inducible lysozomal thiol reductase (GILT). A GILT knock-out mouse was able to respond to determinants with a single cysteine residue, but failed to respond to a tightly folded, double disulfide bond region of HEL which was immunogenic in the wild-type H-2^k mouse. Interestingly, the presentation of the dominant determinant of HEL, 46–61, was affected by GILT, even though it does not contain a cysteine, because in the absence of GILT there was a failure to reduce the nearby disulfide bond, cys64–cys80, and a consequent lack of response to 46–61 (Maric et al., 2001).

A final example with direct relevance for autoimmunity concerns MBP. The enzyme AEP was identified as the endopeptidase (Manoury et al., 1998) that could make the first cut in various proteins at a motif containing a crucial

asparagine. In the region between amino acids 89 and 101 of MBP, there are three overlapping determinants for the SJL mouse, MBP(89–94), MBP(92–98), and MBP(95–101), each binding to the same MHC molecule, and therefore subject to competition for the binding groove. In the absence of AEP the central determinant was dominant; however, AEP was shown to cleave MBP at position asn94, resulting in the destruction of this determinant, which allowed the flanking determinants to gain a degree of freedom from constraints of molecular rigidity and seclusion (Anderton et al., 2002). Accordingly, a flanking cryptic determinant indirectly achieved dominance in this situation.

Another site that is consequential in the race to be dominant concerns the removal of the invariant chain CLIP peptide from its position guarding the MHC class II groove. This is accomplished by two nonpolymorphic MHC molecules, DM and DO, which are special agents that supervise the occupation of the class II binding site. The DM/DO chaperone molecules play a crucial role in fostering the exchange for the CLIP peptide, and DM is also involved in a peptide-editing function, acting to favor the presentation of dominant epitopes and to disfavor recessive epitopes (Nanda and Sant, 2000). In DM knock-out mutants in H-2^d and H-2^k mouse strains, there is a selection away from dominant epitopes and toward the appearance of responses to novel epitopes (Nanda and Bikoff, 1995). There is also a DM-independent pathway which simply involves removal of Ii from MHC class II molecules (Wolf and Ploegh, 1995), followed by the binding of various large multideterminant fragments, which can be termed "prodeterminants" (Sercarz et al., 1993). The binding to class I MHC molecules is entirely different, following enzymatic activity by the proteosome, which cleaves the antigen into much smaller peptides, although these can be somewhat longer than the 8–10 amino acid length that fits snugly into the class I MHC, close-ended groove. Interestingly, a cytosolic pathway for MHC class II-restricted determinants has also been recently discovered (Tewari et al., 2005). This process is proteosome and transporter associated with antigen processing (TAP) dependent. Since the pathway may be restricted to virally infected dendritic cells (DCs), its role in the presentation of self-determinants is still uncertain.

The interactions of processing enzymes are quite complex, one example being the relative activities of cathepsin S and cathepsin L in controlling invariant chain degradation. The latter is active in thymic epithelial cells and kidney epithelium, but not in intestinal epithelium cells (IEC), while cathepsin S predominates in MHC class II maturation and presentation *in vivo* in professional as well as some nonprofessional cell types, such as IEC. Despite the dominance of cathepsin S in the IEC, cathepsin L is present, but is inhibited by cystatin F (Beers et al., 2005).

Thus, the nature of another hierarchy, that arising from the competition between processing enzymes, will determine the all important "first endopeptidic cut" that establishes in turn the hierarchy of peptide choice for the dominant determinant on the antigen. This determinant will be the one that, possibly among others, induces self-tolerance and in that sense, establishes which are the "self-determinants" (those which induce tolerance).

Influence of T-Cell and B-Cell Repertoires on Determinant Dominance

The T-cell repertoire can have a commanding role in the establishment of the dominant elements of a self-antigen. As one illustrative example, the dominant determinant on MBP in the B10.PL mouse strain is the N-terminal Ac1-9 or Ac1-11. This is the case despite the fact that other determinants on MBP (e.g., amino acids 7–16 or 121–140) bind with much higher affinity to the I-A^u MHC molecule. It is likely that those determinants induce tolerance in the complementary T-cell repertoire, but the poorly binding Ac1-9 does not, and because its neighboring, flanking determinants are of higher binding affinity, Ac1-9 is probably unable to compete at all for the I-A^u binding groove (Maverakis et al., 2003; Seamons et al., 2003). This permits the high-affinity clone, 172.10, to evade negative selection and to survive in the periphery to become the dominant clone after the administration of MBP. Accordingly, even though the MHC does play a role in this affair, the high-affinity T cell that evades tolerance is the direct agent that appears to contribute most to the dominance of Ac1-9; in fact, such high-affinity "escapees" are probably very influential in many autoimmune diseases.

In the above example, the strong encephalitogenic type 1 helper T (Th1)-cell response of the 172.10 Ac1-9–specific T-cell clone is likely dictated in part by the cell's high-affinity TCR. The 172.10 receptor is encoded by a Vβ8.2Jβ2.7 genetic recombination resulting in a CDR3 length of nine amino acids (Urban et al., 1988). This receptor has been shown to have a high affinity for the Ac1-9–I-A^u complex and the T cell responds to Ac1-9 in a Th1 fashion (Garcia et al., 2001). Other experimental systems have also investigated the role of the TCR in establishing a Th1 versus Th2 response. Janeway and colleagues (Blander et al., 2000) introduced a single amino acid substitution at a peptide-contacting residue within a TCR. The altered TCR had a 100-fold reduced affinity for the MHC peptide complex and responded in a Th2 fashion to antigenic challenge. Similarly, other investigators have shown that Th1 and Th2 populations can differ with respect to their TCRs (Foucras et al., 2000; Maverakis et al., 2000a). It is noteworthy that the class of T-cell response, Th1 versus Th2, can also be influenced by the antigen–MHC interactions. A very high-affinity ligand leads to a dense array of specific complexes on the APC surface, which favors a Th1 response. Changing a single amino acid on the ligand, to greatly

reduce its MHC-binding affinity, leads to a Th2 response (Kumar et al., 1995). These results are in accord with the idea that dense matrices and broad areas of contact between T cells and APCs strongly influence responsiveness in a Th1 direction.

The B-cell repertoire also can play a somewhat indirect role in the dominance of certain T cells. In this case, the scenario finds a rather predominant member of the B-cell repertoire directed against the self-antigen, which binds to the native antigen at a particular point, which can be called W, in the determinant sequence UVWXY. W, along with its flanking ends, UV and XY, are taken up by the B cell in question. The binding between the antigen and BCR can then lead, for example, to the enhancement of the reactivity of U or V, as well as the lessening of the reactivity of X or Y with the TCR. Such cases have been elegantly described by Watts and Lanzavecchia (1993), as well as in earlier studies (Manca et al., 1985).

STRUCTURAL FEATURES DETERMINING THE IMMUNOGENICITY OF ANTIGENIC DETERMINANTS

In an effort to define the molecular basis of dominance and crypticity of antigenic determinants, we have studied the pattern of immunodominance of determinants within HEL in mice of different MHC haplotypes, as well as in MHC-congenic strains. Results from 19 mouse strains bearing 11 different MHC haplotypes demonstrate that practically every 14–16-mer region within HEL could harbor an immunodominant determinant (Moudgil et al., 1997b). Furthermore, in mice with identical non-MHC genes but different MHC haplotypes, the pattern of dominance was quite different. These results suggest that despite presumably identical antigen-processing machinery, the display of dominant determinants within HEL was primarily determined by the MHC of the host. Thus, there are no inherent structural constraints in display of determinants in any region of HEL. In an earlier study from another laboratory on the relationship between MHC polymorphism and T-cell response to determinants within bacteriophage λ repressor protein, cI, it was observed that cI-reactive hybridomas raised in mice of different MHC haplotypes demonstrated reactivity to determinants spread all over the molecule (Roy et al., 1989). However, in most strains of mice, T-cell responses focused on only a few determinants of cI.

In a similar study, but based on self (mouse)-lysozyme (ML), we have determined the pattern of crypticity of determinants within ML in MHC-congenic mouse strains. Results again indicated that crypticity is MHC-associated and that there are no special structural features that render a determinant cryptic (Moudgil and Sercarz, 1994).

The above results on the association between MHC haplotype and dominance/crypticity of antigenic determinants have important functional significance with regard to induction of autoimmunity. The importance of this association is further underscored by the strong link between a particular MHC allele and the susceptibility to an autoimmune disease (see Chapters 5 and 20).

One of the chief prerequisites for initiation of a T-cell response is the availability and accessibility of the appropriate determinant. Factors that could compromise the immunogenicity of a potential determinant are described below.

Hindering MHC Interaction with Antigen

The presence of hindering residues within a product of processed native antigen was proposed by Brett et al. (1988) in their study on equine myoglobin (Emb). It was suggested that the flanking residues of an Emb determinant hindered its interaction with the Ak molecule but not with the As molecule. Similar observations of either inhibition or stimulation of T-cell reactivity by residue(s) flanking a minimal determinant or even far away from it have also been reported (Shastri et al., 1986; Bhayani et al., 1988; Vacchio et al., 1989; Liu et al., 1991; Kim and Jang, 1992).

In the case of a determinant within region 46–61 of HEL, we have observed that peptide 46–61 (p46–61) is immunogenic in C57BL/6 mice but only when the bulky C-terminal arginine residue (R61) is either deleted or replaced by an alanine residue (Grewal et al., 1995). Thus, p46–61 of HEL is nonimmunogenic in B6 mice, whereas p46–60 and p46–61(R61A) induce a good T-cell response in the same mouse strain. However, T cells primed by p46–60 cannot be recruited by native HEL and vice versa, suggesting that the immunogenic determinant within p46–60 is cryptic in nature. Unlike other cryptic determinants that are usually good immunogens in the peptide form, the cryptic determinant within p46–61 could only be revealed by using p46–60 instead of p46–61. Upon further examination of the mechanism of unresponsiveness of p46–61, we found that p46–61 did not bind at all to the A^b molecule, whereas p46–60 bound quite well. Thus, R61 hindered the binding of p46–61 to A^b. We have termed this determinant a "silent" cryptic determinant because even within the context of the peptide p46–61, it is cryptic in the C57BL/6 mouse.

Interestingly, another H-2^b strain, C3H.SW, could raise a strong proliferative response to p46–61 as well as to p46–60 following immunization with HEL. Thus, in this strain, the determinant within the region 46–61 behaved as a dominant determinant. We further observed that, unlike B6 mice, C3H.SW APCs could "fine-process" p46–61 to remove R61 and thereby display the immunogenic determinant. Thus, the inability of B6 mice to fine-process p46–61, possibly owing to absence of a carboxypeptidase, rendered

this determinant a nonimmunogenic, silent cryptic determinant. Extrapolating this model antigenic determinant to an autoimmune situation, it is conceivable that a defect in the antigen-processing machinery would preempt the induction and/or perpetuation of autoreactivity. On the contrary, if this determinant were involved in immune regulation, the same defect would compromise the host's ability to keep the autoreactive T-cell response under control, and thereby the host could succumb to a chronic, autoimmune disease.

Hindering MHC-Antigen Interaction with Cell Receptor

In another experimental system, we observed that p46–61 of ML, which binds well to the A^k molecule (but not to the E^k molecule), gave a strong proliferative T-cell response in CBA/J and B10.A mice (both A^k, E^k), but was nonimmunogenic in B10.A(4R) mice (A^k, E^o) (Moudgil et al., 1996). There are two arginine residues (R47, R61) in ML p46–61. This peptide could be rendered immunogenic in B10.A(4R) mice either by removal of arginine residues or by their substitution with alanine (or Phe, Leu, Lys, or Asn). Thus, in fact, B10.A(4R) mice have the T-cell repertoire to respond to the core residues between the arginine residues. From these results, we concluded that bulky arginine residues, flanking the core within ML p46–61, hindered the TCR-A^k–p46–61 complex interaction (epitopic hindrance), and thus, rendered this peptide nonimmunogenic in B10.A(4R) mice. Additionally, B10.A and CBA/J mice circumvent this hindrance by recruiting a subset of T cells whose TCRs can accommodate arginine residues within p46–61 bound to the A^k molecule. These T cells were presumably positively selected on peptides from the $E^k/D^d/D^k$ molecules encoded within the MHC E^kD^k region that was deleted in B10.A(4R) mice during the recombination event.

In another study on staphylococcal nuclease (Nase), it was similarly proposed that residues flanking a minimal determinant within Nase might be responsible for interfering with the interaction between the TCR and its complementary MHC–peptide ligand (Liu et al., 1991). The above results demonstrate that a self-/foreign determinant could be rendered nonimmunogenic owing to hindering residues flanking a minimal immunogenic determinant. In the context of autoimmunity, these results predict that susceptibility or resistance to induction of an autoreactive T-cell response could be determined by the availability of appropriate T cells, whose selection might be dependent on a particular MHC haplotype. In this regard, a particular MHC molecule would indirectly behave as a susceptibility allele. Furthermore, in the setting of inflammation, upregulated/altered antigen processing might result in removal of a hindering residue from an autoantigenic determinant, making the host susceptible to autoimmunity.

"Determinant Capture" During the Unfolding of Native Antigen

Another mechanism that could result in a lack of response to a potentially immunogenic determinant is based on preferential, strong binding of a determinant to one of the MHC molecules during unfolding of the native antigen, and concomitant, inadvertent capturing of the neighboring determinant restricted to another MHC molecule ("determinant capture") (Cogswell et al., 1988; Deng et al., 1993). Thus, the neighboring determinant is preempted from inducing a T-cell response. This concept of determinant capture is based on the role of the MHC in guiding processing of determinants within a multideterminant antigen (MHC-guided processing) (Sercarz, 1986; Sercarz et al., 1993; Ojcius et al., 1994a; 1994b; Sercarz and Maverakis, 2003). We gathered evidence for this hypothesis in an experimental system using nonobese diabetic (NOD) mice (I-A^{g7}, E^0) (Deng et al., 1993). We observed that NOD mice immunized with native HEL raised proliferative T-cell responses to determinants having the core sequences 14–20 (dominant determinant) and 95–102 (subdominant determinant), whereas BALB/c mice (A^d, E^d) responded to one immunodominant determinant having the core sequence 108–116, which is E^d restricted. Interestingly, (NOD × BALB/c)F1 mice immunized with HEL raised strong proliferative T-cell responses to determinants 14-20/I-A^{g7} and 108-116/I-A^d, whereas the T-cell response to determinant 95–102/I-A^{g7} was completely lost. We hypothesized that in (NOD × BALB/c)F1 mice, the unfolding HEL molecule bound strongly to the E^d molecule in the region 108–116. Consequently, the neighboring determinant 95–102 was carried along with the determinant 108–116, and thus, was preempted from binding to the A^{g7} molecule, presumably by exopeptidase trimming.

We tested the above hypothesis by using the cyanogen bromide (CNBr)-treated HEL molecule (Deng et al., 1993). CNBr cleaves HEL at positions 12 and 105 and thus, by separating determinants 108–116 and 95–102, should provide increased flexibility and availability for determinants near the cleavage point. Strikingly, we found that F1 mice challenged with CNBr-treated HEL not only responded to determinants 14–20 and 108–116, but also raised a potent response to the determinant 95–102. Thus, cleavage of HEL at position 105 allowed the neighboring determinant, 95–102, to bind freely to the A^{g7} molecule and thereby to induce a T-cell response that was much better in the F1 than that in the NOD mouse itself!

The above results provide a conceptual basis to explain the findings from several studies of protection from autoimmune diabetes in NOD mice by introducing an additional class II MHC molecule (Nishimoto et al., 1987; Slattery et al., 1990), as well as to demonstrate how diabetes-susceptibility/resistance alleles (Nepom, 1990a; 1990b;

1990c) could exert their influence. Presumably, the newly introduced MHC molecule could bind to the diabetogenic autoantigen at a site in close proximity to a major pathogenic determinant. As a consequence, a critical pathogenic determinant would be captured in this process and rendered unable to bind to the A^{g7} molecule for induction of a T-cell response. Projection of the above experimental observations to the heterogeneous human population predicts that one crucial factor in determining susceptibility or resistance to a particular autoimmune disease would be the *combination* of different MHC molecules expressed within the APCs of a given individual. In this context, the mere presence of a "susceptibility" allele might not be a real risk factor. Instead, the presence or absence of a suitable "protective" allele, operating through determinant capture, would determine the final outcome of events.

One mechanism underlying the association between MHC haplotype and particular diseases was recently shown in the case of two HLA-B27 subtypes, only one of which is associated with ankylosing spondylitis. The subtypes differ only at a single amino acid residue, position 116 in the peptide-binding groove. The susceptible one, B*2705, but not the resistant one, B*2709, can bind a disease-associated self-peptide in two distinct conformations, one of which particularly selects a disease-initiating T-cell repertoire. Thus, the differential binding pattern of a dangerous peptide to the two subtypes has a crucial influence on susceptibility to an autoimmune disease (Hulsmeyer et al., 2004).

PUBLIC AND PRIVATE T-CELL REPERTOIRES AND DRIVER CLONES

Public and Private Repertoires

The HEL-specific immune response in the BALB/c mouse is a classical example of T-cell determinant hierarchies and has proved to be an excellent model to characterize the antigen-specific T-cell repertoire. Kourilsky and colleagues (Cibotti et al., 1994) coined the term "public" clonotype to describe an expansion of T cells consisting of a clonotype that can be isolated from all individuals of a particular MHC haplotype after identical antigenic challenge. In contrast to the public response, a "private" repertoire is not found in every animal. For example, adult BALB/c mice immunized with HEL mount a T-cell response utilizing a "public" Vβ8.2Jβ1.5 T-cell clone characterized by a CDR3 length of eight amino acids, GTGNNQAP (Cibotti et al., 1994). [The complementary determining region 3 (CDR3) is a region of the TCR involved in antigen recognition.] The phenomenon of private and public responses has now been observed for several other model antigens and has been shown to exist also in humans and rainbow trout (Saubermann et al., 1999; Boudinot et al., 2001). Owing to the nature of junctional diversity, public responses, although identical at the amino acid level, can differ at the genetic level.

Concept of Driver Clones

The immune response to a particular determinant is usually heterogeneous, with several different clones expanding to a particular antigenic stimulus (Manca et al., 1984). Despite this heterogeneous response, a minority clonotype can play a role as a crucial "driver" in the initiation and propagation of autoimmunity. Our laboratory has been studying driver clones in the MBP B10.PL model of autoimmunity. In this system, experimental autoimmune encephalomyelitis (EAE) can be induced by immunizing B10.PL animals with MBP or its immunodominant determinant Ac1-9. By comparing the peripheral self-directed Ac1-9-specific T cells to those actually infiltrating the spinal cord, it was discovered that some self-directed clones played a more important role in the initiation and propagation of disease than others. For example, some clones that were expanded in response to Ac1-9 never infiltrated the spinal cord or did so only at very late stages of disease. The encephalitogenic potential of the driver response was suggested by: 1) their presence in the spinal cord during the acute phase of disease; 2) their positive reactivity in each animal to the inducing antigen; 3) a proinflammatory cytokine profile; and 4) the appearance of spontaneous disease when their TCR is expressed as a transgene (Menezes, J. et al., personal communication). Following the public "driver" repertoire through the induction and resolution of EAE demonstrates that during the natural process of recovery, the "driver" portion of the self-directed response is selectively purged, resulting in a repertoire shift which leaves behind a set of self-directed, non-central nervous system (CNS)-infiltrating T cells. During recovery, other private myelin-specific T cells remain available for antigen recognition, but they are unable to cause disease. When magnetic beads were used to separate the IFN-γ–secreting cells, the public (Vβ8.2Jβ2.7) response was exclusively found within the IFN-γ–secreting population (Menezes, J. et al., personal communication). Contrariwise, private members could be found secreting either IFN-γ or IL-4. Interestingly, the majority of the IFN-γ-secreting private repertoire never entered the CNS. Thus, not all self-reactive IFN-γ-secreting T cells are pathogenic.

We have identified public autoreactive T cells in other models of autoimmunity as well, including the PLP SJL model of EAE (A. Ametani, unpublished data) and the NOD model of type I diabetes (Quinn et al., personal communication). In addition, in both the SJL and B10.PL mouse models of autoimmunity, we have found self-directed public responses that do not play key roles in the disease process: self-reactive public T-cell clones are not always pathogenic.

Likewise, we have not excluded the possibility that a private response might play an accessory role in the induction and propagation of autoimmunity, although to date we have not found such an example.

In summary, of the vast number of self-reactive T cells, only a small fraction comprise the pathogenic population. In addition, recovery from an autoimmune episode correlates with a repertoire shift in which the pathogenic members of the self-reactive response are purged while a more benign set of T cells remains. It is worth noting that those dominant determinants, T cells specific for which have evaded negative selection, are often the inducers of driver clones.

MOLECULAR MIMICRY AND DEGENERACY

As pointed out earlier, there is a large repertoire in the normal animal specific for self-determinants, which fortunately is rarely successfully engaged for a pathogenic purpose. This repertoire can be activated following exposure to antigens of a pathogen, provided the foreign determinant mimics a self-determinant (molecular mimicry). This margin of safety which reduces the danger of unexpected mimicry might have been predicted because of the necessity for any cross-reactive determinant on a viral or microbial entity to be dominant so as to elicit a response in the first place, and concomitantly, for the self-determinant to be cryptic, so that a repertoire might still exist against it, and not have been negatively selected. But in addition to these ground rules, there is another overriding feature of immune recognition that must be understood with respect to mimicry, and that is degeneracy.

Within the past decade, it has become clear that T lymphocyte recognition is degenerate. Degeneracy, in contemporary immunologic usage, describes the ability of a single receptor to recognize and react to a heterogeneous assortment of ligands, with each lymphocyte clone having its own pattern of degeneracy (i.e., its own set of stimulatory ligands) (Bankovich et al., 2004; Christen and von Herrath, 2004; Cohen et al., 2004; Cunningham, 2004; Fae et al., 2004; Ford and Evavold, 2004; Fourneau et al., 2004; Holler and Kranz, 2004; Im et al., 2004; Judkowski et al., 2004; Nicholson and Wraith, 2004; Nino-Vasquez et al., 2004; Nishimura et al., 2004; Olson et al., 2004; Parnes, 2004; Sercarz and Maverakis, 2004; Shih and Allen, 2004; Shukaliak Quandt et al., 2004; Singh, 2004; Wilson et al., 2004; Wucherpfennig, 2004; Zhou and Hemmer, 2004). A reasonable estimate is that there may be 10^{18} such epitopes that need to be distinguished by the immune system. In a human, there is a finite number of lymphocytes, about 10^{12}, at any one time, so that each lymphocyte would have to recognize 10^6 epitopes in order to cover the universe of pathogenic epitopes (Mason, 2001). Current estimates, especially from combinatorial peptide libraries, suggest that each T cell can probably recognize from 10^6 to 10^8 ligands (Wilson et al., 1999). In the face of such a broad capacity for recognition, why is there not much more molecular mimicry and autoimmune disease?

Two requirements additional to those mentioned above can be suggested. First, it is far more difficult to activate a naïve cell than a primed one, and this feature alone would reduce the frequency of mimicry interactions. However, previous primings by other nonpathogenic cross-reactive stimuli could increase the frequency (Christen et al., 2004). Second, competition between the large number of T cells uniquely specific for the mimicking ligand, and T cells specific for the self-ligand, which are cross-reactive with the mimicking ligand (Maverakis personal communication), will protect the organism in large measure.

Historically, considerations of the relatedness of the mimicking determinant and the self-determinant have passed through three stages. In the first, it appeared that mimicry could occur if stretches of identical amino acids from the determinant on the mimic could be found on the self-determinant (Fujinami and Oldstone, 1985). Later, during the second stage, some studies showed that the determinants need not be identical, but only similar or homologous in several residues. Using a panel of 129 peptides derived from viral and bacterial antigens that matched a molecular mimicry motif, Wucherpfennig and Strominger (1995) showed cross-reactive recognition by MBP-specific T-cell clones from multiple sclerosis patients. Overlapping with this period, other experiments showed that there was no need for any identity between the two determinants, and emphasized the degeneracy of T-cell recognition (Bhardwaj et al., 1993). Several interesting examples of mimicry should be mentioned. Zhao et al. (1998) showed that the inducing mimic actually was processed and a mutational change in the mimic at the appropriate point eliminated the mimicry. In other work, it was shown that two entirely different regions within the same protein, human α-1-antitrypsin, could stimulate the same antigen-reactive T-cell lines and clones (Hagerty and Allen, 1995). Finally, we observed that a T-cell hybridoma directed against HEL could also recognize a determinant on the unrelated antigen, mouse albumin, as well as a third, but undefined, determinant: in this case, three different MHC class II molecules were involved, one for each antigen (H. Deng, unpublished results).

"DETERMINANT SPREADING" DURING THE COURSE OF AUTOIMMUNITY

A T-cell response to the dominant determinant, Ac1-9/Ac1-11, following immunization with whole MBP (Zamvil et al., 1986; Urban et al., 1988; Acha-Orbea et al.,

1988; Lehmann et al., 1992) is crucial for initiation of autoreactivity leading to induction of EAE in certain susceptible mouse strains like B10.PL, PL/J, and (B10.PL × SJL)F1 mice. We observed that when (B10.PL × SJL)F1 mice (Lehmann et al., 1992) were injected with this single dominant peptide of a pathogenic autoimmunogen, spreading of the T-cell response to previously cryptic determinants of MBP occurred, to 35–47, 81–100, and 121–140 ("determinant spreading"). This spreading was attributed to upregulated processing and presentation of MBP within the local inflammatory milieu, leading to efficient display of previously cryptic determinants (Lehmann et al., 1992; 1993). The above results are suggestive of a physiologic role of cryptic self-determinants in the propagation of autoimmunity induced by the dominant determinant of the same autoantigen.

Studies by the Miller laboratory (Weiner et al., 1994; McRae et al., 1995) have shown that both inter- and intramolecular determinant spreading occurred during the course of relapsing EAE. The phenomenon of recruitment of additional T-cell specificities during the course of chronic, relapsing EAE has also been reported by other investigators (Cross et al., 1993; Perry and Barzaga, 1987; Perry et al., 1991), although they initiated response with the native protein, so that intermolecular spreading may have been due to protein contamination. In another study by Tuohy and colleagues (Yu et al., 1996), a predictable, orderly determinant spreading involving determinants within PLP, MBP, and myelin oligodendrocyte glycoprotein (MOG) was observed during the course of EAE in (SWR × SJL)F1 mice. These studies provided functional evidence for the role of determinant spreading in the relapsing pathology of EAE.

The above results on determinant spreading have important implications for immunotherapeutic strategies in the treatment of EAE. In the face of determinant spreading, therapeutic approaches based solely on controlling T-cell responses to the initial disease-inducing determinant may not succeed once spreading has occurred. The timing of tolerization of the initial disease-inducing T cells is critical (McRae et al., 1995; Yu et al., 1996). Furthermore, strategies aimed at blocking the interaction between CD80 and CD28 molecules have also been found to be successful in blocking determinant spreading and thereby preventing relapses during the course of EAE (Miller et al., 1995).

From the viewpoint of initiation of autoimmunity, immunogenic, dominant, and subdominant determinants within an antigen (e.g., Ac 1-9 determinant of MBP) (Zamvil et al., 1986), which are responsible for the induction of autoimmunity, are of utmost importance. Nevertheless, latent cryptic determinants might play a critical role in the broadening of the autoreactive T-cell response and thereby, perpetuation of autoimmunity, e.g., in EAE (Lehmann et al., 1992; 1993; Watts and Lanzavecchia, 1993; Moudgil and Sercarz, 1994).

Diabetes and Arthritis

In another T-cell-mediated autoimmune disease, type 1 diabetes in NOD mice, the disease is characterized by early, spontaneous T-cell responsiveness to glutamic acid decarboxylase (GAD) (Kaufman et al., 1993; Tisch et al., 1993), with the initial response directed against C-terminal determinants. However, during the course of disease, there was spreading of the T-cell response not only to other determinants within GAD (intramolecular spreading), but also to other antigens, namely a mycobacterial hsp65 peptide, carboxypeptidase H, insulin, and others (intermolecular spreading). Interestingly, tolerization of GAD-reactive T cells in NOD mice successfully blocked the development of autoimmunity to the above-mentioned antigens as well as insulitis and clinical diabetes. This was not the case after treatment with the hsp65 peptide. Thus, in this case, understanding of the temporal pattern of appearance of the T-cell response to GAD and other β-cell antigens allowed development of an appropriate therapeutic regimen for prevention and treatment of diabetes.

Using the rat adjuvant-induced arthritis (AA) model of human rheumatoid arthritis (RA), we observed that arthritic Lewis rats in the late phase of AA raised T-cell responses to certain new determinants located at the C-terminus of Bhsp65 (Moudgil et al., 1997a). Furthermore, pretreatment of naïve Lewis rats with peptides comprising these C-terminal epitopes afforded significant protection from subsequently induced AA. These results suggest that diversification and intramolecular spreading of the T-cell response is involved in inducing natural remission or protection from AA ("regulatory diversification") (Moudgil et al., 1997a). Recently, we have shown that the C-terminal epitopes of Bhsp65 involved in this diversification of response are originally cryptic and cross-reactive with the corresponding self-epitopes within rat hsp65 (Rhsp65) (Durai et al., 2004a; 2004b); later, these regulatory determinants become available owing to enhanced processing. Interestingly, the self-homologs of the regulatory cryptic Bhsp65 epitopes are dominant in nature (Durai et al., 2004b). Our results suggest a model for epitope spreading in AA involving the upregulation of the cryptic C-terminal epitopes of Bhsp65, as well as enhanced cellular expression of self-hsp65, leading to display of its dominant C-terminal determinants during the acute phase of AA (Durai et al., 2004a).

CONCLUSION

In summary, there is no common structural characteristic describing antigenic determinants involved in autoimmunity. T lymphocytes directed against such determinants have managed to escape negative selection, and their emergence

into pathogenicity is contextual. The "dominant self" that *does* induce negative selection is created by the ordeal of antigen-processing events. The residual self-reactive potential, inherent in the "cryptic self," may erupt into autoimmune attack when fortuitously cross-reactive, dominant determinants are encountered, or upon interaction with environmental agents that excite Toll-like receptors, or provide unusual cell-activation stimuli (e.g., ultraviolet light). Deficiencies in regulation at a variety of points can alternatively unleash the available repertoire, largely directed against the cryptic self.

As a final word, autoimmune disease is an outcome dependent on a string of chance events, some genetically underwritten and others related to a series of immunologic events in the lifetime of the individual that prey upon the residual self-reactive repertoire. How to efficiently regulate these events is the goal of many current studies.

References

Abromson-Leeman, S., Hayashi, M., Martin, C., Sobel, R., al-Sabbagh, A., Weiner, H., and Dorf, M.E. (1993). T cell responses to myelin basic protein in experimental autoimmune encephalomyelitis-resistant BALB/c mice. *J. Neuroimmunol. 45*, 89–101.

Abromson-Leeman, S., Alexander, J., Bronson, R., Carroll, J., Southwood, S., and Dorf, M. (1995). Experimental autoimmune encephalomyelitis-resistant mice have highly encephalitogenic myelin basic protein (MBP)-specific T cell clones that recognize a MBP peptide with high affinity for MHC class II. *J. Immunol. 154*, 388–398.

Acha-Orbea, H., Mitchell, D.J., Timmermann, L., Wraith, D.C., Tausch, G.S., Waldor, M.K., Zamvil, S.S., McDevitt, H.O., and Steinman, L. (1988). Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell 54*, 263–273.

Anderton, S.M., Viner, N.J., Matharu, P., Lowrey, P.A., and Wraith, D.C. (2002). Influence of a dominant cryptic epitope on autoimmune T cell tolerance. *Nat. Immunol. 3*, 175–181.

Avichezer, D., Grajewski, RS., Chan, C .C., Mattapallil, M.J., Silver, P.B., Raber, J.A., Liou, G.I., Wiggert, B., Lewis, G.M., Donoso, L.A., and Caspi, R.R. (2003). An immunologically privileged retinal antigen elicits tolerance: major role for central selection mechanisms. *J. Exp. Med. 198*, 1665–1676.

Bankovich, A.J., Girvin, A.T., Moesta, A.K., and Garcia, K.C. (2004). Peptide register shifting within the MHC groove: theory becomes reality. *Mol. Immunol. 40*, 1033–1039.

Beers, C., Burich, A., Kleijmeer, M.J., Griffith, J.M., Wong, P., and Rudensky, A.Y. (2005). Cathepsin S controls MHC class II-mediated antigen presentation by epithelial cells *in vivo*. *J. Immunol. 174*, 1205–1212.

Bhardwaj, V., Kumar, V., Geysen, H.M., and Sercarz, E.E. (1993). Degenerate recognition of a dissimilar antigenic peptide by myelin basic protein-reactive T cells. Implications for thymic education and autoimmunity. *J. Immunol. 151*, 5000–5010.

Bhayani, H., Carbone, F.R., and Paterson, Y. (1988). The activation of pigeon cytochrome c-specific T cell hybridomas by antigenic peptides is influenced by non-native sequences at the amino terminus of the determinant. *J. Immunol. 141*, 377–382.

Blander, J.M., Sant'Angelo, D.B., Bottomly, K., and Janeway, C.A., Jr. (2000). Alteration at a single amino acid residue in the T cell receptor alpha chain complementarity determining region 2 changes the differentiation of naive CD4 T cells in response to antigen from T helper cell type 1 (Th1) to Th2. *J. Exp. Med. 191*, 2065–2074.

Boudinot, P., Boubekeur, S., and Benmansour, A. (2001). Rhabdovirus infection induces public and private T cell responses in teleost fish. *J. Immunol. 167*, 6202–6209.

Brett, S.J., Cease, K.B., and Bezofsky, 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.

Castellino, F., Zappacosta, F., Coligan, J.E., and Germain, R.N. (1998). Large protein fragments as substrates for endocytic antigen capture by MHC class II molecules. *J. Immunol. 161*, 4048–4057.

Christen, U. and von Herrath, M.G. (2004). Induction, acceleration or prevention of autoimmunity by molecular mimicry. *Mol. Immunol. 40*, 1113–1120.

Christen, U., Edelmann, K.H., McGavern, D.B., Wolfe, T., Coon, B., Teague, M.K., Miller, S.D., Oldstone, M.B., and von Herrath, M.G. (2004). A viral epitope that mimics a self antigen can accelerate but not initiate autoimmune diabetes. *J. Clin. Invest. 114*, 1290–1298.

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.

Cibotti, R., Cabaniols, J.P., Pannetier, C., Delarbre, C., Vergnon, I., Kanellopoulos, J.M., and Kourilsky, P. (1994). Public and private V beta T cell receptor repertoires against hen egg white lysozyme (HEL) in nontransgenic versus HEL transgenic mice. *J. Exp. Med. 180*, 861–872.

Cogswell, J.P., Gammon, G., Wilbur, S., Shastri, N., Miller, A., and Sercarz, E. (1988). Ia dependent selection of agretopes on the partially unfolded chicken lysozyme molecule affects subsequent processing pathways and the dominance of T cell determinants. In L.B. Sherlock (ed.), *Antigen Presenting Cells* (pp. 115–126). New York: Alan R. Liss.

Cohen, I.R., Hershberg, U., and Solomon, S. (2004). Antigen-receptor degeneracy and immunological paradigms. *Mol. Immunol. 40*, 993–996.

Cross, A.H., Tuohy, V.K., and Raine, C.S. (1993). Development of reactivity to new myelin antigens during chronic relapsing autoimmune demyelination. *Cell Immunol. 146*, 261–269.

Cunningham, M.W. (2004). T cell mimicry in inflammatory heart disease. *Mol. Immunol. 40*, 1121–1127.

Deng, H., Apple, R., Clare-Salzler, M., Trembleau, S., Mathis, D., Adorini, L., and Sercarz, E. (1993). Determinant capture as a possible mechanism of protection afforded by major histocompatibility complex class II molecules in autoimmune disease. *J. Exp. Med. 178*, 1675–1680.

Durai, M., Gupta, R.S., and Moudgil, K.D. (2004a). The T cells specific for the carboxyl-terminal determinants of self (rat) heat-shock protein 65 escape tolerance induction and are involved in regulation of autoimmune arthritis. *J. Immunol. 172*, 2795–2802.

Durai, M., Kim, H.R., and Moudgil, K.D. (2004b). The regulatory C-terminal determinants within mycobacterial heat shock protein 65 are cryptic and cross-reactive with the dominant self homologs: implications for the pathogenesis of autoimmune arthritis. *J. Immunol. 173*, 181–188.

Fae, K., Kalil, J., Toubert, A., and Guilherme, L. (2004). Heart infiltrating T cell clones from a rheumatic heart disease patient display a common TCR usage and a degenerate antigen recognition pattern. *Mol. Immunol. 40*, 1129–1135.

Feng, J.M., Givogri, I.M., Bongarzone, E.R., Campagnoni, C., Jacobs, E., Handley, V.W., Schonmann, V., and Campagnoni, A.T. (2000). Thymocytes express the golli products of the myelin basic protein gene and levels of expression are stage dependent. *J. Immunol. 165*, 5443–5450.

Ford, M.L. and Evavold, B.D. (2004). Degenerate recognition of T cell epitopes: impact of T cell receptor reserve and stability of peptide:MHC complexes. *Mol. Immunol. 40*, 1019–1025.

Foucras, G., Gapin, L., Coureau, C., Kanellopoulos, J.M., and Guery, J.C. (2000). Interleukin 4-producing CD4 T cells arise from different precursors depending on the conditions of antigen exposure *in vivo*. *J. Exp. Med. 191*, 683–694.

Fourneau, J.M., Bach, J.M., van Endert, P.M., and Bach, J.F. (2004). The elusive case for a role of mimicry in autoimmune diseases. *Mol. Immunol. 40*, 1095–1102.

Fujinami, R.S. and Oldstone, M.B. (1985). Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science 230*, 1043–1045.

Gammon, G. and Sercarz, E. (1989). How some T cells escape tolerance induction. *Nature 342*, 183–185.

Garcia, K.C., Radu, C.G., Ho, J., Ober, R.J., and Ward, E.S. (2001). Kinetics and thermodynamics of T cell receptor-autoantigen interactions in murine experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA 98*, 6818–6823.

Grewal, I.S., Moudgil, K.D., and Sercarz, E.E. (1995). Hindrance of binding to class II major histocompatibility complex molecules by a single amino acid residue contiguous to a determinant leads to cryptic-ity of the determinant as well as lack of response to the protein antigen. *Proc. Natl. Acad. Sci. USA 92*, 1779–1783.

Griffith, 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*, 1189–1192.

Hagerty, D.T. and Allen, P.M. (1995). Intramolecular mimicry. Identification and analysis of two cross-reactive T cell epitopes within a single protein. *J. Immunol. 155*, 2993–3001.

Harrington, C.J., Paez, A., Hunkapiller, T., Mannikko, V., Brabb, T., Ahearn, M., Beeson, C., and Goverman, J. (1998). Differential tolerance is induced in T cells recognizing distinct epitopes of myelin basic protein. *Immunity 8*, 571–580.

Holler, P.D. and Kranz, D.M. (2004). T cell receptors: affinities, cross-reactivities, and a conformer model. *Mol. Immunol. 40*, 1027–1031.

Hulsmeyer, M., Fiorillo, M.T., Bettosini, F., Sorrentino, R., Saenger, W., Ziegler, A., and Uchanska-Ziegler, B. (2004). Dual, HLA-B27 subtype-dependent conformation of a self-peptide. *J. Exp. Med. 199*, 271–281.

Im, J.S., Quinn, A., Sercarz, E.E., and Lake, D.F. (2004). Molecular profile of the T cell receptors of regulatory and effector $CD4^+$ T cells recognizing overlapping determinants on glutamic acid decarboxylase (524–543). *Mol. Immunol. 40*, 971–980.

Judkowski, V., Pinilla, C., Alicotti, G., Flodstrom, M., Sanna, P., and Sarvetnick, N. (2004). Strong attraction may be dangerous: parameters for the development of long term commitment. *Mol. Immunol. 40*, 1109–1112.

Katz, M.E., Maizels, R.M., Wicker, L., Miller, A., and Sercarz, E.E. (1982). Immunological focusing by the mouse major histocompatibility complex: mouse strains confronted with distantly related lysozymes confine their attention to very few epitopes. *Eur. J. Immunol. 12*, 535–540.

Kaufman, D.L., Clare-Salzler, M., Tian, J., Forsthuber, T., Ting, G.S., Robinson, P., Atkinson, M.A., Sercarz, E.E., Tobin, A.J., and Lehmann, P.V. (1993). Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature 366*, 69–72.

Kim, B.S. and Jang, Y.S. (1992). Constraints in antigen processing result in unresponsiveness to a T cell epitope of hen egg lysozyme in C57BL/6 mice. *Eur. J. Immunol. 22*, 775–782.

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. *Nat. Med. 6*, 56–61.

Krogsgaard, M. and Davis, M.M. (2005). How T cells 'see' antigen. *Nat. Immunol. 6*, 239–245.

Kumar, V., Bhardwaj, V., Soares, L., Alexander, J., Sette, A., and Sercarz, E. (1995). Major histocompatibility complex binding affinity of an antigenic determinant is crucial for the differential secretion of interleukin 4/5 or interferon gamma by T cells. *Proc. Natl. Acad. Sci. USA 92*, 9510–9514.

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*, 155–157.

Lehmann, P.V., Sercarz, E.E., Forsthuber, T., Dayan, C.M., and Gammon, G. (1993). Determinant spreading and the dynamics of the autoimmune T-cell repertoire. *Immunol. Today 14*, 203–208.

Liu, G.Y., Fairchild, P.J., Smith, R.M., Prowle, J.R., Kioussis, D., and Wraith, D.C. (1995). Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity 3*, 407–415.

Liu, Z.R., Williams, K.P., Chang, Y.H., and Smith, J.A. (1991). Single amino acid substitution alters T cell determinant selection during antigen processing of *Staphylococcus aureus* nuclease. *J. Immunol. 146*, 438–443.

Manca, F., Clarke, J.A., Miller, A., Sercarz, E.E., and Shastri, N. (1984). A limited region within hen egg-white lysozyme serves as the focus for a diversity of T cell clones. *J. Immunol. 133*, 2075–2078.

Manca, F., Kunkl, A., Fenoglio, D., Fowler, A., Sercarz, E., and Celada, F. (1985). Constraints in T–B cooperation related to epitope topology on *E. coli* beta-galactosidase. I. The fine specificity of T cells dictates the fine specificity of antibodies directed to conformation-dependent determinants. *Eur. J. Immunol. 15*, 345–350.

Manoury, B., Hewitt, E.W., Morrice, N., Dando, P.M., Barrett, A.J., and Watts, C. (1998). An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. *Nature 396*, 695–699.

Manoury, B., Mazzeo, D., Fugger, L., Viner, N., Ponsford, M., Streeter, H., Mazza, G., Wraith, D.C., and Watts, C. (2002). Destructive processing by asparagine endopeptidase limits presentation of a dominant T cell epitope in MBP. *Nat. Immunol. 3*, 169–174.

Maric, M., Arunachalam, B., Phan, U.T., Dong, C., Garrett, W.S., Cannon, K.S., Alfonso, C., Karlsson, L., Flavell, R.A., and Cresswell, P. (2001). Defective antigen processing in GILT-free mice. *Science 294*, 1361–1365.

Mason, D. (2001). Some quantitative aspects of T-cell repertoire selection: the requirement for regulatory T cells. *Immunol. Rev. 182*, 80–88.

Maverakis, E., Beech, J.T., Wilson, S.S., Quinn, A., Pedersen, B., and Sercarz, E.E. (2000a). T cell receptor complementarity determining region 3 length analysis reveals the absence of a characteristic public T cell repertoire in neonatal tolerance. The response in the "tolerant" mouse within the residual repertoire is quantitatively similar but qualitatively different. *J. Exp. Med. 191*, 695–702.

Maverakis, E., Mendoza, R., Southwood, S., Raja-Gabaglia, C., Abromson-Leeman, S., Campagnoni, A.T., Sette, A., and Sercarz, E.E. (2000b). Immunogenicity of self antigens is unrelated to MHC-binding affinity: T-cell determinant structure of golli-MBP in the BALB/c mouse. *J. Autoimmun. 15*, 315–322.

Maverakis, E., Beech, J., Stevens, D.B., Ametani, A., Brossay, L., van den Elzen, P., Mendoza, R., Thai, Q., Macias, L.H., and Ethell, D. (2003). Autoreactive T cells can be protected from tolerance induction through competition by flanking determinants for access to class II MHC. *Proc. Natl. Acad. Sci. USA 100*, 5342–5347.

Maverakis E et al. (personal communication)

McRae, B.L., Vanderlugt, C.L., Dal Canto, M.C., and Miller, S.D. (1995). Functional evidence for epitope spreading in the relapsing pathology of experimental autoimmune encephalomyelitis. *J. Exp. Med. 182*, 75–85.

Menezes J. et al. (personal communication)

Miller, S.D., McRae, B.L., Vanderlugt, C.L., Nikcevich, K.M., Pope, J.G., Pope, L., and Karpus, W.J. (1995). Evolution of the T-cell repertoire during the course of experimental immune-mediated demyelinating diseases. *Immunol. Rev. 144*, 225–244.

Moudgil, K.D. and Sercarz, E.E. (1994). The T cell repertoire against cryptic self determinants and its involvement in autoimmunity and cancer. *Clin. Immunol. Immunopathol. 73*, 283–289.

Moudgil, K.D., Grewal, I.S., Jensen, P.E., and Sercarz, E.E. (1996). Unresponsiveness to a self-peptide of mouse lysozyme owing to hindrance of T cell receptor-major histocompatibility complex/peptide interaction caused by flanking epitopic residues. *J. Exp. Med. 183*, 535–546.

Moudgil, K.D., Chang, T.T., Eradat, H., Chen, A.M., Gupta, R.S., Brahn, E., and Sercarz, E.E. (1997a). Diversification of T cell responses to carboxy-terminal determinants within the 65-kD heat-shock protein is involved in regulation of autoimmune arthritis. *J. Exp. Med. 185*, 1307–1316.

Moudgil, K.D., Sekiguchi, D., Kim, S.Y., and Sercarz, E.E. (1997b). Immunodominance is independent of structural constraints: each region within hen eggwhite lysozyme is potentially available upon processing of native antigen. *J. Immunol. 159*, 2574–2579.

Moudgil, K.D., Sercarz, E.E., and Grewal, I.S. (1998). Modulation of the immunogenicity of antigenic determinants by their flanking residues. *Immunol. Today 19*, 217–220.

Nanda, N.K. and Bikoff, E.K. (1995). DM peptide-editing leads to immunodominance in CD4 T cell responses *in vivo*. *J. Immunol. 175*, 1673–1680.

Nanda, N.K. and Sant, A.J. (2000). DM determines the cryptic and immunodominant fate of T cell epitopes. *J. Exp. Med. 192*, 781–788.

Nanda, N.K., Apple, R., and Sercarz, E. (1991). Limitations in plasticity of the T-cell receptor repertoire. *Proc. Natl. Acad. Sci. USA 88*, 9503–9507.

Nepom, G.T. (1990a). HLA and type I diabetes. *Immunol. Today 11*, 314–315.

Nepom, G.T. (1990b). Molecular biology of immune diseases and the immune response. *Immunol. Today 11*, 150–151.

Nepom, G.T. (1990c). Mutagenesis and expression of putative class II susceptibility genes: a "reverse immunogenetics" approach to analysis of HLA and disease. *Autoimmunity 7*, 189–199.

Nicholson, L.B., and Wraith, D.C. (2004). T-cell receptor degeneracy: the dog that did not bark. Adaptation of the self-reactive T-cell response to limit autoimmune disease. *Mol. Immunol. 40*, 997–1002.

Nino-Vasquez, J.J., Allicotti, G., Borras, E., Wilson, D.B., Valmori, D., Simon, R., Martin, R., and Pinilla, C. (2004). A powerful combination: the use of positional scanning libraries and biometrical analysis to identify cross-reactive T cell epitopes. *Mol. Immunol. 40*, 1063–1074.

Nishimoto, H., Kikutani, H., Yamamura, K., and Kishimoto, T. (1987). Prevention of autoimmune insulitis by expression of I-E molecules in NOD mice. *Nature 328*, 432–434.

Nishimura, Y., Chen, Y.Z., Uemura, Y., Tanaka, Y., Tsukamoto, H., Kanai, T., Yokomizo, H., Yun, C., Matsuoka, T., Irie, A., and Matsushita, S. (2004). Degenerate recognition and response of human $CD4^+$ Th cell clones: implications for basic and applied immunology. *Mol. Immunol. 40*, 1089–1094.

Ojcius, D.M., Gapin, L., Kanellopoulos, J.M., and Kourilsky, P. (1994a). Is antigen processing guided by major histocompatibility complex molecules? *FASEB J 8*, 974–978.

Ojcius, D.M., Langlade-Demoyen, P., Gachelin, G., and Kourilsky, P. (1994b). Role for MHC class I molecules in selecting and protecting high affinity peptides in the presence of proteases. *J. Immunol. 152*, 2798–2810.

Olson, J.K., Ludovic Croxford, J., and Miller, S.D. (2004). Innate and adaptive immune requirements for induction of autoimmune demyelinating disease by molecular mimicry. *Mol. Immunol. 40*, 1103–1108.

Opdenakker, G., and Van Damme, J. (1994). Cytokine-regulated proteases in autoimmune diseases. *Immunol. Today 15*, 103–107.

Parnes, O. (2004). From interception to incorporation: degeneracy and promiscuous recognition as precursors of a paradigm shift in immunology. *Mol. Immunol. 40*, 985–991.

Perry, L.L. and Barzaga, M.E. (1987). Kinetics and specificity of T and B cell responses in relapsing experimental allergic encephalomyelitis. *J. Immunol. 138*, 1434–1441.

Perry, L.L., Barzaga-Gilbert, E., and Trotter, J.L. (1991). T cell sensitization to proteolipid protein in myelin basic protein-induced relapsing experimental allergic encephalomyelitis. *J. Neuroimmunol. 33*, 7–15.

Pribyl, T.M., Campagnoni, C.W., Kampf, K., Kashima, T., Handley, V.W., McMahon, J., and Campagnoni, A.T. (1993). The human myelin basic protein gene is included within a 179-kilobase transcription unit: expression in the immune and central nervous systems. *Proc. Natl. Acad. Sci. USA 90*, 10695–10699.

Quinn et al. (personal communication)

Roy, S., Scherer, M.T., Briner, T.J., Smith, J.A., and Gefter, M.L. (1989). Murine MHC polymorphism and T cell specificities. *Science 244*, 572–575.

Saubermann, L.J., Probert, C.S., Christ, A.D., Chott, A., Turner, J.R., Stevens, A.C., Balk, S.P., and Blumberg, R.S. (1999). Evidence of T cell receptor beta-chain patterns in inflammatory and noninflammatory bowel disease states. *Am. J. Physiol. 276*, G613–621.

Schneider, S.C., Ohmen, J., Fosdick, L., Gladstone, B., Guo, J., Ametani, A., Sercarz, E.E., and Deng, H. (2000). Cutting edge: introduction of an endopeptidase cleavage motif into a determinant flanking region of hen egg lysozyme results in enhanced T cell determinant display. *J. Immunol. 165*, 20–23.

Seamons, A., Sutton, J., Bai, D., Baird, E., Bonn, N., Kafsack, B.F., Shabanowitz, J., Hunt, D.F., Beeson, C., and Goverman, J. (2003). Competition between two MHC binding registers in a single peptide processed from myelin basic protein influences tolerance and susceptibility to autoimmunity. *J. Exp. Med. 197*, 1391–1397.

Seidah, N.G. and Chretien, M. (1997). Eukaryotic protein processing: endoproteolysis of precursor proteins. *Curr. Opin. Biotechnol. 8*, 602–607.

Sercarz, E.E. (1986). Hierarchies of epitope preference and the problem of Ir gene control. *Concepts Immunopathol. 3*, 61–73.

Sercarz, E.E. and Maverakis, E. (2003). Mhc-guided processing: binding of large antigen fragments. *Nat. Rev. Immunol. 3*, 621–629.

Sercarz, E.E. and Maverakis, E. (2004). Recognition and function in a degenerate immune system. *Mol. Immunol. 40*, 1003–1008.

Sercarz, E.E., Lehmann, P.V., Ametani, A., Benichou, G., Miller, A., and Moudgil, K. (1993). Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol. 11*, 729–766.

Shastri, N., Gammon, G., Horvath, S., Miller, A., and Sercarz, E.E. (1986). The choice between two distinct T cell determinants within a 23-amino acid region of lysozyme depends on their structural context. *J. Immunol. 137*, 911–915.

Shaw, M.K., Kim, C., Ho, K.L., Lisak, R.P., and Tse, H.Y. (1992). A combination of adoptive transfer and antigenic challenge induces consistent murine experimental autoimmune encephalomyelitis in C57BL/6 mice and other reputed resistant strains. *J. Neuroimmunol. 39*, 139–149.

Shih, F.F. and Allen, P. (2004). T cells are not as degenerate as you think, once you get to know them. *Mol. Immunol. 40*, 1041–1046.

Shimonkevitz, R., Colon, S., Kappler, J.W., Marrack, P., and Grey, H.M. (1984). Antigen recognition by H-2-restricted T cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen. *J. Immunol. 133*, 2067–2074.

Shukaliak Quandt, J., Borras, E., Prat, E., Gelderblom, H., Houghten, R.A., Kashani, A., Pinilla, C., Stuerzebecher, C.S., and Martin, R. (2004). Peptidic complex mixtures as therapeutic agents in CNS autoimmunity. *Mol. Immunol. 40*, 1075–1087.

Singh, R.R. (2004). Prevention and control of reciprocal T-B cell diversification: implications for lupus-like autoimmunity. *Mol. Immunol. 40*, 1137–1145.

Sinha, P., Chi, H.H., Kim, H.R., Clausen, B.E., Pederson, B., Sercare E.E., Forster, I., and Moudiik, D. (2004). Mouse lysozyme-M knockout mice reveal how the self-determinant hierarchy shapes the T cell repertoire

against this circulating self-antigen in the wild-type mice. *J. Immunol. 173*, 1763–1771.

Slattery, R.M., Kjer-Nielsen, L., Allison, J., Charlton, B., Mandel, T.E., and Miller, J.F. (1990). Prevention of diabetes in non-obese diabetic I-Ak transgenic mice. *Nature 345*, 724–726.

Targoni, O.S. and Lehmann, P.V. (1998). Endogenous myelin basic protein inactivates the high avidity T cell repertoire. *J. Exp. Med. 187*, 2055–2063.

Tewari, M.K., Sinnathamby, G., Rajagopal, D., and Eisenlohr, L.C. (2005). A cytosolic pathway for MHC class II-restricted antigen processing that is proteasome and TAP dependent. *Nat. Immunol. 6*, 287–294.

Tisch, R., Yang, X.D., Singer, S.M., Liblau, R.S., Fugger, L., and McDevitt, H.O. (1993). Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature 366*, 72–75.

Urban, J.L., Kumar, V., Kono, D.H., Gomez, C., Horvath, S.J., Clayton, J., Ando, D.G., Sercarz, E.E., and Hood, L. (1988). Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell 54*, 577–592.

Vacchio, M.S., Berzofsky, J.A., Krzych, U., Smith, J.A., Hodes, R.J., and Finnegan, A. (1989). Sequences outside a minimal immunodominant site exert negative effects on recognition by staphylococcal nuclease-specific T cell clones. *J. Immunol. 143*, 2814–2819.

Viner, N.J., Nelson, C.A., and Unanue, E.R. (1995). Identification of a major I-Ek-restricted determinant of hen egg lysozyme: limitations of lymph node proliferation studies in defining immunodominance and crypticity. *Proc. Natl. Acad. Sci. USA 92*, 2214–2218.

Watts, C. and Lanzavecchia, A. (1993). Suppressive effect of antibody on processing of T cell epitopes. *J. Exp. Med. 178*, 1459–1463.

Weiner, H.L., Friedman, A., Miller, A., Khoury, S.J., al-Sabbagh, A., Santos, L., Sayegh, M., Nussenblatt, R.B., Trentham, D.E., and Hafler, D.A. (1994). Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu. Rev. Immunol. 12*, 809–837.

Wilson, D.B., Pinilla, C., Wilson, D.H., Schroder, K., Boggiano, C., Judkowski, V., Kaye, J., Hemmer, B., Martin, R., and Houghten, R.A. (1999). Immunogenicity. I. Use of peptide libraries to identify epitopes that activate clonotypic CD4+ T cells and induce T cell responses to native peptide ligands. *J. Immunol. 163*, 6424–6434.

Wilson, D.B., Wilson, D.H., Schroder, K., Pinilla, C., Blondelle, S., Houghten, R.A., and Garcia, K.C. (2004). Specificity and degeneracy of T cells. *Mol. Immunol. 40*, 1047–1055.

Wolf, P.R. and Ploegh, H.L. (1995). How MHC class II molecules acquire peptide cargo: biosynthesis and trafficking through the endocytic pathway. *Annu. Rev. Cell. Dev. Biol. 11*, 267–306.

Wucherpfennig, K.W. (2004). T cell receptor crossreactivity as a general property of T cell recognition. *Mol. Immunol. 40*, 1009–1017.

Wucherpfennig, K.W. and Strominger, J.L. (1995). Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell 80*, 695–705.

Yoshizawa, I., Bronson, R., Dorf, M.E., and Abromson-Leeman, S. (1998). T-cell responses to myelin basic protein in normal and MBP-deficient mice. *J. Neuroimmunol. 84*, 131–138.

Yu, M., Johnson, J.M., and Tuohy, V.K. (1996). A predictable sequential determinant spreading cascade invariably accompanies progression of experimental autoimmune encephalomyelitis: a basis for peptide-specific therapy after onset of clinical disease. *J. Exp. Med. 183*, 1777–1788.

Zamvil, S.S., Mitchell, D.J., Moore, A.C., Kitamura, K., Steinman, L., and Rothbard, J.B. (1986). T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature 324*, 258–260.

Zhao, Z.S., Granucci, F., Yeh, L., Schaffer, P.A., and Cantor, H. (1998). Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science 279*, 1344–1347.

Zhou, D. and Hemmer, B. (2004). Specificity and degeneracy: T cell recognition in CNS autoimmunity. *Mol. Immunol. 40*, 1057–1061.

CHAPTER

15

Apoptotic Cells as a Source of Autoantigens

LIVIA CASCIOLA-ROSEN*

*Department of Medicine, Johns Hopkins University School of Medicine

ANTONY ROSEN*‡§

‡Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine; and §Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Systemic autoimmune diseases are a heterogeneous group of genetically complex diseases in which the immune system targets a diverse, but very specific, group of molecules. Each disease is associated with the production of high-titer autoantibodies directed against a group of ubiquitously expressed, but phenotype-specific, autoantigens (von Muhlen and Tan, 1995; Hall et al., 2004). The autoantibody response in patients and animals with autoimmune disease exhibits clonal expansion, affinity maturation, and class switching, and provides evidence that the immune responses in these processes are antigen driven and T-cell dependent (Diamond et al., 1992; Burlingame et al., 1993; Radic and Weigert, 1995). Such studies have focused attention on the autoantigens that drive these immune responses, and the circumstances in which these molecules might have satisfied the criteria for initiation and propagation of a T-cell-dependent immune response. The most important criterion for initiation of a primary T-cell response is that the molecule targeted has not been previously presented in that form during development of T-cell tolerance.

Approximately a decade ago, we observed that the autoantigens targeted in systemic autoimmune diseases are unified by their clustering and concentration within surface blebs on apoptotic cells, and we proposed that such molecules were unified by their propensity to undergo structural modifications during some forms of cell death, generating novel structures not previously tolerized by the host (Casciola-Rosen et al., 1994a). Accumulating experimental evidence indicates that most autoantigens are indeed unified by their susceptibility to structural modification during cell death (Utz and Anderson, 1998; Rosen and Casciola-Rosen, 1999; Doyle and Mamula, 2002). Although autoantigens have been demonstrated to undergo several types of modification during cell death, we focus only on the proteolytic cleavage of autoantigens, and the mechanisms whereby such changes might lead to initiation of an autoimmune response.

APOPTOTIC CELLS MAY INDUCE TOLERANCE OR IMMUNITY

Apoptosis [reviewed extensively by Hengartner (2000)] is a sequence of morphologic and biochemical changes characterized by nuclear condensation and membrane blebbing (Kerr et al., 1972). During the apoptotic process, cleavage and activation of a series of cysteine proteases (called

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

caspases) results in the activation of a proteolytic cascade that cleaves downstream molecules that function in pathways essential to cell survival (Thornberry and Lazebnik, 1998; Green and Kroemer, 2004). Apoptotic cell death occurs throughout development and during tissue homeostasis; in these circumstances, the immune consequences are predominantly noninflammatory and tolerance inducing (Voll et al., 1997; Fadok et al., 1998; Gershov et al., 2000; Huynh et al., 2002). Furthermore, apoptosis occurs with great frequency during lymphocyte development and education in the thymus and bone marrow (Surh and Sprent, 1994). Significant experimental evidence indicates that apoptotic cells themselves constitute a prominent source of toleragen in these settings (Cocca et al., 2001; Li et al., 2003). A role of apoptotic cells in the establishment and maintenance of tolerance to peripheral tissues has also been established (Miller et al., 1998; Huang et al,. 2000; Belz et al., 2002; Hugues et al., 2002; Liu et al., 2002; Steinman and Nussenzweig, 2002), providing the immune system with the appropriate forms of autoantigens that will likely be encountered during homeostatic cell death of self-tissues. Consequently, abnormalities in the processes regulating clearance of, and tolerance induction by, apoptotic cells play important roles in rendering individuals susceptible to initiation of systemic autoimmunity (Botto et al., 1998; Scott et al., 2001; Cohen et al., 2002; Hanayama et al., 2004; Manderson et al., 2004). Furthermore, the structural changes that autoantigens undergo during various forms of non-tolerance-inducing cell death may be particularly relevant in selecting the molecular targets of such autoimmunity. An excellent construct within which to view such changes is that proposed by Sercarz et al. (1993), in which altered autoantigen structure modifies antigen processing and presentation such that previously cryptic epitopes are presented to the immune system and activate autoreactive T cells. This paradigm is reviewed in more detail below.

DOMINANCE AND CRYPTICITY

That the macromolecular structure of an antigen may influence its subsequent processing and presentation by MHC class II has become increasingly clear over the past two decades (Streicher et al., 1984; Lipham et al*.,* 1991; Mamula et al., 1992; Mamula, 1993; Schneider et al., 2000). Indeed, several studies have persuasively demonstrated this for various model antigens (see below). In Sercarz's construct of immunologic dominance and crypticity (Lanzavecchia, 1995; Sercarz et al., 1993; Sercarz, 2002), not all determinants on an intact protein antigen are equally immunogenic. Due to certain structural features of the antigen itself, specific determinants are preferentially presented. For a given antigen conformation and antigen-processing machinery, the processing pathway is relatively constant and predictable, and preferentially selects a few determinants that are effectively loaded onto major histocompatibility complex (MHC) class II molecules (dominant epitopes). Several recent studies demonstrating that intact antigens are also the preferential substrates for cross-priming suggest that a similar paradigm is possible for presentation of exogenous antigens on MHC class I (Norbury et al., 2004; Wolkers et al., 2004). Naïve T cells recognizing dominant epitopes of self-antigens in the thymus or periphery are deleted. In contrast, cryptic epitopes in self-antigens are not presented in significant amounts, and potentially autoreactive T cells recognizing such cryptic epitopes are therefore not deleted. Since the products of antigen processing are constant under homeostatic conditions, cryptic epitopes are not revealed under most circumstances. However, were they to be generated during some unusual antigen-processing event, autoreactive T cells recognizing these cryptic epitopes could conceivably be activated and drive the autoimmune response (Sercarz et al., 1993).

The dominance or crypticity of an epitope may be influenced by many intrinsic and extrinsic properties. For example, the sequence of unfolding and specific cleavage of antigens along the endosome/lysozome pathway strikingly influences which peptides finally get loaded onto a particular MHC molecule (Manoury et al., 1998; Antoniou et al., 2000). Also, the intrinsic affinity of a peptide for MHC class II proteins, as well as the properties of neighboring structural determinants, may influence the binding of this peptide to the binding groove (Sercarz et al., 1993; Sercarz, 2002). Understanding how the structure and processing of self-antigens might be altered during different physiologic and pathologic states is therefore significant. Of particular importance are early proteolytic events (Mamula, 1993; Bockenstedt et al., 1995; Manoury et al., 2002) and the effects of high-affinity binding of antibody or other molecules (Watts and Lanzavecchia, 1993; Salemi et al., 1995; Simitsek et al., 1995) on subsequent antigen processing.

Since potentially autoreactive T cells that recognize the cryptic self abound in the immune repertoire, they provide the capacity to respond to novel antigen forms generated through non-standard processing pathways. Changes in autoantigen structure that occur during cell death are therefore potentially of relevance.

MODIFICATION OF AUTOANTIGEN STRUCTURE DURING APOPTOSIS

Autoantigens in systemic autoimmune diseases are clustered and concentrated within the surface blebs on apoptotic cells (Casciola-Rosen et al., 1994a; Rosen et al., 1995; Korb

and Ahearn, 1997; Cocca et al., 2002; Caricchio et al., 2003). Whether structure (and potentially antigen processing) of naturally-selected autoantigens is altered during different forms of cell death has been a subject of significant experimental effort over the past several years. Such studies have demonstrated a striking susceptibility of autoantigens to structural modification, particularly specific proteolysis, during cell death (Utz and Anderson, 1998; Rosen and Casciola-Rosen, 1999; Doyle and Mamula, 2001). Recent studies have also demonstrated that membranes of cells undergoing apoptosis contain increased levels of biologically active oxidized phospholipids, which activate endothelial cells to induce monocyte adhesion. Furthermore, immunization of mice with syngeneic apoptotic cells induced high autoantibody titers to various oxidation-specific epitopes of oxidized phospholipids (Chang et al., 2004). Such structural changes during apoptotic cell death might play a role in the induction of autoimmune responses to apoptotic cells. The different forms of apoptotic proteolysis and some potential mechanisms whereby these changes may be relevant to human autoimmunity are reviewed below.

Cleavage of Autoantigens by Aspartic Acid-Specific Apoptotic Proteases

Proteases play a critical role in the apoptotic process through targeted cleavage of a limited group of downstream substrates that are functionally important in the maintenance of life (Nunez et al., 1998; Thornberry and Lazebnik, 1998). The caspases, a family of cysteine proteases with an absolute requirement for cleavage after aspartic acid, constitute the most prominent apoptotic protease family (Thornberry and Lazebnik, 1998; Boyce et al., 2004). In initial studies looking for modification of autoantigen structure in apoptotic cells, we and others noted that although caspases only have a limited number of substrates during cell death (perhaps a few hundred at maximum), autoantigens are highly represented among these molecules (Casciola-Rosen et al., 1994b; 1995; Casiano et al., 1996; Utz et al., 1998; Rutjes et al., 1999; Ayukawa et al., 2000; Malmegrim de Farias et al., 2003). In a minority of cases (e.g., NuMA, CENP-C), the cleaved form of the antigen is better recognized by patient autoantibodies than the intact protein, suggesting that cleavage may alter accessibility of the relevant epitopes (Schachna et al., 2002). There are also reports of some autoantibodies to U1-70-kDa, which specifically recognize the apoptotically modified form of this molecule. The observation that such antibodies are associated with distinct clinical features suggests that caspase-mediated cleavage may play a role in driving the immune response to U1-70-kDa in specific circumstances (Greidinger et al., 2004). To date, no persuasive experiments directly addressing the effects of caspase cleavage on immunogenicity of naturally-selected autoantigens have been performed. While it is possible that cleavage of autoantigens influences immunogenicity, it is equally likely that the presence and structural features of the cleavage site itself influence antigen processing and epitope selection, and that different mechanisms apply to different autoantigens.

In this regard, a series of experiments using tetanus toxin c fragment as a model antigen, which has demonstrated that a hierarchy of antigen-processing events exists, is relevant (Manoury et al., 1998; Antoniou et al., 2000). In these studies, processing of tetanus toxin is initiated upon cleavage by a lysozomal enzyme, asparaginyl endopeptidase (AEP), at a single defined site. There is an absolute requirement for this cleavage event, as elimination of the specific processing sites through mutagenesis dramatically alters the efficiency of epitope presentation to an antigen-specific T-cell clone. As a result of a single conservative mutation at the enzyme cleavage site, novel epitopes are generated during antigen processing. Inhibiting the AEP protease also alters processing of the whole antigen, resulting in different peptides being loaded onto MHC class II molecules. Thus, in this example, antigen processing occurs in a stepwise manner, with cleavage by a single, initial protease being necessary for the subsequent processing steps that generate the dominant T-cell epitopes. Subtle changes in antigen structure, which alter such key upstream processing sites, can dramatically alter processing of the intact antigen. Where such changes cause the revelation of epitopes not normally seen by the immune system, the subsequent development of an autoimmune response is enabled (Moudgil and Sercarz, 1993) (see Chapter 14).

Recent studies from Crow and colleagues (Chernysheva et al., 2002) have demonstrated the importance of caspase-dependent events in the initiation of an autologous mixed lymphocyte reaction (AMLR). Incubation of apoptotic non-T-cell stimulator cells with autologous T cells induces proliferation of the T cells, an event that is enhanced when apoptosis is promoted in the stimulator cell population by treatment with either γ-irradiation or staurosporine. This proliferation is almost completely abrogated when a number of different caspase inhibitors are preincubated with the apoptotic stimulator cell population. In contrast, preincubation of the responder T cells with caspase inhibitors did not alter T-cell proliferation, indicating that caspase activity is required in the stimulator population to initiate a response (Chernysheva et al., 2002). It remains unclear whether this effect is due to caspase-mediated cleavage of autoantigens themselves, or occurs indirectly, through pathways downstream of caspases that modify autoantigen structure (e.g., activation of kinases, nucleases) or otherwise enhance autoantigen presentation (e.g., enhanced phagocytosis or clearance).

Novel Autoantigen Structure during Cytotoxic Lymphocyte Granule-Induced Death

The initiation of a primary immune response is dependent upon the generation of suprathreshold concentrations of antigen with a structure not previously tolerized by the host, occurring within a proinflammatory environment. Since most forms of homeostatic apoptotic cell death are profoundly tolerance inducing (Voll et al., 1997; Fadok et al., 1998; Gershov et al., 2000; Huynh et al., 2002), it is likely that initiation of autoimmunity is associated with a more proinflammatory type of cell death. The killing of virally-infected or transformed cells is of particular relevance in this regard, both due to the proimmune context of such killing, as well as to alterations in autoantigen expression, structure, and conformation in such settings. For example, viral infection may directly alter the structure of self-antigens (Dong et al., 1994; Duncan and Nakhasi, 1997). It is also possible that viral infection initiates cytokine secretion and immune effector pathways that alter the expression and/or structure of self-antigens. Similarly, there is also significant evidence that autoantigen expression and conformation may be strikingly altered in transformed cells and tumors (Corradi et al., 1997; Zhang et al., 1999; Ulanet et al., 2003). Interestingly, both virus-infected and transformed cells are targets of similar immune effector pathways unleashed by cytotoxic T cells and natural killer (NK) cells (Russell and Ley, 2002; Trapani and Sutton, 2003).

Cytotoxic Lymphocyte Granule-Induced Death Pathways

Cytotoxic lymphocytes induce target cell death through several different pathways, including ligation of the Fas receptor on the surface of the target cell, as well as release of proteases contained in lytic granules within the cytotoxic cell (Andrade et al., 2004). Transduction of the Fas signal occurs through multiple protein–protein interactions that activate the caspase cascade by inducing processing of caspases-8 and -10 (Siegel et al., 2000). Thus, ligation of the death receptor Fas (CD95) results in recruitment of the adaptor molecule FADD. Death effector domains within FADD recruit and induce oligomerization of group 3 caspase precursors, which undergo autocatalytic cleavage and activation of the receptor complex known as DISC (death-inducing signaling complex) (Tibbetts et al., 2003). Cytolysis induced via the Fas pathway is thought to be predominantly associated with immune regulatory processes (Lenardo, 2003). Granzyme-mediated killing utilizes several granule components with unique activities. The most abundant components present in cytotoxic granules are perforin (a pore-forming protein) and a family of serine proteases termed granzymes (Henkart, 1994). Perforin has long been considered crucial for the entry of granzymes into the target cell, but the mechanisms underlying its activity remain controversial (Russell and Ley, 2002). Following release into the cytosol with the help of perforin, granzyme B (GrB), a rapidly-acting apoptotic enzyme, catalyzes the cleavage and activation of several downstream substrates, inducing apoptotic changes in the target cell. Prominent among the upstream mediators of the GrB effect are Bid and procaspases, which are directly cleaved by GrB to generate their active forms. Thus, through the early cleavage and activation of Bid, GrB rapidly recruits the mitochondrial amplification loop of caspase activation (Sutton et al., 2000; 2003; Pinkoski et al., 2001). GrB similarly cleaves and activates effector caspases that further amplify the apoptotic proteolytic cascade, resulting in cleavage of multiple downstream substrates, and generation of the apoptotic phenotype. In addition to the recruitment of caspase-dependent pathways, GrB also directly cleaves several downstream signature death substrates, constituting an important caspase-independent death pathway that can lead to cell death even in the absence of caspase activity (Andrade et al., 1998; Casciola-Rosen et al., 1999). For example, GrB directly cleaves and activates the apoptotic nuclease DFF45/ICAD; such cleavage is required for optimal granzyme-induced target cell death (Thomas et al., 2000). Additionally, autoantigens targeted in human autoimmune diseases are highly represented among these substrates (Casciola-Rosen et al., 1994a; 1996; 1999; 2001; Mancini et al., 2000; Nagaraju et al., 2001; Mahoney et al., 2002) (see Figure 15.1 for examples).

Granzyme B Cleavage of Autoantigens

The number of autoantigens known to be cleaved by GrB is steadily growing. The observation that many autoantigens targeted across the spectrum of human autoimmune diseases are directly and efficiently cleaved by GrB is remarkable for several reasons: 1) susceptibility to cleavage by GrB is a highly specific feature of autoantigens, and nonautoantigens are not similarly susceptible to cleavage by GrB at sites not cleaved by caspases (Casciola-Rosen et al., 1999); 2) a very similar group of autoantigens is cleaved by caspases and GrB, although at distinct sites, thus generating different fragments (Andrade et al., 1998); 3) the cleavage sites in autoantigens are uniquely suited to cleavage by GrB and are resistant to cleavage by the upstream activating caspases (caspases-8 and -9) due to the enrichment of residues in the P2 and P3 substrate positions that are preferred by GrB but not tolerated by caspases (Casciola-Rosen et al., 1999); 4) GrB activation of caspases leads to significant amplification of caspase cleavages, and when efficiency of cleavage of a substrate by caspases and GrB is similar, products of caspase cleavage generally predominate. Generation of GrB frag-

ments is thus enhanced by inhibition of the caspase pathway (Andrade et al., 1998; Casciola-Rosen et al., 1999). Also of particular note, GrB and other components of the lytic granule pathway are not expressed in the thymus (Shresta et al., 1995), and cleavage products generated by such proteases are therefore unlikely to have been tolerized.

The striking association between susceptibility of a substrate to cleavage by GrB and autoantigen status strongly suggests that these properties are mechanistically related. As noted for the caspases above, it remains unknown whether it is the properties of the cleavage site or cleavage itself that plays a role in selecting molecules against which autoimmune responses are generated. If cleavage is the important parameter, the reciprocal relationship between caspase activity and generation of granzyme-specific autoantigen fragments suggests that initial immunization occurs in a setting in which caspases are under endogenous (e.g., inhibitors of apoptosis (IAP) proteins) or exogenous inhibition (e.g., viral caspases inhibitors) (Kobzik et al., 1994; Deveraux et al,. 1997; 1998; Roy et al., 1997; Li et al., 1998).

The effect of autoantigen cleavage on recognition by autoantibodies has been addressed in part using an immunoblotting approach; however, there is, as yet, no common feature that unifies all the antigens in terms of antibody recognition. Indeed, it is striking that GrB cleavage can have various effects, described below, on recognition of different molecules by autoantibodies. For example, although infrequently observed, GrB-generated fragments of autoantigens may be recognized better by autoantibodies than the intact molecule. A striking example of this is the scleroderma autoantigen CENP-C (Schachna et al., 2002), for which GrB cleavage greatly enhances autoantibody recognition. Interestingly, such CENP-C fragment-preferential autoantibodies are particularly enriched in patients with scleroderma and ischemic digital loss. Similar findings have also been made for nuclear mitotic apparatus protein (NuMA) (Andrade et al., 1998). Enhanced autoantibody recognition of GrB-cleaved autoantigens may be indicative of several things: 1) exposure of a neoepitope at the site of granzyme cleavage; or 2) a conformational change at a site more distant from the scissile bond, which improves antibody access to the epitope. Either way, these data indicate that the intact cleavage site suppresses or restrains access to the epitope, which becomes dominant in the autoantibody response. How this relates to the driving T-cell response is still unknown, but is of significant importance. There are also clear examples where GrB cleavage of an autoantigen greatly inhibits recognition by autoantibodies. For example, Gershwin and colleagues (Matsumura et al., 2002) demonstrated that autoantibodies recognizing the intact pyruvate dehyrdrogenase E2 subunit (PDC-E2) fail to recognize the GrB-cleaved form of PDC-E2. Since previous studies had demonstrated that the dominant B- and T-cell epitopes in PDC-E2 coincide, and include the GrB cleavage site, these data demonstrate that the intact cleavage site itself forms part of the dominant B-cell epitope. Similar findings have also been made for XRCC4 (Lee et al., 2002). Lastly, for most autoantigens studied, cleavage by GrB has no effect on recognition by autoantibodies.

The fact that cleavage can enhance or destroy recognition of some molecules by autoantibodies demonstrates that the GrB cleavage site can either enhance or suppress accessibility of epitopes, occasionally at the level of the autoantibody, but potentially more generally at the level of the T cell. Although autoantibodies are excellent probes of the targets of the autoimmune response, they are less well suited to defining the particular conformation of the antigen that initiated the T-cell response. Interestingly, studies by Mamula et al. (1999) examining the generation of autoimmune responses to an isoaspartyl-containing Sm-D or cytochrome c peptide have demonstrated that T cells recognize only the isoaspartyl form of the antigen, while autoantibodies bind both modified and unmodified forms. Defining the effects of the intact GrB cleavage site, and its destruction after proteolysis, on the processing and presentation of antigens to T cells therefore remains a major priority.

Cleavage of Tissue-Specific Autoantigens

Structural changes of autoantigens induced by post-translational modification can alter the processing of self-antigens and influence the ability to generate an immune response against self. For example, studies have shown that the neuronal glutamate receptor subunit 3 is an autoantigen in patients with a severe form of pediatric epilepsy, Rasmussen encephalitis (Rogers et al., 1994). Autoantibodies tend to be of low affinity, and many recognize a well-defined extracellular epitope residing in the receptor-activating epitope, which is aligned on the surface of the folded protein (Twyman et al., 1995). Of note, the GrB cleavage site contains an asparagine that is normally N-glycosylated, and is found within a major epitope that is recognized by autoantibodies in Rasmussen encephalitis (Gahring et al., 2001). Interestingly, Gharing et al. (2001) showed that deglycosylated GluR3 is susceptible to cleavage by GrB, whereas the glycosylated form of this receptor is poorly cleaved, possibly because the N-linked sugar sterically hinders the ability of GrB to bind to its substrate. The authors postulate that inflammatory events may inhibit the glycosylation of GluR3, thus rendering it susceptible to cleavage by GrB, and potentially to initiation of GluR3-directed autoimmunity.

Novel Conformation of Disease-Specific Autoantigens

In spite of the fact that many autoantigens in systemic autoimmune diseases are ubiquitously expressed, there is

nevertheless a striking association of specific antibody responses with unique clinical phenotypes. One potential explanation for this observation is that changes in autoantigen structure may be limited to the relevant disease microenvironment and may contribute to initiation of an autoimmune response. As several autoantigens that are targeted in systemic autoimmune diseases can also be targeted in patients with hepatocellular carcinoma (HCC), we have sought to define microenvironment-specific changes in autoantigens in liver tissue of patients with HCC. Interestingly, the nucleolar HCC autoantigen B23 exists in a truncated form in HCC liver lacking six amino acids at the N-terminus. While full-length B23 (expressed in all other tissues) is very resistant to cleavage by GrB, HCC B23 is strikingly sensitive to such cleavage (Ulanet et al., 2003). Whether exposure of the GrB cleavage site or cleavage itself plays a role in selecting B23 as an HCC antigen remains unknown, but the striking restriction of this novel conformation to the likely site of immunization is of great importance.

Additional Post-Translational Modifications that Affect Autoantigen Structure

Although this chapter has focused on apoptosis-specific proteolysis as a primary mechanism of structural change during cell death, other post-translational changes (e.g., phosphorylation/dephosphorylation, isoaspartyl modification, transglutamination, and deimination) occur frequently during various death processes, and may profoundly affect antigen processing and presentation (Utz et al., 2000; Doyle and Mamula, 2002). It should also be noted that autoantigens may undergo novel proteolytic modifications distinct from those observed during apoptosis during necrotic death (Casiano et al., 1998).

MODEL OF ANTIGEN SELECTION DURING CELL DEATH

We propose that apoptotic cells are sources of cleaved antigens that can either tolerize or activate lymphocytes, depending on the type of cell death (homeostatic or proimmune), the species of aspartic acid-specific protease that preferentially process the self-antigen, and the efficiency of clearance of the apoptotic cell. Under normal homeostatic circumstances, apoptotic cells are efficiently cleared and actively induce tolerance (Figure 15.1A, left side). These apoptotic cells are highly enriched in autoantigen fragments generated by caspase cleavage. Such peptide fragments generated by caspases are likely the form of the autoantigen that is tolerized during development and homeostasis. Where apoptotic cells are efficiently cleared and captured by immature dendritic cells, tolerance to caspase-cleaved antigens is fully established, and autoreactive T cells recognizing caspase-cleaved antigens are likely deleted. In contrast, where clearance of, or tolerance induction by, apoptotic cells is abnormal, establishment of tolerance to caspase-cleaved antigens will be incomplete, and may later allow activation of autoreactive T cells recognizing such caspase-generated fragments during a major apoptosis-inducing event

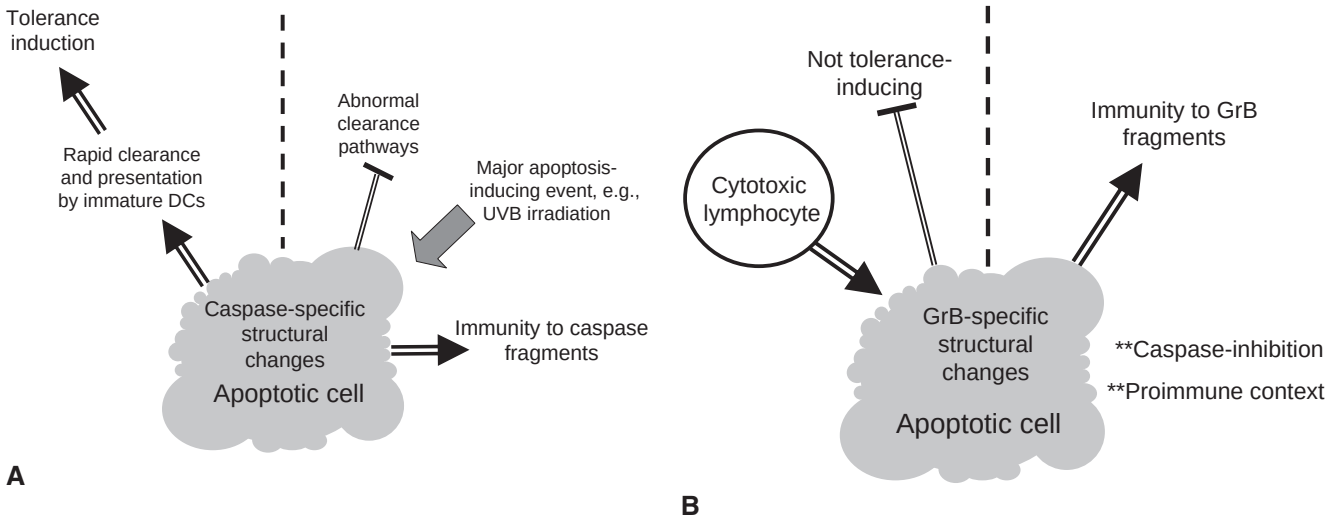


FIGURE 15.1 Model of antigen selection during cell death. *A,* Homeostatic death. The predominant form of the molecule tolerized during development and homeostasis is the caspase-induced form. Tolerance to caspase-cleaved fragments results when apoptotic clearance is normal. *B,* Proimmune death. Certain proimmune circumstances allow preferential activity of the granzyme (GrB) pathway, generating novel autoantigen fragments not previously tolerized by the host. DC, dendritic cell.

(Figure 15.1A, right side). Even where apoptotic cell clearance is normal, and tolerance to the default caspase-induced death pathways fully established, some proinflammatory forms of cell death (e.g., cytotoxic lymphocyte granule pathway-induced death of virus-infected or transformed cells) may generate novel forms of autoantigens not usually generated during tolerance induction (Figure 15.1B). This will most likely occur under circumstances in which caspases are under exogenous or endogenous inhibition, maximizing the generation of granzyme-induced fragments. Such fragments may allow the activation of autoreactive T cells recognizing nontolerized epitopes. Direct demonstration of the differential immunogenicity of different forms of caspase/granzyme-cleaved apoptosis is a major priority.

Acknowledgments

This work was supported by grants from the NIH AR-44684 (to LCR), DE-12354 (to AR), the MARRC Program of the Arthritis Foundation, Maryland Chapter, the Vernon Lynch Memorial Fellowship in Arthritis Research, and a Burroughs Wellcome Fund Translational Research Award.

References

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.

Andrade, F., Casciola-Rosen, L.A., and Rosen, A. (2004). Granzyme B-induced cell death. *Acta Haematol. 111*, 28–41.

Antoniou, A.N., Blackwood, S.L., Mazzeo, D., and Watts, C. (2000). Control of antigen presentation by a single protease cleavage site. *Immunity 12*, 391–398.

Ayukawa, K., Taniguchi, S., Masumoto, J., Hashimoto, S., Sarvotham, H., Hara, A., Aoyama, T., and Sagara, J. (2000). La autoantigen is cleaved in the COOH terminus and loses the nuclear localization signal during apoptosis. *J. Biol. Chem. 275*, 34465–34470.

Belz, G.T., Behrens, G.M., Smith, C.M., Miller, J.F., Jones, C., Lejon, K., Fathman, C.G., Mueller, S.N., Shortman, K., Carbone, F.R., and Heath, W.R. (2002). The CD8alpha(+) dendritic cell is responsible for inducing peripheral self-tolerance to tissue-associated antigens. *J. Exp. Med.* 196, 1099–1104.

Bockenstedt, L.K., Gee, R.J., and Mamula, M.J. (1995). Self-peptides in the initiation of lupus autoimmunity. *J. Immunol. 154*, 3516–3524.

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. *Nat. Genet. 19*, 56–59.

Boyce, M., Degterev, A., and Yuan, J. (2004). Caspases: an ancient cellular sword of Damocles. *Cell Death Differ. 11*, 29–37.

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.

Caricchio, R., McPhie, L., and Cohen, P.L. (2003). Ultraviolet B radiation-induced cell death: critical role of ultraviolet dose in inflammation and lupus autoantigen redistribution. *J. Immunol.* 171, 5778–5786.

Casciola-Rosen, L.A., Anhalt, G., and Rosen, A. (1994a). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med. 179*, 1317–1330.

Casciola-Rosen, L.A., Miller, D.K., Anhalt, G.J., and Rosen, A. (1994b). 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.

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.

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.

Casciola-Rosen, L., Andrade, F., Ulanet, D., Wong, W.B., and Rosen, A. (1999). Cleavage by granzyme B is strongly predictive of autoantigen status: Implications for initiation of autoimmunity. *J. Exp. Med. 190*, 815–825.

Casciola-Rosen, L., Pluta, A.F., Plotz, P.H., Cox, A.E., Morris, S., Wigley, F.M., Petri, M., Gelber, A.C., and Rosen, A. (2001). The DNA mismatch repair enzyme PMS1 is a myositis-specific autoantigen. *Arthritis Rheum. 44*, 389–396.

Casiano, C.A., Martin, S.J., Green, D.R., and Tan, E.M. (1996). Selective cleavage of nuclear autoantigens during CD95 (Fas/APO-1)-mediated T cell apoptosis. *J. Exp. Med. 184*, 765–770.

Casiano, C.A., Ochs, R.L., and Tan, E.M. (1998). Distinct cleavage products of nuclear proteins in apoptosis and necrosis revealed by autoantibody probes. *Cell Death Differ. 5*, 183–190.

Chang, M.K., Binder, C.J., Miller, Y.I., Subbanagounder, G., Silverman, G.J., Berliner, J.A., and Witztum J.L. (2004). Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. *J. Exp. Med. 200*, 1359–70.

Chernysheva, A.D., Kirou, K.A., and Crow, M.K. (2002). T cell proliferation induced by autologous non-T cells is a response to apoptotic cells processed by dendritic cells. *J. Immunol. 169*, 1241–1250.

Cocca, B.A., Seal, S.N., D'Agnillo, P., Mueller, Y.M., Katsikis, P.D., Rauch, J., Weigert, M., and Radic, M.Z. (2001). Structural basis for autoantibody recognition of phosphatidylserine-beta 2 glycoprotein I and apoptotic cells. *Proc. Natl. Acad. Sci. USA* 98, 13826–13831.

Cocca, B.A., Cline, A.M., and Radic, M.Z. (2002). Blebs and apoptotic bodies are B cell autoantigens. *J. Immunol.* 169, 159–166.

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*, 135–140.

Corradi, J.P., Yang, C., Darnell, J.C., Dalmau, J., and Darnell, R.B. (1997). A post-transcriptional regulatory mechanism restricts expression of the paraneoplastic cerebellar degeneration antigen cdr2 to immune privileged tissues. *J. Neurosci. 17*, 1406–1415.

Deveraux, Q.L., Takahashi, R., Salvesen, G.S., and Reed, J.C. (1997). X-linked IAP is a direct inhibitor of cell-death proteases. *Nature 388*, 300–304.

Deveraux, Q.L., Roy, N., Stennicke, H.R., Van Arsdale, T., Zhou, Q., Srinivasula, S.M., Alnemri, E.S., Salvesen, G.S., and Reed, J.C. (1998). IAPs block apoptotic events induced by caspase-8 and cytochrome *c* by direct inhibition of distinct caspases. *EMBO J. 17*, 2215–2223.

Diamond, B., Katz, J.B., Paul, E., Aranow, C., Lustgarten, D., and Scharff, M.D. (1992). The role of somatic mutation in the pathogenic anti-DNA response. *Annu. Rev. Immunol. 10*, 731–757.

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.

Doyle, H.A. and Mamula, M.J. (2001). Post-translational protein modifications in antigen recognition and autoimmunity. *Trends Immunol. 22*, 443–449.

Doyle, H.A. and Mamula, M.J. (2002). Posttranslational protein modifications: new flavors in the menu of autoantigens. *Curr. Opin. Rheumatol. 14*, 244–249.

Duncan, R.C. and Nakhasi, H.L. (1997). La autoantigen binding to a 5′ cis-element of rubella virus RNA correlates with element function *in vivo*. *Gene 201*, 137–149.

Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y., and Henson, P.M. (1998). Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J. Clin. Invest.* 101, 890–898.

Gahring, L., Carlson, N.G., Meyer, E.L., and Rogers, S.W. (2001). Granzyme B proteolysis of a neuronal glutamate receptor generates an autoantigen and is modulated by glycosylation. *J. Immunol.* 166, 1433–1438.

Gershov, D., Kim, S., Brot, N., and Elkon, K.B. (2000). C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. *J. Exp. Med. 192*, 1353–1364.

Green, D.R. and Kroemer, G. (2004). The pathophysiology of mitochondrial cell death. *Science 305*, 626–629.

Greidinger, E.L., Foecking, M.F., Magee, J., Wilson, L., Ranatunga, S., Ortmann, R.A., and Hoffman, R.W. (2004). A major B cell epitope present on the apoptotic but not the intact form of the U1-70-kDa ribonucleoprotein autoantigen. *J. Immunol. 172*, 709–716.

Hall, J.C., Casciola-Rosen, L., and Rosen, A. (2004). Altered structure of autoantigens during apoptosis. *Rheum. Dis. Clin. North Am. 30*, 455–71, vii.

Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K, Uchiyama, Y, and Nagata, S. (2004). Science. Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science 304*, 1147–1150.

Hengartner, M.O. (2000). The biochemistry of apoptosis. *Nature 407*, 770–776.

Henkart, P.A. (1994). Lymphocyte-mediated cytotoxicity: Two pathways and multiple effector molecules. *Immunity 1*, 343–346.

Huang, F.P., Platt, N., Wykes, M., Major, J.R., Powell, T.J., Jenkins, C.D., and MacPherson, G.G. (2000). A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J. Exp. Med. 191*, 435–444.

Hugues, S., Mougneau, E., Ferlin, W., Jeske, D., Hofman, P., Homann, D., Beaudoin, L., Schrike, C., Von Herrath, M., Lehuen, A., and Glaichenhaus, N. (2002). Tolerance to islet antigens and prevention from diabetes induced by limited apoptosis of pancreatic beta cells. *Immunity 16*, 169–181.

Huynh, M.L., Fadok, V.A., and Henson, P.M. (2002). Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. *J. Clin. Invest. 109*, 41–50.

Kerr, J.F.R., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer 26*, 239–257.

Kobzik, L., Reid, M.B., Bredt, D.S., and Stamler, J.S. (1994). Nitric oxide in skeletal muscle. *Nature 372*, 546–548.

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, 4525–4528.

Lanzavecchia, A. (1995). How can cryptic epitopes trigger autoimmunity? *J. Exp. Med. 181*, 1945–1948.

Lee, K.J., Dong, X., Wang, J., Takeda, Y., and Dynan, W.S. (2002). Identification of human autoantibodies to the DNA ligase IV/XRCC4 complex and mapping of an autoimmune epitope to a potential regulatory region.PG. *J. Immunol. 169*, 3413–3421.

Lenardo, M.J. (2003). Molecular regulation of T lymphocyte homeostasis in the healthy and diseased immune system. *Immunol. Res.* 27, 387–398.

Li, F., Ambrosini, G., Chu, E.Y., Plescia, J., Tognin, S., Marchisio, P.C., and Altieri, D.C. (1998). Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature 396*, 580–584.

Li, H., Jiang, Y., Cao, H., Radic, M., Prak, E.L., and Weigert, M. (2003). Regulation of anti-phosphatidylserine antibodies. *Immunity 18*, 185–192.

Lipham, W.J., Redmond, T.M., Takahashi, H., Berzofsky, J.A., Wiggert, B., Chader, G.J., and Gery, I. (1991). Recognition of peptides that are immunogenic but cryptic: Mechanisms that allow lymphocytes sensitized against cryptic peptides to initiate pathogenic autoimmune processes. *J. Immunol. 146*, 3757–3762.

Liu, K., Iyoda, T., Saternus, M., Kimura, Y., Inaba, K., and Steinman, R.M. (2002). Immune tolerance after delivery of dying cells to dendritic cells *in situ*. *J. Exp. Med. 196*, 1091–1097.

Mahoney, J.A., Odin, J.A., White, S.M., Shaffer, D., Koff, A., Casciola-Rosen, L., and Rosen, A. (2002). The human homologue of the yeast polyubiquitination factor Ufd2p is cleaved by caspase 6 and granzyme B during apoptosis. *Biochem. J. 361*, 587–595.

Malmegrim de Farias, K.C., Saelens, X., Pruijn, G.J., Vandenabeele, P., and van Venrooij, W.J. (2003). Caspase-mediated cleavage of the U snRNP-associated Sm-F protein during apoptosis. *Cell Death Differ. 10*, 570–579.

Mamula, M.J. (1993). The inability to process a self-peptide allows autoreactive T cells to escape tolerance. *J. Exp. Med. 177*, 567–571.

Mamula, M.J., Lin, R.-H., Janeway, C.A. Jr., and Hardin, J.A. (1992). Breaking T cell tolerance with foreign and self co-immunogens. A study of autoimmune B and T cell epitopes of cytochrome c. *J. Immunol. 149*, 789–795.

Mamula, M.J., Gee, R.J., Elliott, J.I., Sette, A., Southwood, S., Jones, P.J., and Blier, P.R. (1999). Isoaspartyl post-translational modification triggers autoimmune responses to self-proteins. *J. Biol. Chem. 274*, 22321–22327.

Mancini, M., Machamer, C.E., Roy, S., Nicholson, D.W., Thornberry, N.A., Casciola-Rosen, L.A., and Rosen, A. (2000). Caspase-2 is localized at the Golgi complex and cleaves golgin-160 during apoptosis. *J. Cell Biol. 149*, 603–612.

Manderson, A.P., Botto, M., and Walport, M.J. (2004). The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol. 22*, 431–456.

Manoury, B., Hewitt, E.W., Morrice, N., Dando, P.M., Barrett, A.J., and Watts, C. (1998). An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation. *Nature 396*, 695–699.

Manoury, B., Mazzeo, D., Fugger, L., Viner, N., Ponsford, M., Streeter, H., Mazza, G., Wraith, D.C., and Watts, C. (2002). Destructive processing by asparagine endopeptidase limits presentation of a dominant T cell epitope in MBP. *Nat. Immunol. 3*, 169–174.

Matsumura, S., Van De, W.J., Kita, H., Coppel, R.L., Tsuji, T., Yamamoto, K., Ansari, A.A., and Gershwin, M.E. (2002). Contribution to antimitochondrial antibody production: cleavage of pyruvate dehydrogenase complex-E2 by apoptosis-related proteases. *Hepatology 35*, 14–22.

Miller, J.F., Kurts, C., Allison, J., Kosaka, H., Carbone, F., and Heath, W.R. (1998). Induction of peripheral CD8+ T-cell tolerance by cross-presentation of self antigens. *Immunol. Rev.* 165, 267–277.

Moudgil, K.D. and Sercarz, E.E. (1993). Dominant determinants in hen eggwhite lysozyme correspond to the cryptic determinants within its self-homologue mouse lysozyme: implications in shaping of the T-cell repertoire and autoimmunity. *J. Exp. Med. 178*, 2131–2138.

Nagaraju, K., Cox, A., Casciola-Rosen, L., and Rosen, A. (2001). Novel fragments of the Sjögren's syndrome autoantigens alpha-fodrin and

type 3 muscarinic acetylcholine receptor are generated during cytotoxic lymphocyte granule-induced cell death. *Arthritis Rheum. 44*, 2376–2386.

Norbury, C.C., Basta, S., Donohue, K.B., Tscharke, D.C., Princiotta, M.F., Berglund, P., Gibbs, J., Bennink, J.R., and Yewdell, J.W. (2004). CD8+ T cell cross-priming via transfer of proteasome substrates. *Science 304*, 1318–1321.

Nunez, G., Benedict, M.A., Hu, Y., and Inohara, N. (1998). Caspases: the proteases of the apoptotic pathway. *Oncogene 17*, 3237–3245.

Pinkoski, M.J., Waterhouse, N.J., Heibein, J.A., Wolf, B.B., Kuwana, T., Goldstein, J.C., Newmeyer, D.D., Bleackley, R.C., and Green, D.R. (2001). Granzyme B-mediated apoptosis proceeds predominantly through a Bcl-2-inhibitable mitochondrial pathway. *J. Biol. Chem. 276*, 12060–12067.

Radic, M.Z. and Weigert, M. (1995). Origins of anti-DNA antibodies and their implications for B-cell tolerance. *Ann. N. Y. Acad. Sci. 764*, 384–396.

Rogers, S.W., Andrews, P.I., Gahring, L.C., Whisenand, T., Cauley, K., Crain, B., Hughes, T.E., Heinemann, S.F., and McNamara, J.O. (1994). Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science 265*, 648–651.

Rosen, A. and Casciola-Rosen, L. (1999). Autoantigens as substrates for apoptotic proteases: Implications for the pathogenesis of systemic autoimmune disease. *Cell Death Differ.* 6, 6–12.

Rosen, A., Casciola-Rosen, L., and Ahearn, J. (1995). Novel packages of viral and self-antigens are generated during apoptosis. *J. Exp. Med. 181*, 1557–1561.

Roy, N., Deveraux, Q.L., Takahashi, R., Salvesen, G.S., and Reed, J.C. (1997). The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J. 16*, 6914–6925.

Russell, J.H. and Ley, T.J. (2002). Lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol. 20*, 323–370.

Rutjes, S.A., Utz, P.J., van der Heijden, A., Broekhuis, C., van Venrooij, W.J., and Pruijn, G.J. (1999). The La (SS-B) autoantigen, a key protein in RNA biogenesis, is dephosphorylated and cleaved early during apoptosis. *Cell Death Differ. 6*, 976–986.

Salemi, S., Caporossi, A.P., Boffa, L., Longobardi, M.G., and Barnaba, V. (1995). HIVgp120 activates autoreactive CD4-specific T cell responses by unveiling of hidden CD4 peptides during processing. *J. Exp. Med. 181*, 2253–2257.

Schachna, L., Wigley, F.M., Morris, S., Gelber, A.C., Rosen, A., and Casciola-Rosen, L. (2002). Recognition of Granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. *Arthritis Rheum. 46*, 1873–1884.

Schneider, S.C., Ohmen, J., Fosdick, L., Gladstone, B., Guo, J., Ametani, A., Sercarz, E.E., and Deng, H. (2000). Cutting edge: introduction of an endopeptidase cleavage motif into a determinant flanking region of hen egg lysozyme results in enhanced T cell determinant display. *J. Immunol. 165*, 20–23.

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*, 207–211.

Sercarz, E.E. (2002). Processing creates the self. *Nat. Immunol. 3*, 110–112.

Sercarz, E.E., Lehmann, P.V., Ametani, A., Benichou, G., Miller, A., and Moudgil, K. (1993). Dominance and crypticity of T cell antigenic determinants. *Annu. Rev. Immunol. 11*, 729–766.

Shresta, S., Heusel, J.W., Macivor, D.M., Wesselschmidt, R.L., Russell, J.H., and Ley, T.J. (1995). Granzyme B plays a critical role in cytotoxic lymphocyte-induced apoptosis. *Immunol. Rev. 146*, 211–221.

Siegel, R.M., Chan, F.K., Chun, H.J., and Lenardo, M.J. (2000). The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. *Nat. Immunol. 1*, 469–474.

Simitsek, P.D., Campbell, D.G., Lanzavecchia, A., Fairweather, N., and Watts, C. (1995). Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. *J. Exp. Med. 181*, 1957–1963.

Steinman, R.M. and Nussenzweig, M.C. (2002). Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA 99*, 351–358.

Streicher, H.Z., Berkower, I.J., Busch, M., Gurd, F.R.N., and Berzofsky, J.A. (1984). Antigen conformation determines processing requirements for T cell activation. *Proc. Natl. Acad. Sci.USA 81*, 6831–6835.

Surh, C.D. and Sprent, J. (1994). T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature 372*, 100–103.

Sutton, V.R., Davis, J.E., Cancilla, M., Johnstone, R.W., Ruefli, A.A., Sedelies, K., Browne, K.A., and Trapani, J.A. (2000). Initiation of apoptosis by granzyme B requires direct cleavage of bid, but not direct granzyme B-mediated caspase activation. *J. Exp. Med. 192*, 1403–1414.

Sutton, V.R., Wowk, M.E., Cancilla, M., and Trapani, J.A. (2003). Caspase activation by granzyme B is indirect, and caspase autoprocessing requires the release of proapoptotic mitochondrial factors. *Immunity 18*, 319–329.

Thomas, D.A., Du, C., Xu, M., Wang, X., and Ley, T.J. (2000). DFF45/ICAD can be directly processed by granzyme B during the induction of apoptosis. *Immunity 12*, 621–632.

Thornberry, N.A. and Lazebnik, Y. (1998). Caspases: Enemies within. *Science 281*, 1312–1316.

Tibbetts, M.D., Zheng, L., and Lenardo, M.J. (2003). The death effector domain protein family: regulators of cellular homeostasis. *Nat. Immunol. 4*, 404–409.

Trapani, J.A. and Sutton, V.R. (2003). Granzyme B: pro-apoptotic, antiviral and antitumor functions. *Curr. Opin. Immunol. 15*, 533–543.

Twyman, R.E., Gahring, L.C., Spiess, J., and Rogers, S.W. (1995). Glutamate receptor antibodies activate a subset of receptors and reveal an agonist binding site. *Neuron 14*, 755–762.

Ulanet, D.B., Torbenson, M., Dang, C.V., Casciola-Rosen, L., and Rosen, A. (2003). Unique conformation of cancer autoantigen B23 in hepatoma: a mechanism for specificity in the autoimmune response. *Proc. Natl. Acad. Sci. USA 100*, 12361–12366.

Utz, P.J. and Anderson, P. (1998). Posttranslational protein modifications, apoptosis, and the bypass of tolerance to autoantigens. *Arthritis Rheum. 41*, 1152–1160.

Utz, P.J., Hottelet, M., Le, T.M., Kim, S.J., Geiger, M.E., van Venrooij, W.J., and Anderson, P. (1998). The 72-kDa component of signal recognition particle is cleaved during apoptosis. *J. Biol. Chem. 273*, 35362–35370.

Utz, P.J., Gensler, T.J., and Anderson, P. (2000). Death, autoantigen modifications, and tolerance. *Arthritis Res. 2*, 101–114.

Voll, R.E., Herrmann, M., Roth, E.A., Stach, C., Kalden, J.R., and Girkontaite, I. (1997). Immunosuppressive effects of apoptotic cells. *Nature 390*, 350–351.

von Muhlen, C.A. and Tan, E.M. (1995). Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin. Arthritis Rheum. 24*, 323–358.

Watts, C. and Lanzavecchia, A. (1993). Suppressive effect of antibody on processing of T cell epitopes. *J. Exp. Med. 178*, 1459–1463.

Wolkers, M.C., Brouwenstijn, N., Bakker, A.H., Toebes, M., and Schumacher, T.N. (2004). Antigen bias in T cell cross-priming. *Science 304*, 1314–1317.

Zhang, J.Y., Chan, E.K., Peng, X.X., and Tan, E.M. (1999). A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. *J. Exp. Med. 189*, 1101–1110.

CHAPTER

16

Effector Mechanisms of Autoimmunity: Antibodies and Immune Complexes

LARS RÖNNBLOM*

**Department of Medical Sciences, Section of Rheumatology, Uppsala University*

GUNNAR V. ALM†

†Department of Molecular Biosciences, Section of Veterinary Immunology and Virology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Immune complex (IC) formation occurs in all immune responses associated with antibody production, as long as antigen is available. Such ICs principally occur in two forms, either as antibodies bound to antigens that are part of intact cells or tissues (including extracellular matrix), or as antibodies bound to antigens that are freely diffusible molecules or subcellular particles. The ICs are involved in many different diseases with very diverse clinical manifestations. This is due both to the many different actions by ICs and the actual constituents of the IC. In immune reactions against various types of microorganisms, formation of ICs is considered important in the pathogenesis of infections (Mims et al., 2001). In many acute infections, ICs contribute to inflammatory reactions, such as skin rashes and conjunctivitis, which subside as production of microbial antigens ceases and ICs are eliminated. In persistent infections, such as hepatitis C virus, with long-term production of antigens, ICs are continuously formed and may result in a chronic inflammatory disease (Trendelenburg and Schifferli, 2003).

In autoimmunity, antibodies to a vast number of autoantigens have been described, and moreover, many of these antigens are continuously available for binding autoantibodies. This sometimes results in a very high load of ICs in autoimmune rheumatic diseases, such as circulating ICs in systemic lupus erythematosus (SLE) (see Chapter 28) and ICs in the joint compartment of patients with rheumatoid arthritis (RA) (see Chapter 32; Jarvis et al., 1995). Such ICs contribute to clinical manifestations that resemble those in some infections, with IC deposits causing inflammatory changes in organs and tissues, e.g., vasculitis in blood vessels and glomerulonephritis in kidneys. It is, however, important to distinguish IC-mediated disease due to infections or to autoimmunity, not least when appropriate treatment for a certain condition is discussed.

The pathogenic effects of antibodies is often regarded in the context of the original Coombs and Gell classification of hypersensitivity reactions, where type II hypersensivity is due to antibodies binding to cells, tissues, and organs, while type III hypersensitivity is due to the effects of soluble ICs. In both cases antibody and ICs can be inflammatory and promote consumption and/or destruction of cells. However, the actual underlying mechanisms behind inflammation and tissue destruction are not always clear. Furthermore, it is less well recognized that the combination of antigens and antibodies, especially as soluble ICs, in addition can have profound immunoregulatory effects and in this way either suppress or promote immune responses.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

EFFECTS OF ANTIBODIES ON LIVING CELLS, TISSUES, AND ORGANS

Autoantibodies binding to cells can occasionally directly affect cell function and viability, for instance, if they are directed to molecules involved in Fas/CD95 activation or in ion transport (see Chapter 17). Because of the fluidity of cell membranes and the fact that many autoantibodies are directed to separate epitopes on cell-surface autoantigens, the latter can be cross-linked in cell membranes, resulting in clustering and capping of ICs (Latif et al., 2001; Kwiatkowska et al., 2003). The antibodies in such cell-associated ICs can in turn efficiently interact with Fc-receptors (FcRs) that are present on many effector cells in the immune system and can also activate the complement system. The principal modes of action of these antibodies are described in Figure 16.1.

The properties and functions of both the FcRs and complement receptors (CRs) have been extensively reviewed (Ravetch and Bolland, 2001; Hogarth, 2002; Takai, 2002; Monteiro and van de Winkel, 2003; Prodinger et al., 2003). Several human FcRs are present (Table 16.1), and of particular relevance here are the FcγR, that is FcγRI (CD64), FcγRIIa, b, and c (CD32), FcγRIIIa and b (CD16), as well as the FcεRI, FcεRII, FcαRI (CD89), and Fcα/μR. Table 16.1 also indicates the affinities of FcRs for immunoglobulin isotypes and subclasses and their expression on different types of cells. Other FcRs include the neonatal FcR (FcRn) that is involved in transport of immunoglobulins across the placenta. The FcRn is also present on endothelial cells and serves to prolong the lifespan of serum IgG (Ober et al., 2004). The poly-IgR transports IgA and IgM across intestinal epithelium (Monteiro and van de Winkel, 2003).

When the extracellular parts of the α chains of FcR bind to the Fc portions of immunoglobulin, cross-linking of FcR can occur and signals are then generated by activation of tyrosine kinases noncovalently interacting with intracellular FcR domains. In all FcRs, except the FcγRII, FcγRIIIb, FcRn, and poly-IgR, this is due to the activation of FcR-γ chains, present as disulfide-linked dimers. The γ chains contain the immunoreceptor tyrosine-based activation motif (ITAM), also present in several other proteins in the immune system that are associated with activation of cells, such as the B-cell receptor (BCR) for antigen (Billadeau and Leibson, 2002; Takai, 2002). The ITAM consists of a conserved amino acid sequence and is also present in the α chain of the single-chain FcγRIIa, an FcR present in man and other primates but not in mice. Upon FcR cross-linking ITAMs are tyrosine-phosphorylated by activation of Src-family kinases, creating docking sites for the SH2 domain

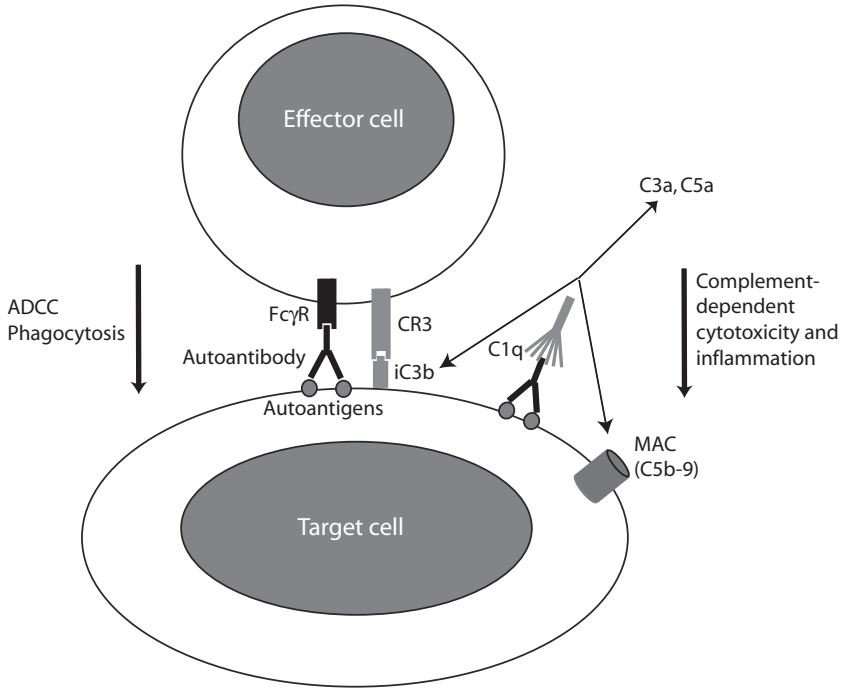


FIGURE 16.1 Basic mechanisms whereby autoantibodies bind to and damage cells or tissues. Autoantibodies bind to cell-surface antigens and activate the complement system, with deposition of iC3b, generation of the inflammatory split products C3a and C5a, and formation of the membrane attack complex (MAC). Effector cells, such as monocytes/macrophages, neutrophils, and natural killer cells use the FcγR to bind to IgG autoantibodies and the complement receptor 3 (CR3) to bind to iC3b on target cell membranes. This activates mechanisms involved in antibody-dependent cellular cytotoxicity (ADCC). Alternatively, target cells can be phagocytosed.

TABLE 16.1 Summary of human Fc receptors involved in inflammation and immunoregulation

Designation (CD number)	General structure	Affinity for immunoglobulin	Expression
FcγRI (CD64)	α,γ2 (ITAM)	Medium (10^7–10^9/M) IgG1, 3 > 4 > 2	Monocytes/macrophages, dendritic cells (DCs), mast cells, neutrophils (inducible)
FcγRIIa (CD32)*	α (ITAM)	Low (<10^7/M) IgG3 > 1 >> 2, 4	Platelets, myeloid cells, DCs, plasmacytoid DCs (PDCs), B cells (?)
FcγRIIb (CD32)*†	α (ITIM)	Low (<10^7/M) IgG3 > 1 > 4 > 2	B cells, mast cells, basophils, eosinophils, neutrophils, DCs, macrophages
FcγRIIc (CD32)	α (ITAM)	Low	Macrophages, natural killer (NK) cells, neutrophils
FcγRIIIa (CD16)*	α,γ2 (ITAM)	Medium (2×10^7/M) IgG1, 3 >> 2, 4	NK cells, macrophages, mast cells
FcγRIIIb (CD16)*	α (GPI)	Low (<10^7/M) IgG1, 3 >> 2, 4	Neutrophils, eosinophils
FcεRI	α,γ2,β (ITAM)	High (10^{10}/M) IgE	Monocytes, DCs, PDCs (no β chain). Mast cells, basophils, eosinophils
FcεRII (CD23)	Lectin-like trimer	Low (≤10^7/M) IgE	Ubiquitous, platelets
FcαRI (CD89)	α,γ2,β (ITAM)	Medium (2×10^7/M) IgA	Macrophages, neutrophils, eosinophils
Fcα/μR	α	Medium (10^8/M) IgA, IgM	Mesangial cells, B cells, macrophages
FcRn	α,β_2m	IgG	Placenta, endothelium, monocytes, intestine
Poly-IgR	α	High, transport of IgA dimers, IgM	Epithelium in intestine and lung, liver

GpI, Glycosylphosphatidylinositol-anchored; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif; β_2m, β_2-microglobulin; α,γ,β refer to the different chains; γ2 dimer of γ-chain.

*Allelic variants of FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb exist and differ in their expression and/or function (see text).

†Alternative splicing of FcγRIIb results in FcR isoforms that differ with respect to function and expression in different cell types.

of the cytosolic protein kinase Syk. The docking and activation of Syk kinases leads to the phosphorylation and recruitment of several intracellular proteins, which results in stimulation of, for instance, phospholipase Cγ, phosphatidylinositol 3-kinase (PI3K), the Ras pathway with activation of, for example, MAP kinase and GTPases of the Rac and Rho families (Takai, 2002). This results in activated functions in different cells, including phagocytosis, production of cytokines, an oxidative burst, degranulation of mast cells, and antibody-dependent cellular cytotoxicity (ADCC). This FcR-mediated activation can be achieved by antibodies attached to cell surfaces or by soluble ICs.

The activating effects of FcR can be inhibited by the FcγRIIb, which is present on B cells and most myeloid cells, but absent in natural killer (NK) cells, T cells, and plasmacytoid dendritic cells (PDCs) (Ravetch and Bolland, 2001; Båve et al., 2003). This is due to the presence of the immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic domain of this single-chain FcR. Upon activation by coaggregation with, for example, BCR or other FcR, Src-family kinases tyrosine-phosphorylate the ITIM, which results in recruitment of SH2-domain containing phosphatases, primarily protein tyrosine phosphatase (SHP1, SHP2) and the inositol polyphosphate 5′ phosphatase (SHIP). These enzymes, especially SHIP, inhibit the pathways triggered by activating receptors, preventing, for example, intracellular calcium mobilization, activation of MAPK, and antiapoptotic signals. In addition, homoaggregation of FcR produces ITIM-independent apoptotic signals. Thus, the FcγRIIb can downregulate the many effects caused by ITAM-containing FcR and other molecules, such as BCR (Ravetch and Bolland, 2001; Takai, 2002).

A number of functionally important polymorphisms have been described in the genes encoding the low-affinity FcRs in man (van Sorge et al., 2003; Reefman et al., 2003; Magnusson et al., 2004; Takai, 2005). The most investigated FcγRIIa polymorphism is a point mutation affecting the amino acid at position 131, coding for either arginine (R131) or histidine (H131). The FcγRIIa-131R has been shown to bind IgG in ICs less efficiently than the 131H genotype, especially IgG2. The FcγRIIIa polymorphism, which results in either valine (158V) or phenylalanine (158F) in amino acid position 158, alters the ligand affinity. Thus, the 158V genotype has higher affinity for IgG1 and IgG3 than does the 158F genotype. The lower-affinity FcRs allele 131R and 158F have both been associated with an

increased risk to develop systemic lupus erythematosus (SLE). At least three alleles of the FcγRIIIb gene have been identified, designated NA1, NA2, and SH. The NA2 allele appears to have lower capacity to mediate phagocytosis than NA1 and has been reported to be associated with SLE in Japanese and Spanish patients. Finally, polymorphisms occur in both the coding and promotor regions of the FcγRIIb gene, affect the function or expression of this important inhibitory FcR, and have been reported to be associated with SLE (Takai, 2005).

Whenever autoantibodies bind to cell-surface autoantigens, their Fc portions can interact with and activate FcR on effector cells, such as NK cells, neutrophils, eosinophils, and macrophages (Figure 16.1). All three FcγR types can be involved, but in many human *in vitro* experimental systems, FcγRIIIa, and to some extent FγRIIa, are important. The FcγRI, which has the highest binding affinity for IgG, seems to be less important, although it is expressed on monocytes/macrophages. It is poorly expressed on granulocytes, but the expression can be increased by exposure of cells to interferon (IFN)-γ (Pan et al., 1990). The affinities of FcR for IgG subclasses differ, the IgG1 and IgG3 showing higher affinity, while IgG2 and IgG4 have lower affinity. As part of activation via FcRs, the effector cells secrete a variety of mediators that differ depending on the type of cells, e.g., proinflammatory cytokines derived from monocytes/macrophages (see below) and cytotoxic molecules, such as perforin and granzyme B derived from NK cells (Russell and Ley, 2002; Lieberman, 2003; Wagner et al., 2004). At least NK cells also upregulate FasL, which can activate apoptosis in Fas-expressing target cells (Eischen et al., 1996). The killing of antibody-coated target cells by the different effector cells is termed antibody-dependent cellular cytotoxicity (ADCC). Its relative importance in autoimmunity *in vivo* needs to be clarified. Alternatively, cells coated by autoantibodies can be phagocytosed, which should be especially important with free cells, e.g. erythrocytes and leukocytes.

Depending on immunoglobulin isotype and IgG subclass, autoantibodies bound to cells may activate the classical pathway of the complement system (Figure 16.1) (Walport, 2001a; 2001b; Prodinger et al., 2003). Here, IgM, IgG1, and IgG3 are efficient activators, while IgG2 and IgG4 are poor activators. Cross-linking with resulting close proximity of antibody molecules is important both for FcR-mediated activation of, for example, phagocytes, and for initiation of complement activation upon binding of C1q to the antibodies. The ensuing activation of factors C4, C2, and C3 results in the formation of membrane attack complex (MAC) that consists of C5-9, resulting in some damage to target cells. The deposition of C3b and iC3b on cells sets them up for interactions with CR present on a variety of cells, including neutrophils and macrophages (Prodinger et al., 2003; Gelderman et al., 2004). Several membrane-bound complement regulatory proteins (CD46, CD55, and CD59) and soluble proteins inhibit activation and deposition of complement factors on mammalian cells, thus reducing complement-mediated cytotoxicity. However, the interaction between iC3b deposited on target cells and CR3 on effector cells can enhance ADCC, although not causing cytotoxicity alone (van Spriel et al., 2001; Gelderman et al., 2004; Salio et al., 2004). Furthermore, activation of the complement system results in the inflammatory split products C3a and C5a that act via the complement receptors C3aR and C5aR, present on many different types of cells (Drouin et al., 2001; Prodinger et al., 2003).

Many autoantibodies are directed against intracellular antigens, in which case they may be incapable of binding to living cells. There are exceptions to this rule, because antibodies to DNA can penetrate cells and even enter the nuclei, where they might affect cellular functions (Portales-Perez et al., 1998; Ruiz-Arguelles et al., 2003). The situation is different with cells dying by apoptosis, because several important intracellular autoantigens (e.g., RNA- and DNA-binding proteins, calreticulin, heat-shock proteins) are concentrated in apoptotic blebs and/or are exposed on the cell surface (Casciola-Rosen et al., 1994; Rosen and Casciola-Rosen, 1999). To some extent these autoantigens are modified during the apoptotic process and autoantibodies can recognize such modifications (see Chapter 15).

FORMATION AND PROPERTIES OF SOLUBLE IMMUNE COMPLEXES

Many of the effects of soluble ICs in autoimmune diseases are due to mechanisms that are also involved in the direct effects of autoantibodies on cells. However, ICs present many unique features with regard to their immunoregulatory and pathogenic effects. The properties of ICs, including lattice size, depend on the relative concentrations and net charge of antigen and antibodies, the antibody affinity, and immunoglobulin isotype and subclass, as well as on the size and number of epitopes of the antigen (Doekes et al., 1984; Jarvis et al., 1995; 1999; Voice and Lachmann, 1997). Obviously, IgM theoretically has the possibility to bind 10 antigen epitopes, secretory IgA binds four epitopes, while, for instance, IgG only binds two epitopes. However, IgG3 has been reported to self-associate and this might increase the propensity to form ICs (Panka, 1997). Antigen excess in combination with high concentrations of high-affinity antibodies may result in the formation of very large ICs. Such ICs are more easily trapped, or even precipitate, in tissues. Furthermore, ICs that contain anti-DNA antibodies with cationic charge and DNA have much increased propensity to bind to the polyanionic parts of glomerular basal membranes, and are therefore more prone to cause nephritis (Mohan and Datta, 1995). A special form

of IC is the cryoglobulins, which are immunoglobulins precipitating at a temperature below 37°C. Such ICs are classified into types I, II or III, and usually contain IgM. The IgM in cryoglobulinemia type II and III show rheumatoid factor activity and are also in complex with IgG. Their existence is associated with malignant, infectious, and autoimmune diseases (Dammacco et al., 2001; see Chapter 65).

The biologic activity of ICs is, however, a much more complex issue, and to a large extent related to the ability of IC-associated antibodies to interact with FcR and also to activate the complement system. Also important is the intrinsic ability of the antigen in ICs to interact with cellular receptors, such as Toll-like receptors (TLRs) (Takeda and Akira, 2004). The principal functional parts of ICs are shown in Figure 16.2A. The fact that the interaction between TLR and soluble IC containing DNA or RNA actually occurs in an endosomal/phagosomal compartment in B cells (Figure 16.2B) and in PDCs (Figure 16.2C) is discussed below.

The major immunoglobulin isotype in soluble ICs is IgG and the FcγRI (CD64), II (CD32), and III (CD16) are therefore important for the interaction of ICs with different cells, resulting in activation or inhibition of various cell functions. The low affinity of FcγRII and FcγRIII for Fc-portions of IgG requires the multiple Fc portions present in IC for binding. Thus, only IC can interact with these FcRs, which are expressed on many different cell types (Table 16.1), ensuring that only physiologically relevant ICs, and not monomeric immunoglobulin, activate the effector responses. The FcγRI has in contrast a high affinity for the Fc portions of IgG and can therefore bind solitary immunoglobulin molecules, but such FcRs must still be cross-linked in order to become activated. Antibodies in ICs can activate the classical pathway of the complement system, and IgM, IgG1, and IgG3 are efficient at this, as mentioned above. However, IgG2 also can activate the complement system at higher epitope density; both this subclass and IgA can activate the alternative complement pathway, and IgA can activate the lectin pathway (Endo et al., 1998; Roos et al., 2001). Therefore, complement components C1q, C4b, C3b, C3d, and iC3b are usually present in ICs and are functionally relevant in several ways, via interactions with the complement receptors C1qR and CR1-4 (Prodinger

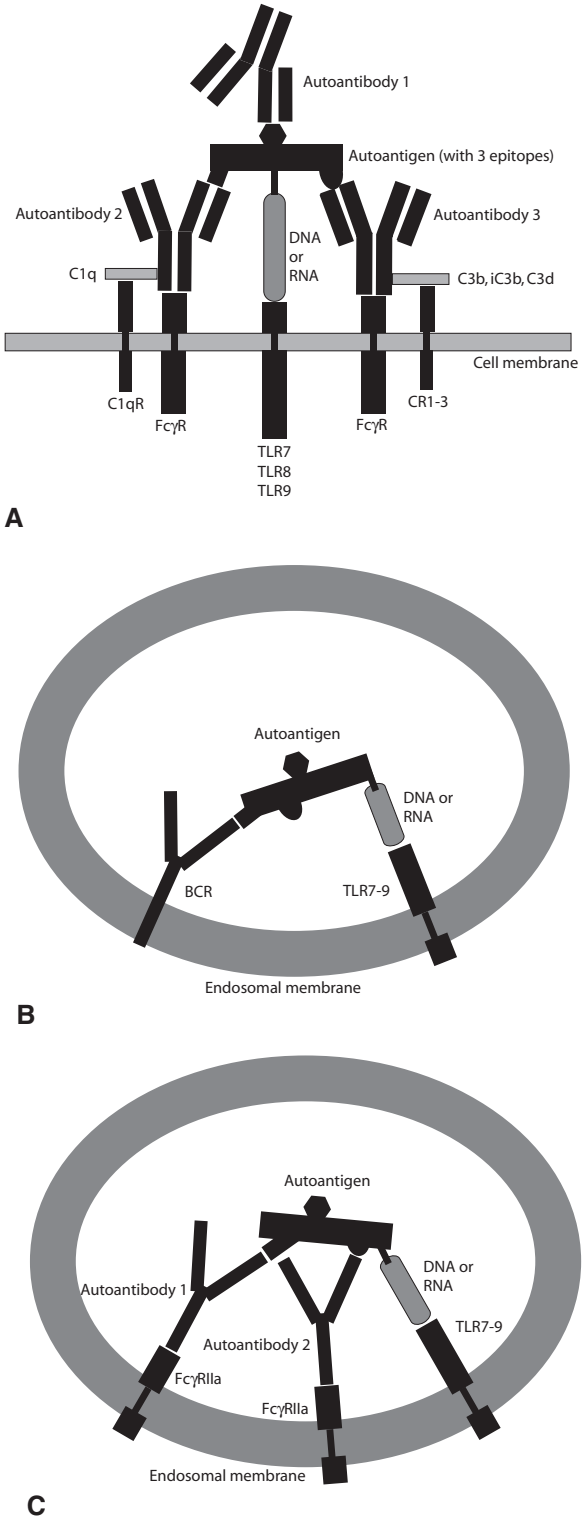


FIGURE 16.2 Composition of immune complexes (ICs) and their interaction with cellular receptors. *A*, Principal components of ICs are autoantigens, autoantibodies (e.g., IgG isotype) that bind to Fc-receptors (e.g., FcγR), as well as complement components (e.g., C1q, C3, iC3b and C3d) that bind to complement receptors (CR). Autoantigens can contain biologically active components, such as DNA or RNA that bind and activate Toll-like receptors (e.g., TLR7-9). *B*, In B cells, autoantigens that contain DNA or possibly RNA can be internalized by binding to the B-cell receptor (BCR) for antigen and then activate TLR7-9. *C*, In immature plasmacytoid dendritic cells (PDCs), also termed natural type I interferon-producing cells (NIPCs), ICs consisting of autoantibodies (IgG isotype) and RNA/DNA-containing autoantigens are internalized by the FcγRIIa and then activate TLR7-9. The DNA/RNA and TLR interact in an intracellular endoplasmatic reticulum–endosomal/phagosomal compartment.

et al., 2003). When ICs activate the complement system via the classical pathway, the split products C3a and C5a form and are highly inflammatory (Baumann et al., 2001).

Antigen can contribute to the biologic activity of ICs in other ways than influencing IC size, charge, and number of available Fc portions and complement factors. Thus, portions of the antigen can directly interact with receptors on cells, including the TLR1-10, scavenger receptors, and macrophage mannose receptors (Gordon, 2002). For instance, when ICs contain DNA and DNA-binding proteins (even nucleosomes) or RNA and RNA-binding proteins, the nucleic acids can interact with TLRs (see below) (Akira and Hemmi, 2003; Takeda and Akira, 2004). Furthermore, proteins that include calreticulins, heat-shock proteins (hsp), and high-mobility group 1 protein (HMGB1) may interact with yet other receptors, e.g., HMGB1 with RAGE (Huttunen and Rauvala, 2004), hsp with CD91 and TLR2/4 (Akira and Hemmi, 2003), and calreticulin with scavenger receptor-A (Berwin et al., 2003). Consequently, an IC can bind and activate different receptors on cells, e.g. FcR, CR, and TLR, resulting in responses that vary depending on the particular cell types and receptors engaged.

ELIMINATION OF IMMUNE COMPLEXES

The inflammatory and immunostimulatory effects of ICs (see below) are in part inhibited by systems that eliminate ICs (Prodinger et al., 2003). Activation of the complement system with deposition of C3b in ICs prevents precipitation and maintains ICs in a soluble form. In primates, such ICs can bind to the CR1 on erythrocytes and lysis of these cells is prevented because of the presence of the complement regulatory protein CD59. The ICs are then transported to the liver and spleen especially where they are released by the degradation of C3b to iC3b and C3dg. The ICs subsequently bind to CR3, CR4, and FcγRI-III on macrophages, are endocytosed, and finally degraded. In the autoimmune disease SLE, this function may be deficient (Manderson et al., 2004). The low levels of CR1 and complement components seen in SLE especially is probably secondary to the great load of circulating ICs in this disease, but may still be of pathogenic significance (Sturfelt et al., 2000). It is interesting that there is a striking increase in the incidence of SLE in individuals with hereditary deficiencies in the early complement components C1, C4, and C2 (Manderson et al., 2004). This may be due to decreased elimination of immunostimulatory ICs (see below), but the early components of the complement system, especially C1q, are also involved in the elimination of apoptotic and perhaps also necrotic cells. A reduced clearance of dead cells therefore increases the amount of autoantigens that are available for autoimmunization and IC formation.

A special situation pertains with regard to IgA-containing ICs, which can be transported across, for example, intestinal epithelia by the poly-IgR. This may be one way of eliminating antigens that otherwise may cause local inflammation in the intestine and immunization of the gut-associated lymphoid tissue especially (Monteiro and van de Winkel, 2003). Also the FcRn has functions that suggest involvement in some autoimmune diseases. This FcR prolongs the lifespan of serum IgG by preventing its uptake and degradation in endothelial and perhaps other cells (Ober et al., 2004). In a murine autoimmune arthritis model, the presence of FcRn afforded partial or complete protection against disease (Akilesh et al., 2004). The relation of the FcRn to ICs of different types remains to be evaluated.

INFLAMMATORY RESPONSES CAUSED BY IMMUNE COMPLEXES

The classical Arthus reaction was described as a dermal inflammatory reaction due to local IC formation after repeated subcutaneous or intradermal injections of foreign antigens. It has served as a model for IC-induced inflammation and the mechanism has been thought to involve activation of the complement system. Recent data indicate that, at least in mice, complement plays no major part. Instead, activation of FcγRIII by ICs is the essential trigger of the Arthus reaction, while activation of FcγRIIb is inhibitory (Ierino et al., 1993; Ravetch and Clynes, 1998; Ravetch, 2002). The complement system may still play an important accessory role, because the inflammatory complement component C5a acts by upregulating FcγRIII (Shushakova et al., 2002). In humans, FcγRIIa is also relevant in IC-induced inflammation (Tan Sardjono et al., 2003; Hart et al., 2004). This activating FcγR is lacking in mice and is expressed on many cells in the human immune system, including monocytes/macrophages, DCs, neutrophils, and thrombocytes, but not clearly on T and B cells. Importantly, mice transgenic for the human FcγRIIa can be made to display an immune thrombocytopenia that resembles that in man (McKenzie et al., 1999) and furthermore develop autoimmune diseases resembling human rheumatoid arthritis and SLE (Tan Sardjono et al., 2003).

ICs in the circulation, or formed in tissues, activate many key cells in inflammatory processes (Figure 16.3). Endothelial cells express FcγRIIa and become activated by ICs to increase expression of cell-adhesion molecules, such as VCAM-1 and E-selectin, and to produce several chemokines (Groger et al., 1996; Norman et al., 2003; Christopherson and Hromas, 2004). Thrombocytes can be directly activated via FcγRIIa, resulting in their aggregation and adherence to the blood vessel wall, blood coagulation, and endothelial cell proliferation. Granulocytes and monocytes/macrophages express all three FcγR and can, via FcγRIIa or

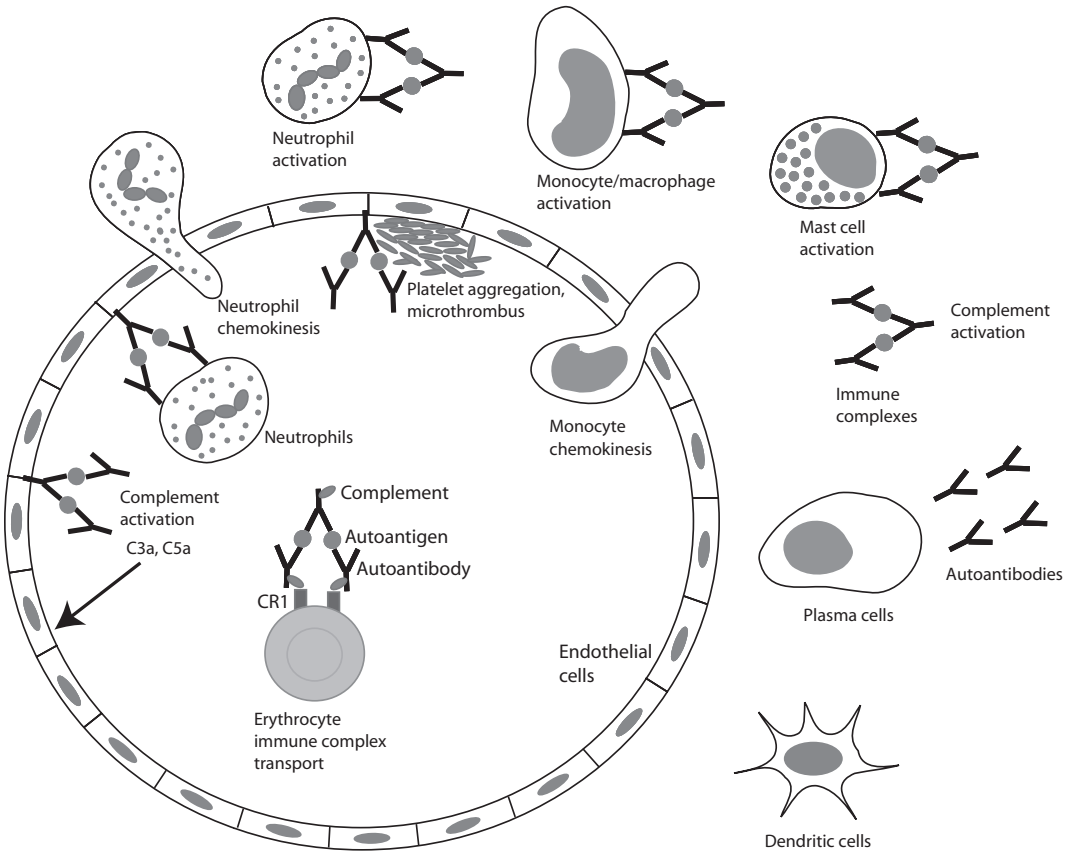


FIGURE 16.3 Inflammation in blood vessels and tissues caused by immune complexes (ICs). The ICs can activate the complement system, resulting in production of inflammatory C3a and C5a. The ICs can cause aggregation of platelets on the endothelium and also trigger release of inflammatory and cytotoxic mediators from indicated effector cells (e.g., neutrophils, monocytes/macrophages and mast cells), as well as endothelial cells. Migration of blood leukocytes to tissues occurs by chemokinesis, governed by induced expression of chemokines and cell-adhesion molecules. Migrating cells include B cells that, as plasma cells, produce autoantibodies, resulting in local IC formation, and dendritic cells that can be loaded with autoantigens and aid in the propagation of the autoimmune response.

FcγRIIIa, be activated by ICs to produce a wide range of proinflammatory cytokines and chemokines (see below). Other cells that are involved in IC-mediated inflammation are mast cells and basophilic granulocytes that, besides being triggered by antigen via cross-linking of the FcεRI-associated IgE, also can become activated by IgG-containing ICs via FcγRI or FcγRIIa (Takai, 2002; Tkaczyk et al., 2004). Inflammatory cytokines and chemokines, and expression of cell adhesion molecules on endothelial cells and leukocytes, result in adhesion of the latter to blood vessel walls and migration into tissues. Besides the cell types mentioned so far, B and T cells, as well as type 1 DCs (e.g., monocyte-derived DCs) and PDCs, express different FcRs and are involved in the inflammatory process. B cells mature into autoantibody-forming plasma cells that can give rise to local formation of ICs that sustain the inflammatory process. Thus, all cells that are considered important in the development of inflammatory changes in blood vessels and tissues can interact with ICs and become activated in various ways.

IMMUNOINHIBITORY EFFECTS OF IMMUNE COMPLEXES

Antibodies can regulate feedback their production, being either suppressive or enhancing. A wealth of experimental data, especially in mice, have revealed suppressions of more than 99% and enhancements of more than 100-fold (Heyman, 2000; 2003). In both cases, IC formation appears to be a prerequisite for these effects. Suppression of immune responses to particulate antigens, often erythrocytes, has primarily been seen with IgG antibodies, although IgM and IgE antibodies can also suppress. Attempts to demonstrate involvement of, for example, FcγR have failed and led to the conclusion that the suppressive effects of antibodies are due

to masking of antigens. The situation appears different with ICs containing soluble antigens. It seems that such ICs normally enhance antibody responses, but there is a simultaneous regulation of negative feedback that tends to restrict the enhancement and which involves the FcγRIIb. Thus, greatly increased antibody titers were seen in FcγRIIb knock-out mice after injection with IgG1-, IgG2a- or IgG2b-complexed antigens (Wernersson et al., 1999). This FcR is inhibitory in nature via its content of ITIM; is expressed on B cells, for example; and inhibits BCR activation when cross-linked with the BCR (Ravetch and Bolland, 2001). The FcγRIIb is also expressed on many other cells, including DCs and mast cells, and can inhibit the functions of such cells in immune responses (Kepley et al., 2003; 2004; Tkaczyk et al., 2004). The observation that FcγRIIb knock-out mice develop autoimmune diseases supports the contention that ICs interacting with this FcR are involved in the inhibition of autoimmune responses *in vivo* (Bolland and Ravetch, 2000).

Another way by which ICs can inhibit immune responses is via induction of interleukin (IL)-10 (see below). This cytokine has potent inhibitory effects on the production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, and immunostimulatory cytokines, such as IL-12 and IFN-α (Moore et al., 2001). For instance, experimental IC-induced pulmonary disease in rats is aggravated upon neutralization of endogenous IL-10 (Shanley et al., 1995).

IMMUNOSTIMULATORY EFFECTS OF IMMUNE COMPLEXES

Immunostimulatory ICs can have an important role in the normal immunizing process, because antibodies appear sufficiently early in the local environment of the lymphoid organs to have a major effect on the early immune response to exogenous antigens. In fact, preformed natural antibodies may be important for the initiation of immune responses in mice (Diaz de Stahl et al., 2003; Zinkernagel, 2003). Classical complement pathway activation and FcRs are also important for normal primary immune responses, supporting a crucial role for ICs (Diaz de Stahl et al., 2003). Against this background, it is likely that ICs should also have a major impact on the immunization against autoantigens, and consequently in autoimmune diseases. Regarding possible mechanisms for this, the IC-mediated enhancement of antibody production is dependent on FcγR with respect to murine IgG1 and IgG2, and CR for IgG3 and IgM (Heyman, 2003). On the other hand, the immunostimulatory effect of IgE is due to the low-affinity FcεRII (Getahun and Heyman, 2004).

ICs consisting of aggregated immunoglobulin, or ICs containing antibodies and proteins such as ovalbumin (OVA), have at least two major effects on monocytes and DCs. They can trigger production of cytokines, such as IL-10, IL-6, and TNF-α, by monocytes/macrophages and by monocyte-derived DCs (Rönnelid et al., 2003; Radstake et al., 2004). The IC-activated DCs produce IL-10 but not IL-12, despite findings that ICs induce DC maturation (Kalergis and Ravetch, 2002; Radstake et al., 2004). Consequently, ICs have been suggested to promote type 2 helper T (Th2)-cell responses and even tolerance. A further important property of ICs is their ability to enhance uptake of exogenous antigens for presentation not only by major histocompatibility complex (MHC) class II molecules, but also MHC class I molecules (Villinger et al., 2003; Gil-Torregrosa et al., 2004). Maturation of DCs, together with MHC class I- and II-associated presentation of antigen, sets the stage for activation of Th1 cells and cytotoxic T lymphocytes (CTLs). However, this requires costimulation by appropriate cytokines, such as IL-12 and/or IFN-α, the production of which is not readily triggered by simple ICs.

Another mechanism of action of ICs is their ability to activate B cells via their content of C3b/d and antigen (Carter and Barrington, 2004). This is achieved by cross-linking the BCR and the B-cell coreceptor CD21(=CR2)–CD19 complex, resulting in decrease in the threshold for B-cell activation of more than 1000-fold. This function of ICs is at least in part exerted by follicular DCs (FDCs) that reside in the primary and secondary follicles (germinal centers) in lymphoid organs (Haberman and Shlomchik, 2003; Kosco-Vilbois, 2003). The FDCs trap ICs via FcγRII and CR1/CR2, sequester antigen in the form of ICs for long time periods, and present antigen to B cells that have hypermutated and switched class. Recognition of the antigen has been proposed to prevent apoptosis of B cells and to favor survival and selection of memory B cells and pre-plasma cells that produce antibodies with higher affinity for antigen. This dogma has, however, been questioned, primarily because affinity maturation of B cells and memory development proceeds in the absence of FDCs in certain murine experimental systems.

FUNCTION OF IMMUNE COMPLEXES CONTAINING DNA OR RNA

Nucleic acids, DNA or RNA, as part of autoantigens or ICs may be important in the etiology and pathogenesis of autoimmune diseases, because they can activate key cells in the immune system, in particular B cells (Leadbetter et al., 2002; Viglianti et al., 2003) and immature PDCs, also termed natural IFN-producing cells (NIPC) (Vallin et al., 1999a; 1999b). In order to be activating, the DNA usually needs to contain nonmethylated CpG motifs in a certain base context (Krieg, 2002), while the requirements for RNA are less well studied, but may be dependent on GU-rich single-stranded RNA (Diebold et al., 2004; Heil et al., 2004). The

RNA probably activates cells via binding to TLR7 and TLR8, and the DNA activates via TLR9. However, these TLRs are intracellular in resting cells, and the RNA or DNA must therefore enter cells in order to interact with them (Latz et al., 2004). B cells can internalize nucleic acid complexed with proteins via BCRs that recognize antigenic epitopes on the complex (Figure 16.2B). The NIPCs/PDCs can internalize ICs containing nucleic acids via interaction with FcγRIIa (Figure 16.2C) (Båve et al., 2003). In fact, TLR7-9 appears to react with DNA and RNA in the same endoplasmic reticulum–endosomal/phagosomal compartment that is used to load MHC class I molecules with peptides of an external antigen (i.e., cross-presentation) in DCs (Latz et al., 2004; Gil-Torregrosa et al., 2004).

The findings that proliferation and immunoglobulin secretion in B cells can be activated via dual engagement of BCR and TLR9, either by ICs that contain chromatin or CpG-rich dsDNA, has implications for autoimmunity (Leadbetter et al., 2002; Viglianti et al., 2003). Thus, any autoantigen that associates either with DNA that can activate TLR9, or with RNA that activates TLR7/TLR8, may stimulate production of autoantibodies with specificity for proteins or for associated DNA or RNA. Such autoantibodies are in fact common, especially in systemic autoimmune diseases. This T-cell-independent activation of B cells by means of BCR and TLR9 presents an obvious danger, giving rise to potentially pathogenic autoantibodies, and should be efficiently controlled in order to maintain self-tolerance. One such controlling mechanism may involve prevention of the response to antigen and CpG-DNA in anergic B cells by uncoupling the BCR-activated calcineurin-dependent NFκB pathway, which inhibits their proliferation, as well as by continuous stimulation of the ERK MAP kinase pathway, which inhibits their differentiation to plasma cells (Rui et al., 2003).

With regard to the NIPCs/PDCs, the activation of TLR7-9 triggers the immediate production of cytokines and the further maturation of these immature cells into efficient antigen-presenting cells (APCs) (Coccia et al., 2004; Salio et al., 2004). The NIPCs/PDCs are the principal and extremely efficient producers of type I IFN (IFN-α, -β, -ω, -ε, and -κ), the 12 different IFN-α subtypes being dominant (Fitzgerald-Bocarsly, 2002; Izaguirre et al., 2003). Besides IFN-α, NIPCs/PDCs produce other cytokines under certain conditions, including IL-6, IL-12, IP-10, MIP-1α, and IL-8. During viral infections, the produced IFN-α directly inhibits virus replication as part of the innate immunity, but also promotes development of adaptive antiviral immunity and sometimes also autoimmunity with production of autoantibodies (Hunziker et al., 2003). In recent years, IFN-α has been shown to have a variety of actions on cells in the immune system that can be important in the promotion of autoimmunity, e.g., inducing maturation of potent monocyte-derived DCs; promoting survival, proliferation, and CD40-independent immunoglobulin class switching in B cells; as well as preventing apoptosis and stimulating development of Th1 cells and cytotoxic T cells (Rönnblom et al., 2003). Autoantibodies with specificity for DNA or RNA or associated proteins can form IFN-inducing (interferogenic) ICs when combined with DNA/RNA-containing material released by apoptotic or necrotic cells (Lövgren et al., 2004), and act as endogenous IFN-α inducers that can result in continued triggering of the IFN-α production in NIPCs/PDCs. In fact, small DNA-containing ICs with the capacity to induce IFN-α production in NIPCs/PDCs have been identified in the circulation of SLE patients (Vallin et al., 1999a).

Several findings support the view that ongoing IFN-α production driven by interferogenic ICs may have a pivotal role in the etiology and pathogenesis of some autoimmune diseases (Rönnblom and Alm, 2001; Rönnblom et al., 2003). The latter include SLE, Sjögren syndrome, dermatomyositis, type 1 diabetes, and psoriasis, which all have an activated type I IFN system, and this, at least in SLE, correlates to disease activity and severity. The possible etiopathogenic role for DNA/RNA-containing ICs in such autoimmune diseases, notably SLE, is outlined in Figure 16.4. Initially, autoantibodies to antigens that contain RNA or DNA are produced, perhaps as a result of a viral infection. These autoantibodies then form ICs that serve as endogenous IFN-α inducers, activating NIPCs/PDCs via FcγRIIa and TLR. The IFN-α produced stimulates the immune system as outlined above, so as to promote an autoimmune response. This action of IFN-α is made more efficient by the fact that presentation of antigens and production of type I IFNs occur in DCs that are similar, if not identical, and located primarily in the lymphoid organs. B cells are activated by T cells, but can also be stimulated directly by chromatin–IgG complexes, which will favor production of antibodies that form IFN-α-inducing ICs. A process that has the features of a vicious circle is thus established, which maintains the autoimmune process by continuously exposing the immune system to endogenous IFN-α inducers and to IFN-α produced by the NIPCs/PDCs (Rönnblom and Alm, 2001; Rönnblom et al., 2003). This process is facilitated by increased apoptosis and reduced clearance of apoptotic material in SLE. Antibodies against autoantigens that are not associated with material containing nucleic acids are expected to occur with time.

IMMUNE COMPLEXES AND DISEASES

The prototypic autoimmune IC-mediated disease is SLE (see Chapters 27 and 28), but ICs are also involved in several other rheumatic diseases. Among the most common is RA (see Chapter 32), where IC formation can occur both in

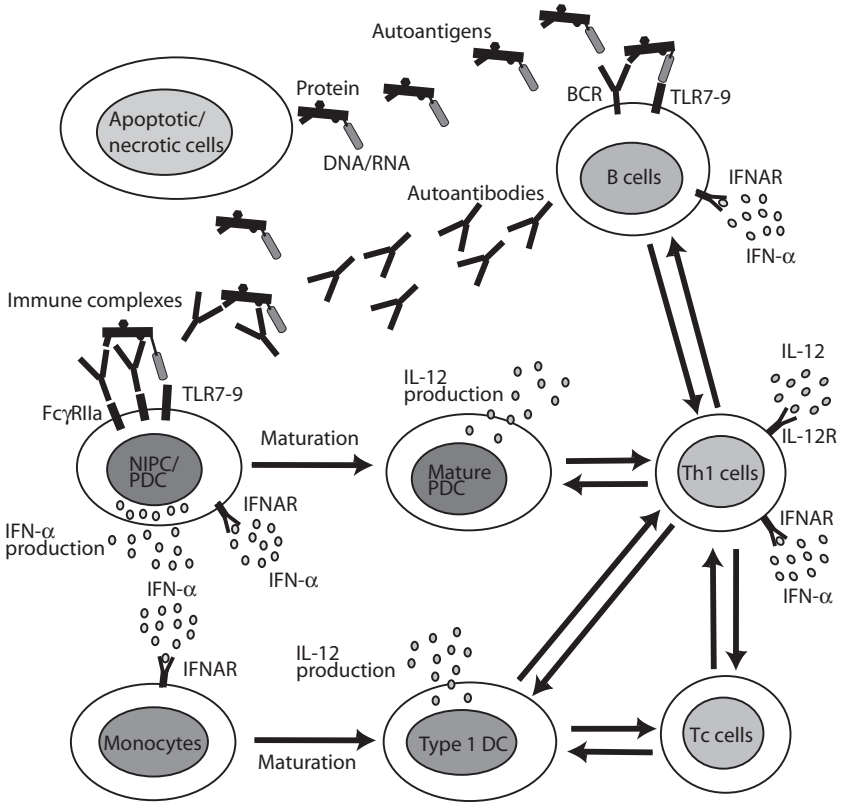


FIGURE 16.4 Role of DNA/RNA-containing immune complexes (ICs) in the induction and maintenance of autoimmune disease. Apoptotic or necrotic cells release DNA- or RNA-containing autoantigens that can activate IgG autoantibody-producing B cells. Such autoantibodies can occur during viral infections, due to stimulatory effects on B cells and induced type I interferon (IFN) production in immature plasmacytoid dendritic cells (NIPCs/PDCs). Autoantibodies and DNA/RNA-containing autoantigens form ICs that act as endogenous inducers of type I IFN production in NIPCs/PDCs. The type I IFN, especially IFN-α, has multiple immunostimulatory actions that include induced maturation of monocyte-derived DCs (type 1 DCs), development of type 1 helper T (Th1) cells and cytotoxic T (Tc) cells, as well as facilitation of B-cell activation. The resulting further autoantibody production causes formation of more IFN-inducing ICs, IFN-α production, and IFN-mediated immunostimulation. The autoimmune process is consequently sustained by a mechanism with the features of a vicious circle. IFNAR, denotes the type I IFN receptor.

tissues (Jarvis et al., 1995) and in the circulation, the latter especially in conjunction with extra-articular disease (Zlabinger et al., 1990). Several of the vasculitides affecting small vessels are IC mediated, and among these are Henöch-Schönlein purpura, cryoglobulinemic vasculitis, and polyarteritis nodosa (see Chapter 65). It is also important to note that circulating ICs may not only cause dramatic diseases such as vasculitis, but may contribute to such a common disease as atherosclerosis (see Chapters 64 and 71). Here, ICs that contain antibodies against low-density lipoprotein (LDL) and oxLDL can trigger cholesterol ester accumulation in macrophages, activation of these cells, inflammation, and plaque formation (Virella et al., 2002; Wick et al., 2004). In several organ-specific autoimmune diseases, ICs can be detected in affected tissues and/or in the circulation. Among these are type 1 diabetes mellitus (Ahmed et al., 1999), Hashimoto's thyroiditis (Nielsen et al., 2004), and pemphigus vulgaris (Wang et al., 2004). The exact role of the ICs in the disease process is unclear, but once formed they may play an important role in the perpetuation of the autoimmune reaction, as discussed above. Consequently, several procedures aimed at reducing the levels of circulating ICs or their biologic effects have been explored. Plasmapheresis or plasma exchange has thus been used for many years with some benefit mainly in severe vasculitis (Braun-Moscovici and Furst, 2003b). To more specifically remove IgG-containing ICs, a staphylococcus protein A column has been developed and used, for example, in severe refractory RA (Kunkel et al., 2002; Braun-Moscovici and Furst, 2003b) and a C1q column in SLE (Hiepe et al., 1999). Furthermore, high-dose intravenous IgG treatment can inhibit FcR-mediated effects by ICs, and has been used in several autoimmune diseases, such as autoimmune thrombocytopenic purpura, vasculitis, and SLE with glomeru-

lonephritis (Braun-Moscovici and Furst, 2003a). In future, more specific inhibitors of IC actions and efficient methods to eliminate detrimental ICs can be anticipated.

Acknowledgments

We thank Professor Birgitta Heyman and Dr Johan Rönnelid for reviewing the manuscript and providing many good suggestions. This work was supported by grants from the Swedish Research Council, the Swedish Rheumatism Foundation, the King Gustav V 80-year Foundation and the Uppsala University Hospital Research and Development Fund.

References

Ahmed, E., Nityanand, S., Mustafa, A., Brismar, K., and Lefvert, A.K. (1999). Anti-cardiolipin antibodies and circulating immune complexes in type 1 diabetes mellitus: Increased prevalence and relation to vascular complications. *Clin. Exp. Immunol. 115*, 255–259.

Akilesh, S., Petkova, S., Sproule, T.J., Shaffer, D.J., Christianson, G.J., and Roopenian, D. (2004). The MHC class I-like Fc receptor promotes humorally mediated autoimmune disease. *J. Clin. Invest. 113*, 1328–1333.

Akira, S. and Hemmi, H. (2003). Recognition of pathogen-associated molecular patterns by TLR family. *Immunol. Lett. 85*, 85–95.

Baumann, U., Chouchakova, N., Gewecke, B., Kohl, J., Carroll, M.C., Schmidt, R.E., and Gessner, J.E. (2001). Distinct tissue site-specific requirements of mast cells and complement components C3/C5a receptor in IgG immune complex-induced injury of skin and lung. *J. Immunol. 167*, 1022–1027.

Båve, U., Magnusson, M., Eloranta, M.L., Perers, A., Alm, G.V., and Rönnblom, L. (2003). Fc gamma RIIa is expressed on natural IFN-α-producing cells (plasmacytoid dendritic cells) and is required for the IFN-α production induced by apoptotic cells combined with lupus IgG. *J. Immunol. 171*, 3296–3302.

Berwin, B., Hart, J.P., Rice, S., Gass, C., Pizzo, S.V., Post, S.R., and Nicchitta, C.V. (2003). Scavenger receptor-a mediates Gp96/Grp94 and calreticulin internalization by antigen-presenting cells. *EMBO J. 22*, 6127–6136.

Billadeau, D.D. and Leibson, P.J. (2002). ITAMs versus ITIMs: Striking a balance during cell regulation. *J. Clin. Invest. 109*, 161–168.

Bolland, S. and Ravetch, J.V. (2000). Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. *Immunity 13*, 277–285.

Braun-Moscovici, Y. and Furst, D.E. (2003a). Immunoglobulin for rheumatic diseases in the twenty-first century: Take it or leave it? *Curr. Opin. Rheumatol. 15*, 237–245.

Braun-Moscovici, Y. and Furst, D.E. (2003b). Plasmapheresis for rheumatic diseases in the twenty-first century: take it or leave it? *Curr. Opin. Rheumatol. 15*, 197–204.

Carter, R.H. and Barrington, R.A. (2004). Signaling by the CD19/CD21 complex on B cells. *Curr. Dir. Autoimmun. 7*, 4–32.

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.

Christopherson, K.W., 2nd and Hromas, R.A. (2004). Endothelial chemokines in autoimmune disease. *Curr. Pharm. Des. 10*, 145–154.

Coccia, E.M., Severa, M., Giacomini, E., Monneron, D., Remoli, M.E., Julkunen, I., Cella, M., Lande, R., and Uze, G. (2004). Viral infection and toll-like receptor agonists induce a differential expression of Type I and λ interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur. J. Immunol. 34*, 796–805.

Dammacco, F., Sansonno, D., Piccoli, C., Tucci, F.A., and Racanelli, V. (2001). The cryoglobulins: an overview. *Eur. J. Clin. Invest. 31*, 628–638.

Diaz de Stahl, T., Dahlstrom, J., Carroll, M.C., and Heyman, B. (2003). A role for complement in feedback enhancement of antibody responses by IgG3. *J. Exp. Med. 197*, 1183–1190.

Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., and Reis e Sousa, C. (2004). Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science 303*, 1529–1531.

Doekes, G., van Es, L.A., and Daha, M.R. (1984). Binding and activation of the first complement component by soluble immune complexes: Effect of complex size and composition. *Scand. J. Immunol. 19*, 99–110.

Drouin, S.M., Kildsgaard, J., Haviland, J., Zabner, J., Jia, H.P., McCray, P.B., Jr., Tack, B.F., and Wetsel, R.A. (2001). Expression of the complement anaphylatoxin C3a and C5a receptors on bronchial epithelial and smooth muscle cells in models of sepsis and asthma. *J. Immunol. 166*, 2025–2032.

Eischen, C.M., Schilling, J.D., Lynch, D.H., Krammer, P.H., and Leibson, P.J. (1996). Fc receptor-induced expression of Fas ligand on activated NK cells facilitates cell-mediated cytotoxicity and subsequent autocrine NK cell apoptosis. *J. Immunol. 156*, 2693–2699.

Endo, M., Ohi, H., Ohsawa, I., Fujita, T., and Matsushita, M. (1998). Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy. *Nephrol. Dial. Transplant. 13*, 1984–1990.

Fitzgerald-Bocarsly, P. (2002). Natural interferon-α producing cells: The plasmacytoid dendritic cells. *Biotechniques 33*, S16-S29.

Gelderman, K.A., Tomlinson, S., Ross, G.D., and Gorter, A. (2004). Complement function in mAb-mediated cancer immunotherapy. *Trends Immunol. 25*, 158–164.

Getahun, A., and Heyman, B. (2004). IgG- and IgE-mediated antigen presentation on MHC class II. *Immunol. Lett. 92*, 33–38.

Gil-Torregrosa, B.C., Lennon-Dumenil, A.M., Kessler, B., Guermonprez, P., Ploegh, H.L., Fruci, D., van Endert, P., and Amigorena, S. (2004). Control of cross-presentation during dendritic cell maturation. *Eur. J. Immunol. 34*, 398–407.

Gordon, S. (2002). Pattern recognition receptors: Doubling up for the innate immune response. *Cell 111*, 927–930.

Groger, M., Sarmay, G., Fiebiger, E., Wolff, K., and Petzelbauer, P. (1996). Dermal microvascular endothelial cells express CD32 receptors *in vivo* and *in vitro*. *J. Immunol. 156*, 1549–1556.

Haberman, A.M. and Shlomchik, M.J. (2003). Reassessing the function of immune-complex retention by follicular dendritic cells. *Nat. Rev. Immunol. 3*, 757–764.

Hart, S.P., Alexander, K.M., and Dransfield, I. (2004). Immune complexes bind preferentially to FcγRIIa (CD32) on apoptotic neutrophils, leading to augmented phagocytosis by macrophages and release of proinflammatory cytokines. *J. Immunol. 172*, 1882–1887.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., and Bauer, S. (2004). Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. *Science 303*, 1526–1529.

Heyman, B. (2000). Regulation of antibody responses via antibodies, complement, and Fc receptors. *Annu. Rev. Immunol. 18*, 709–737.

Heyman, B. (2003). Feedback regulation by IgG antibodies. *Immunol. Lett. 88*, 157–161.

Hiepe, F., Pfuller, B., Wolbart, K., Bruns, A., Leinenbach, H.P., Hepper, M., Schossler, W., and Otto, V. (1999). C1q: A multifunctional ligand for a new immunoadsorption treatment. *Ther. Apher. 3*, 246–251.

Hogarth, P.M. (2002). Fc receptors are major mediators of antibody based inflammation in autoimmunity. *Curr. Opin. Immunol. 14*, 798–802.

Hunziker, L., Recher, M., Macpherson, A.J., Ciurea, A., Freigang, S., Hengartner, H., and Zinkernagel, R.M. (2003). Hypergamma-

globulinemia and autoantibody induction mechanisms in viral infections. *Nat. Immunol. 4*, 343–349.

Huttunen, H.J. and Rauvala, H. (2004). Amphoterin as an extracellular regulator of cell motility: From discovery to disease. *J. Intern. Med. 255*, 351–366.

Ierino, F.L., Powell, M.S., McKenzie, I.F.C., and Hogarth, P.M. (1993). Recombinant soluble human FcγRII: Production, characterization, and inhibition of the Arthus reaction. *J. Exp. Med. 178*, 1617–1628.

Izaguirre, A., Barnes, B.J., Amrute, S., Yeow, W.S., Megjugorac, N., Dai, J., Feng, D., Chung, E., Pitha, P.M., and Fitzgerald-Bocarsly, P. (2003). Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. *J. Leukoc. Biol. 74*, 1125–1138.

Jarvis, J.N., Diebold, M.M., Chadwell, M.K., Iobidze, M., and Moore, H.T. (1995). Composition and biological behaviour of immune complexes isolated from synovial fluid of patients with juvenile rheumatoid arthritis (JRA). *Clin. Exp. Immunol. 100*, 514–518.

Jarvis, J.N., Xu, C., Wang, W., Petty, H.R., Gonzalez, M., Morssy, N., Waxman, F., and Quintero del Rio, A. (1999). Immune complex size and complement regulate cytokine production by peripheral blood mononuclear cells. *Clin. Immunol. 93*, 274–282.

Kalergis, A.M. and Ravetch, J.V. (2002). Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. *J. Exp. Med. 195*, 1653–1659.

Kepley, C.L., Zhang, K., Zhu, D., and Saxon, A. (2003). FcεRI-FcγII Coaggregation inhibits Il-16 production from human Langerhans-like dendritic cells. *Clin. Immunol. 108*, 89–94.

Kepley, C.L., Taghavi, S., Mackay, G.A., Zhu, D., Morel, P.A., Zhang, K., Ryan, J.J., Satin, L.S., Zhang, M., Pandolfi, P.P., and Saxon, A. (2004). Co-aggregation of FcγRII with FcεRI on human mast cells inhibits antigen-induced secretion and involves SHIP-Grb2-Dok complexes. *J. Biol. Chem.* 279, 35139–35149.

Kosco-Vilbois, M.H. (2003). Are follicular dendritic cells really good for nothing? *Nat. Rev. Immunol.* 3, 764–769.

Krieg, A.M. (2002). CpG motifs in bacterial DNA and their immune effects. *Annu. Rev. Immunol.* 20, 709–760.

Kunkel, S., Holtz, M., Grossjohann, B., Schuett, W., Klinkmann, H., Kong, D., Yamada, R., Kimura, H., Matic, G.B., Ramlow, W., and Boeden, H.F. (2002). Selective removal of circulating immune complexes from patient plasma. *Artif. Organs 26*, 124–132.

Kwiatkowska, K., Frey, J., and Sobota, A. (2003). Phosphorylation of FcγRIIA is required for the receptor-induced actin rearrangement and capping: The role of membrane rafts. *J. Cell Sci. 116*, 537–550.

Latif, R., Graves, P., and Davies, T.F. (2001). Oligomerization of the human thyrotropin receptor: Fluorescent protein-tagged hTSHR reveals post-translational complexes. *J. Biol. Chem. 276*, 45217–45224.

Latz, E., Schoenemeyer, A., Visintin, A., Fitzgerald, K.A., Monks, B.G., Knetter, C.F., Lien, E., Nilsen, N.J., Espevik, T., and Golenbock, D.T. (2004). TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat. Immunol. 5*, 190–198.

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature 416*, 603–607.

Lieberman, J. (2003). Mechanisms of granule-mediated cytotoxicity. *Curr. Opin. Immunol. 15*, 513–515.

Lövgren, T., Eloranta, M.-L., Båve, U., Alm, G.V., and Rönnblom, L. (2004). Induction of interferon-α production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum. 50*, 1861–1872.

Magnusson, V., Johanneson, B., Lima, G., Odeberg, J., Alarcon-Segovia, D., and Alarcon-Riquelme, M.E. (2004). Both risk alleles for FcγRIIA and FcγRIIIA are susceptibility factors for SLE: A unifying hypothesis. *Genes Immun. 5*, 130–137.

Manderson, A.P., Botto, M., and Walport, M.J. (2004). The role of complement in the development of systemic lupus erythematosus. *Annu. Rev. Immunol. 22*, 431–456.

McKenzie, S.E., Taylor, S.M., Malladi, P., Yuhan, H., Cassel, D.L., Chien, P., Schwartz, E., Schreiber, A.D., Surrey, S., and Reilly, M.P. (1999). The role of the human Fc receptor FcγRIIA in the immune clearance of platelets: A transgenic mouse model. *J. Immunol. 162*, 4311–4318.

Mims, C., Nash, A., and Stephen, J. (2001) *Mim's Pathogenesis of Infectious Disease.* London: Academic Press.

Mohan, C., and Datta, S.K. (1995). Lupus: key pathogenic mechanisms and contributing factors. *Clin. Immunol. Immunopathol. 77*, 209–220.

Monteiro, R.C. and van de Winkel, J.G. (2003). IgA Fc receptors. *Annu. Rev. Immunol. 21*, 177–204.

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol. 19*, 683–765.

Nielsen, C.H., Hegedus, L., and Leslie, R.G. (2004). Autoantibodies in autoimmune thyroid disease promote immune complex formation with self antigens and increase B cell and $CD4^+$ T cell proliferation in response to self antigens. *Eur. J. Immunol. 34*, 263–272.

Norman, M.U., van de Velde, N.C., Timoshanko, J.R., Issekutz, A., and Hickey, M.J. (2003). Overlapping roles of endothelial selectins and vascular cell adhesion molecule-1 in immune complex-induced leukocyte recruitment in the cremasteric microvasculature. *Am. J. Pathol. 163*, 1491–1503.

Ober, R.J., Martinez, C., Vaccaro, C., Zhou, J., and Ward, E.S. (2004). Visualizing the site and dynamics of IgG salvage by the MHC class I-related receptor, FcRn. *J. Immunol. 172*, 2021–2029.

Pan, L.Y., Mendel, D.B., Zurlo, J., and Guyre, P.M. (1990). Regulation of the steady state level of FcγRI mRNA by IFN-γ and dexamethasone in human monocytes, neutrophils, and U-937 cells. *J. Immunol. 145*, 267–275.

Panka, D.J. (1997). Glycosylation is influential in murine IgG3 self-association. *Mol. Immunol. 34*, 593–598.

Portales-Perez, D., Alarcon-Segovia, D., Llorente, L., Ruiz-Arguelles, A., Abud-Mendoza, C., Baranda, L., de la Fuente, H., Ternynck, T., and Gonzalez-Amaro, R. (1998). Penetrating anti-DNA monoclonal antibodies induce activation of human peripheral blood mononuclear cells. *J. Autoimmun. 11*, 563–571.

Prodinger, W.M., Würzner, R., Stoiber, H., and Dierich, M.P. (2003). Complement. In W. E. Paul (ed.), *Fundamental Immunology* (pp. 1077–1103). Philadelphia: Lippincott Williams & Wilkins.

Radstake, T.R., Van Lent, P.L., Pesman, G.J., Blom, A.B., Sweep, F.G., Rönnelid, J., Adema, G.J., Barrera, P., and van den Berg, W.B. (2004). High production of proinflammatory and Th1 cytokines by dendritic cells from patients with rheumatoid arthritis, and down regulation upon FcγR triggering. *Ann. Rheum. Dis. 63*, 696–702.

Ravetch, J.V. (2002). A full complement of receptors in immune complex diseases. *J. Clin. Invest. 110*, 1759–1761.

Ravetch, J.V. and Bolland, S. (2001). IgG Fc receptors. *Annu. Rev. Immunol. 19*, 275–290.

Ravetch, J.V. and Clynes, R.A. (1998). Divergent roles for Fc receptors and complement *in vivo*. *Annu. Rev. Immunol. 16*, 421–432.

Reefman, E., Dijstelbloem, H.M., Limburg, P.C., Kallenberg, C.G., and Bijl, M. (2003). Fcγ receptors in the initiation and progression of systemic lupus erythematosus. *Immunol. Cell Biol. 81*, 382–389.

Rönnblom, L. and Alm, G.V. (2001). An etiopathogenic role for the type I IFN system in SLE. *Trends Immunol. 22*, 427–431.

Rönnblom, L., Eloranta, M.L., and Alm, G.V. (2003). Role of natural interferon-α producing cells (plasmacytoid dendritic cells) in autoimmunity. *Autoimmunity 36*, 463–472.

Rönnelid, J., Tejde, A., Mathsson, L., Nilsson-Ekdahl, K., and Nilsson, B. (2003). Immune complexes from SLE sera induce IL10 production from normal peripheral blood mononuclear cells by an FcγRII dependent mechanism: Implications for a possible vicious cycle maintaining B cell hyperactivity in SLE. *Ann. Rheum. Dis. 62*, 37–42.

Roos, A., Bouwman, L.H., van Gijlswijk-Janssen, D.J., Faber-Krol, M.C., Stahl, G.L., and Daha, M.R. (2001). Human IgA activates the complement system via the mannan-binding lectin pathway. *J. Immunol. 167*, 2861–2868.

Rosen, A. and Casciola-Rosen, L. (1999). Autoantigens as substrates for apoptotic proteases: implications for the pathogenesis of systemic autoimmune disease. *Cell Death Differ. 6*, 6–12.

Rui, L., Vinuesa, C.G., Blasioli, J., and Goodnow, C.C. (2003). Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling. *Nat. Immunol. 4*, 594–600.

Ruiz-Arguelles, A., Rivadeneyra-Espinoza, L., and Alarcon-Segovia, D. (2003). Antibody penetration into living cells: pathogenic, preventive and immuno-therapeutic implications. *Curr. Pharm. Des. 9*, 1881–1887.

Russell, J.H. and Ley, T.J. (2002). Lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol. 20*, 323–370.

Salio, M., Palmowski, M.J., Atzberger, A., Hermans, I.F., and Cerundolo, V. (2004). CpG-matured murine plasmacytoid dendritic cells are capable of *in vivo* priming of functional CD8 T cell responses to endogenous but not exogenous antigens. *J. Exp. Med. 199*, 567–579.

Shanley, T.P., Schmal, H., Friedl, H.P., Jones, M.L., and Ward, P.A. (1995). Regulatory effects of intrinsic IL-10 in IgG immune complex-induced lung injury. *J. Immunol. 154*, 3454–3460.

Shushakova, N., Skokowa, J., Schulman, J., Baumann, U., Zwirner, J., Schmidt, R.E., and Gessner, J.E. (2002). C5a anaphylatoxin is a major regulator of activating versus inhibitory FcγRs in immune complex-induced lung disease. *J. Clin. Invest. 110*, 1823–1830.

Sturfelt, G., Bengtsson, A., Klint, C., Nived, O., Sjöholm, A., and Truedsson, L. (2000). Novel roles of complement in systemic lupus erythematosus—hypothesis for a pathogenetic vicious circle. *J. Rheumatol. 27*, 661–663.

Takai, T. (2002). Roles of Fc receptors in autoimmunity. *Nat. Rev. Immunol. 2*, 580–592.

Takai, T. (2005). Fc receptors and their role in immune regulation and autoimmunity. *J. Clin. Immunol. 25*, 1–18.

Takeda, K., and Akira, S. (2004). TLR signaling pathways. *Semin. Immunol. 16*, 3–9.

Tan Sardjono, C., Mottram, P.L., and Hogarth, P.M. (2003). The role of FcγRIIa as an inflammatory mediator in rheumatoid arthritis and systemic lupus erythematosus. *Immunol. Cell Biol. 81*, 374–381.

Tkaczyk, C., Okayama, Y., Metcalfe, D.D., and Gilfillan, A.M. (2004). Fcγ receptors on mast cells: Activatory and inhibitory regulation of mediator release. *Int. Arch. Allergy Immunol. 133*, 305–315.

Trendelenburg, M. and Schifferli, J.A. (2003). Cryoglobulins in chronic hepatitis C virus infection. *Clin. Exp. Immunol. 133*, 153–155.

Vallin, H., Blomberg, S., Alm, G.V., Cederblad, B., and Rönnblom, L. (1999a). Patients with systemic lupus erythematosus (SLE) have a circulating inducer of interferon-alpha (IFN-α) production acting on leucocytes resembling immature dendritic cells. *Clin. Exp. Immunol. 115*, 196–202.

Vallin, H., Perers, A., Alm, G.V., and Rönnblom, L. (1999b). Anti-double-stranded DNA antibodies and immunostimulatory plasmid DNA in combination mimic the endogenous IFN-α inducer in systemic lupus erythematosus. *J. Immunol. 163*, 6306–6313.

van Sorge, N.M., van der Pol, W.L., and van de Winkel, J.G. (2003). FcγR polymorphisms: implications for function, disease susceptibility and immunotherapy. *Tissue Antigens 61*, 189–202.

van Spriel, A.B., Leusen, J.H., van Egmond, M., Dijkman, H.B., Assmann, K.J., Mayadas, T.N., and van de Winkel, J.G. (2001). Mac-1 (CD11b/CD18) is essential for Fc receptor-mediated neutrophil cytotoxicity and immunologic synapse formation. *Blood 97*, 2478–2486.

Viglianti, G.A., Lau, C.M., Hanley, T.M., Miko, B.A., Shlomchik, M.J., and Marshak-Rothstein, A. (2003). Activation of autoreactive B cells by CpG dsDNA. *Immunity 19*, 837–847.

Villinger, F., Mayne, A.E., Bostik, P., Mori, K., Jensen, P.E., Ahmed, R., and Ansari, A.A. (2003). Evidence for antibody-mediated enhancement of Simian immunodeficiency virus (SIV) GAG antigen processing and cross presentation in SIV-infected Rhesus macaques. *J. Virol.* 77, 10–24.

Virella, G., Atchley, D., Koskinen, S., Zheng, D., and Lopes-Virella, M.F. (2002). Proatherogenic and proinflammatory properties of immune complexes prepared with purified human OxLDL antibodies and human OxLDL. *Clin. Immunol. 105*, 81–92.

Voice, J.K. and Lachmann, P.J. (1997). Neutrophil Fcγ and complement receptors involved in binding soluble IgG immune complexes and in specific granule release induced by soluble IgG immune complexes. *Eur. J. Immunol. 27*, 2514–2523.

Wagner, C., Iking-Konert, C., Denefleh, B., Stegmaier, S., Hug, F., and Hansch, G.M. (2004). Granzyme B and perforin: Constitutive expression in human polymorphonuclear neutrophils. *Blood 103*, 1099–1104.

Walport, M.J. (2001a). Complement. First of two parts. *N. Engl. J. Med. 344*, 1058–1066.

Walport, M.J. (2001b). Complement. Second of two parts. *N. Engl. J. Med. 344*, 1140–1144.

Wang, X., Bregegere, F., Frusic-Zlotkin, M., Feinmesser, M., Michel, B., and Milner, Y. (2004). Possible apoptotic mechanism in epidermal cell acantholysis induced by pemphigus vulgaris autoimmunoglobulins. *Apoptosis 9*, 131–143.

Wernersson, S., Karlsson, M.C., Dahlström, J., Mattsson, R., Verbeek, J.S., and Heyman, B. (1999). IgG-mediated enhancement of antibody responses is low in Fc receptor γ chain-deficient mice and increased in FcγRII-deficient mice. *J. Immunol. 163*, 618–622.

Wick, G., Knoflach, M., and Xu, Q. (2004). Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu. Rev. Immunol. 22*, 361–403.

Zinkernagel, R.M. (2003). On natural and artificial vaccinations. *Annu. Rev. Immunol. 21*, 515–546.

Zlabinger, G.J., Haberhauer, G., Dax, K., Menzel, E.J., and Broll, H. (1990). Rheumatoid factor isotypes and circulating immune complexes in rheumatoid arthritis. *Clin. Exp. Rheumatol. 8*, 113–119.

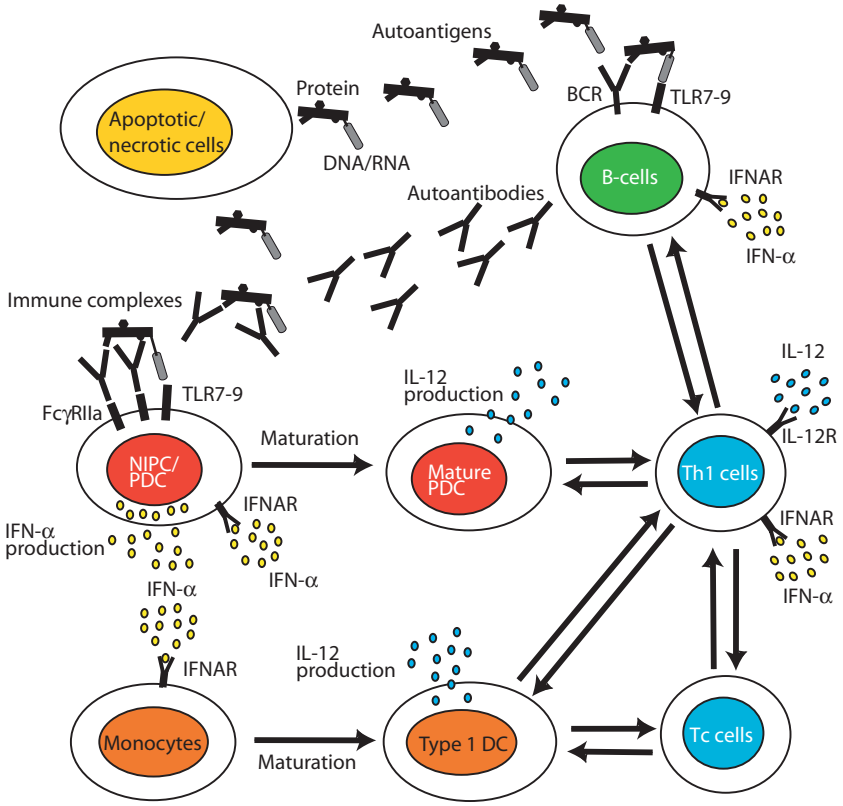


FIGURE 16.4 Role of DNA/RNA-containing immune complexes (ICs) in the induction and maintenance of autoimmune disease. Apoptotic or necrotic cells release DNA- or RNA-containing autoantigens that can activate IgG autoantibody-producing B cells. Such autoantibodies can occur during viral infections, due to stimulatory effects on B cells and induced type I interferon (IFN) production in immature plasmacytoid dendritic cells (NIPCs/PDCs). Autoantibodies and DNA/RNA-containing autoantigens form ICs that act as endogenous inducers of type I IFN production in NIPCs/PDCs. The type I IFN, especially IFN-α, has multiple immunostimulatory actions that include induced maturation of monocyte-derived DCs (type 1 DCs), development of type 1 helper T (Th1) cells and cytotoxic T (Tc) cells, as well as facilitation of B-cell activation. The resulting further autoantibody production causes formation of more IFN-inducing ICs, IFN-α production, and IFN-mediated immunostimulation. The autoimmune process is consequently sustained by a mechanism with the features of a vicious circle. IFNAR, denotes the type I IFN receptor.

CHAPTER

17

Functional Effects of Autoantibodies

SALLY A. WATERMAN, MICHAEL W. JACKSON, AND TOM P. GORDON

Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, Bedford Park, South Australia, Australia

Functional autoantibodies may be defined as antibodies that produce a pharmacologic-type effect on whole organs, tissues or cells *in vivo* and *in vitro*, in the absence of activation of immune mechanisms. These antibodies recognize extracellular epitopes, frequently on neurotransmitter receptors or ion channels, and may contribute to the pathogenesis of the autoimmune disease. They contrast with the majority of classical autoantibodies, such as anticentromere autoantibodies, which are useful as diagnostic markers, recognize intracellular epitopes, and are unlikely to contribute to disease pathogenesis (Table 17.1). The first functional autoantibodies to be characterized were anti-thyroid stimulating hormone (TSH) receptor autoantibodies in the 1950s (see Chapter 35; Adams and Purves, 1956; Lessof et al., 1959), followed by autoantibodies to skeletal muscle nicotinic receptors in myasthenia gravis in the 1970s (see Chapter 48; Almon et al., 1974; Lindstrom et al., 1976) and autoantibodies to neuronal voltage-gated calcium channels (VGCCs) in Lambert–Eaton myasthenic syndrome (LEMS) in the 1980s (see Chapter 48; Prior et al., 1985; Lang et al., 1987; Leys et al., 1989). Since then, new functional autoantibodies have been described in cardiac, endocrine, gastroenterologic, neurologic, and rheumatologic disorders (Table 17.2); a better understanding of these autoantibodies is likely to enhance greatly our ability to diagnose, monitor, and treat the associated autoimmune diseases.

GENERAL FEATURES OF FUNCTIONAL AUTOANTIBODIES

Epitopes

Most functional autoantibodies appear to recognize epitopes on large molecules that are either receptors or ion channels (Figure 17.1) (see Chapter 48). These molecules have complex conformations that alter depending, for example, on ligand binding or changes in membrane potential. Thus, autoantibodies may bind only under highly specific conditions. This necessitates the development of new strategies for studying and identifying functional antibodies.

Neurotransmitter Receptors

The first major class of autoantibody target is the neurotransmitter receptors. These are classified as either metabotropic (usually with a seven-transmembrane domain structure and coupled to intracellular second messenger systems; Figure 17.1B) or ionotropic (containing an intrinsic ion channel and usually comprising multiple subunits; Figure 17.1C). Neurotransmitters such as acetylcholine and glutamate have both metabotropic and ionotropic receptors, and in each case, functional autoantibodies exist to both the metabotropic and ionotropic forms (Table 17.1). Nonpeptide

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 17.1 Comparison of the features of functional versus classical autoantibodies

Functional autoantibodies	Classical autoantibodies
Recognize extracellular epitopes such as neurotransmitter receptors and ion channels	Recognize intracellular epitopes
Very useful as diagnostic markers in some diseases, but simple assays remain to be developed for some autoantibodies	Useful diagnostic markers
Have a pharmacologic effect on cell function	No detectable effect on cell function
Activation of immune mechanisms, such as complement is not required for autoantibody-mediated effect, but may occur	May activate immune mechanisms or have no effect on function
Mimic disease symptoms following passive transfer	No effect on passive transfer, e.g. Sutton and Winer (2002)
Can be difficult to detect by standard immunochemical methods	Detectable by standard immunochemical methods, including ELISA, immunoblot, immunofluorescence
Recognize conformational, fixation-sensitive epitopes	Can recognize linear peptides
Detectable by functional assays and effective in passive transfer	No effect on functional assays or by passive transfer
Examples include anticalcium channel antibodies in LEMS and antimuscarinic receptor antibodies in Sjögren syndrome	Examples include anticentromere and anti-Sc170 antibodies in scleroderma, and anti-Hu and anti-Yo antibodies in paraneoplastic syndromes

LEMS, Lambert–Eaton myasthenic syndrome.

neurotransmitters that act at metabotropic receptors bind to a complex binding pocket composed of multiple amino acids from different transmembrane domains, but which includes part of the second extracellular loop (Zeng and Wess, 1999; Hulme et al., 2003). There has been variable success in detecting functional autoantibodies using short peptides corresponding to the second extracellular loop (see below). *In vivo*, this region of the receptor is linked by disulfide bonds to the first extracellular loop, and may be involved in the formation of receptor multimers (Zeng and Wess, 1999; Figure 17.1B). Thus, linear peptides are unlikely to mimic the conformation of the epitope *in vivo*. However, extracting whole receptors in their native conformation for use in detecting autoantibodies may be difficult, if not impossible, due to the importance of associated molecules in retaining the appropriate conformation of the receptor. Extraction of lipid rafts containing the receptor may be a compromise between using purified receptor and obtaining receptors in the correct conformation.

Ion Channels

The second major class of autoantibody target besides receptors is ion channels (Figure 17.1A). To date, autoantibodies against VGCCs and voltage-gated potassium channels (VGKCs) have been well described (Table 17.1). VGCCs are composed of four major subunits: α_1, β, $\alpha_2\delta$, and in some regions, a γ subunit. The α_1 subunit is the pore-forming subunit and spans the cell membrane. Several subtypes of α_1 subunit exist, each with different pharmacologic and electrophysiologic properties, and each present in different cell types (Jones, 1998). Neurotransmitter release depends principally on calcium influx into nerve terminals through α_{1A} or α_{1B} subunit-containing channels, and autoantibodies recognizing these subunits are present in LEMS, where they inhibit transmitter release (Lang et al., 1987; Motomura et al., 1997). Voltage-gated channels change conformation in response to membrane depolarization; thus, different epitopes may be present depending on the membrane potential of the cell. The α_{1C} subunit that forms the pore of L-type VGCCs in smooth muscle has a binding site for the dihydropyridine class of drugs. Dihydropyridine agonists bind preferentially when the cell is depolarized and the calcium channel is open, whereas the dihydropyridine antagonists bind preferentially to the closed channel at resting membrane potential (Hockerman et al., 1997). Similarly, functional autoantibodies in type 1 diabetes that mimic dihydropyridine agonists may recognize only the depolarized state of the α_{1C} calcium channel subunit (Jackson et al., 2004). Such specificity of binding is likely to explain why some autoantibodies are difficult to detect using standard techniques (see below). VGKCs have a similar structure to that of calcium channels except that four pore-forming α subunits form a complex with four intracellular β subunits (Benatar, 2000). Autoantibodies in patients with neuromyotonia recognize one of the subtypes of α subunit: Kv1.1a, Kv1.2 or Kv1.6 (Hart et al., 1997).

A small group of functional autoantibodies recognizes other structures, including gangliosides and membrane-bound enzymes. Gangliosides are sialic acid-containing glycolipids present in the outer leaflet of cell membranes, and are highly enriched in the nervous system. Bacterial

TABLE 17.2 Functional autoantibodies and their targets in autoimmune disease

Autoantibody target	Disease	Reference
N-type voltage-gated calcium channel (α_{1B} subunit)	Lambert–Eaton myasthenic syndrome	Prior et al. (1985), Lang et al. (1987), Motomura et al. (1997), Waterman et al. (1997)
P/Q-type voltage-gated calcium channel (α_{1A} subunit)	Lambert–Eaton myasthenic syndrome	Prior et al. (1985), Lang et al. (1987), Motomura et al. (1997), Waterman et al. (1997)
	Paraneoplastic cerebellar degeneration	Mason et al. (1997), Graus et al. (2002), Fukuda et al. (2003)
	Amyotrophic lateral sclerosis	Llinas et al. (1993)
L-Type voltage-gated calcium channel (α_{1C} subunit, smooth muscle isoform)	Diabetes	Jackson et al. (2004)
L-Type voltage-gated calcium channel (α_{1S} subunit)	Amyotrophic lateral sclerosis	Kimura et al. (1994)
L-type voltage-gated calcium channel (α_{1C} subunit, cardiac isoform)	Neonatal lupus congenital heart block	Xiao et al. (2001a)
Voltage-gated potassium channel	Neuromyotonia and immune-mediated peripheral nerve hyperexcitability	Shillito et al. (1995), Hart et al. (1997; 2002), Vernino and Lennon (2002)
	Slow transit constipation	Knowles et al. (2002)
	Idiopathic epilepsy and epilepsy with cerebral malaria	Lang et al. (2001), Williams et al. (2001)
	Morvan syndrome	Lee et al. (1998), Barber et al. (2000), Liguori et al. (2001)
	Reversible limbic encephalitis	Buckley et al. (2001), Pozo-Rosich et al. (2003), Schott et al. (2003), Vincent et al. (2004)
Metabolic receptors		
α_1-Adrenoreceptor	Hypertension	Luther et al. (1997), Liao et al. (2002)
β_1-Adrenoreceptor	Idiopathic dilated cardiomyopathy (with ventricular arrhythmia)	Limas et al. (1991), Fu et al. (1993), Matsui et al. (1995), Wallukat et al. (1995), Atger et al. (1999), Chiale et al. (2001), Christ et al. (2001)
	Chagas' disease (with ventricular arrhythmia)	Sterin-Borda and Borda (2000), Chiale et al. (2001)
β_2-Adrenoreceptor	Chagas' disease	Sterin-Borda and Borda (2000)
Angiotensin-1 receptor	Pre-eclampsia	Wallukat et al. (1999b)
	Hypertension	Fu et al. (2000), Liao et al. (2002)
Metabotropic glutamate GluR1α receptor	Paraneoplastic cerebellar ataxia	Sillevis Smitt et al. (2000), Cosesmans et al. (2003)
M1-Muscarinic receptor	Congenital heart block	Borda et al. (1999)
	Sjögren syndrome	Perez Leiros et al. (1999)
M2-Muscarinic receptor	Idiopathic dilated cardiomyopathy (with sinus node dysfunction)	Fu et al. (1993), Matsui et al. (1995), Wallukat et al. (1999a), Chiale et al. (2001)
	Chagas' disease (with sinus node dysfunction)	Goin et al. (1994a), Sterin-Borda and Borda (2000), Chiale et al. (2001), Hernandez et al. (2003)
M3-Muscarinic receptor	Primary and secondary	Bacman et al. (1996),
	Sjögren syndrome	Waterman et al. (2000)
	Scleroderma	Goldblatt et al. (2002)
Melanin-concentrating hormone receptor 1 (MCHR1)	Vitiligo	Kemp et al. (2002)
TSH receptor	Graves' disease	Atger et al. (1999)
Ionotropic receptors		
Glutamate GluR2 receptor*	Cerebellar degeneration	Gahring et al. (1997)
Glutamate GluR3 receptor*	Rasmussen encephalitis	Rogers et al. (1994), Wiendl et al. (2001), Mantegazza et al. (2002)
NMDA receptor (NR2 receptor)	Neuropsychiatric lupus	DeGiorgio et al. (2001)
Skeletal muscle nicotinic receptor	Myasthenia gravis	Appel et al. (1975), Lindstrom et al. (1976)
Neuronal nicotinic receptor	Dysautonomia	Vernino et al. (1998; 2000; 2001)
Other molecules		
Cardiac troponin I	Dilated cardiomyopathy	Okazaki et al. (2003)
Gangliosides GQ1b, GM1	Guillain–Barré syndrome and Fisher syndrome	McCombe et al. (1992), Roberts et al. (1994), Schwerer (2002)
Lysoganglioside GM1	Sydenham chorea	Kirvan et al. (2003)
Muscle-specific serine kinase (MuSK)	Myasthenia gravis	Hoch et al. (2001), Evoli et al. (2003), Sanders et al. (2003)

*AMPA receptor.

NMDA, *N*-methyl-D-aspartate; TSH, thyroid-stimulating hormone.

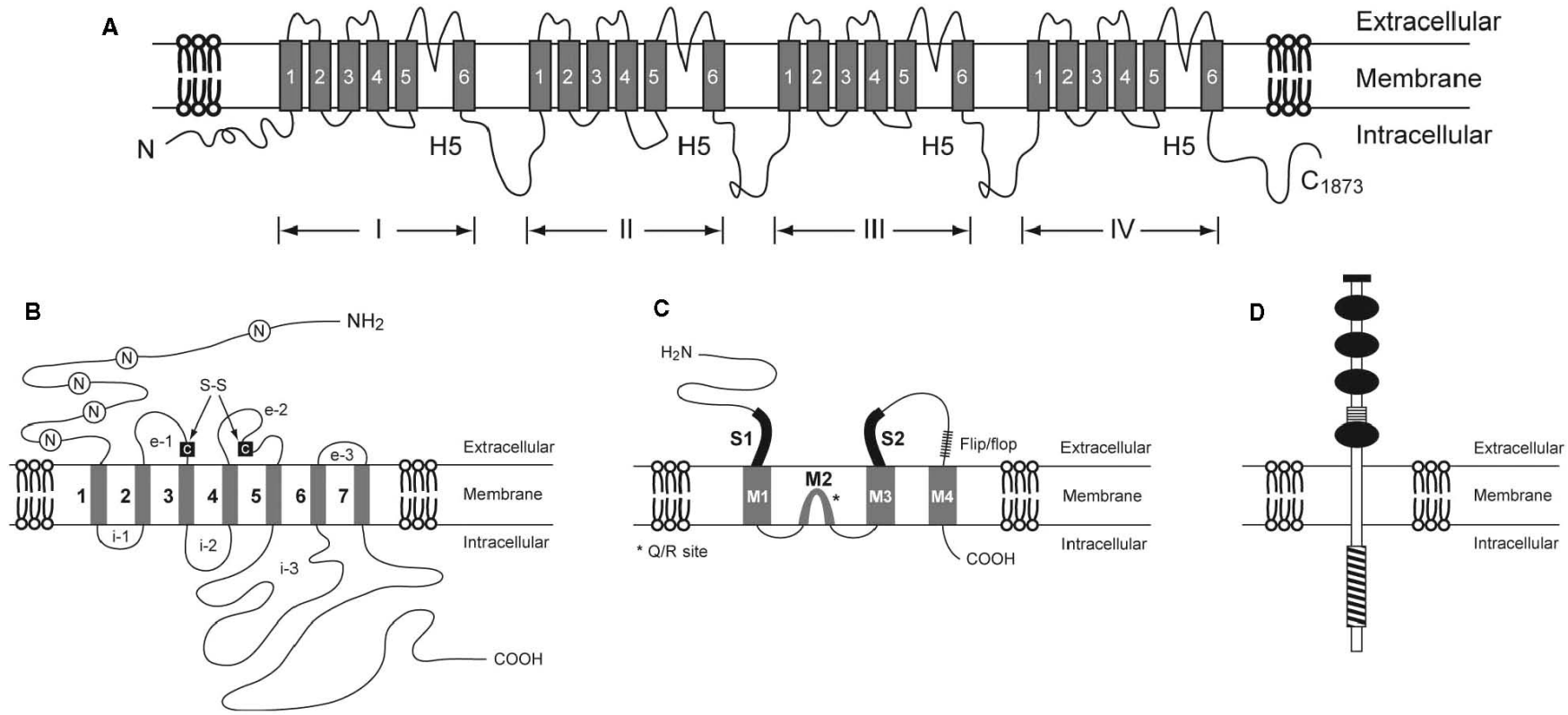


FIGURE 17.1 Structures of some channels and receptors targeted by functional autoantibodies. *A*, α Subunit of a voltage-gated calcium channel, with four repeats (I–IV) each with six hydrophobic transmembrane segments (S1–6). The fourth transmembrane domain in each repeat contains the voltage sensor, and the S5-6 hairpin regions form the lining of the channel pore and confer ion selectivity. *B*, Muscarinic receptor with seven transmembrane domains. The extracellular N-terminal has a number of glycosylation sites (N). A disulfide bond (S–S) occurs between the first and second extracellular loops (e-1 and e-2). i-1–i-3, intracellular loops. *C*, One subunit of an ionotropic glutamate receptor of the AMPA subtype, with its three transmembrane domains (M1, M3, and M4) and a cytoplasm-facing re-entrant membrane loop (M2). Each receptor is composed of four such subunits. S1 and S2 are ligand-binding domains. *D*, Muscle-specific tyrosine kinase (MuSK), with its immunoglobulin-like domains indicated by filled ovals, an N-terminal signal sequence (filled rectangle), extracellular cysteine-rich domain (shaded), and an intracellular kinase domain (hatched).

Adapted from Muth et al. (2001), Smith (1996), Dingledine et al. (1999), and Hoch et al. (2001).

lipopolysaccharides can mimic gangliosides, and most functional autoantibodies in this group of diseases have been proposed to arise as a result of molecular mimicry. Thus, autoantibodies to GM1 and GQ1b in Guillain–Barré syndrome and Miller–Fisher syndrome may arise following infection by *Campylobacter jejuni* (Schwerer, 2002), and antibodies to lysoganglioside GM1 in Sydenham chorea may follow *Streptococcus pyogenes* infection (Kirvan et al., 2003).

Muscle-Specific Tyrosine Kinase

Recently, functional autoantibodies to muscle-specific tyrosine kinase (MuSK) have been described in seronegative myasthenia gravis (Hoch et al., 2001). MuSK is required for agrin-induced clustering of skeletal muscle nicotinic receptors. It has four extracellular immunoglobulin-like domains at its N-terminus, to which autoantibodies bind (Figure 17.1D; Hoch et al., 2001). Anti-MuSK antibodies inhibit receptor clustering, thereby inhibiting skeletal neuromuscular transmission (Hoch et al., 2001).

Tumor Cell Antigens

A significant number of diseases with functional autoantibodies occur as paraneoplastic syndromes. In these diseases, the autoantigen has usually been identified in the tumor cells. It has therefore been proposed that an immune response initially directed against the tumor produces antibodies that cross-react with receptors and ion channels in normal tissues (Vincent, 2002). Myasthenia gravis can occur in association with thymoma, and skeletal muscle nicotinic receptors have been identified in thymoma cells (Newsom-Davis, 1997a). Thymoma can also be present in patients with neuromyotonia (Hart et al., 2002), reversible limbic encephalitis (Buckley et al., 2001) or Morvan syndrome (Lee et al., 1998), all with anti-VGKC autoantibodies. Whether these channels are expressed by thymoma cells is not yet known. Small cell lung carcinoma cells have a neuroectodermal origin and express many neuronal proteins, including N-type and P/Q-type VGCCs, and the α3 subunit of neuronal nicotinic receptors. Small cell lung carcinoma occurs in association with LEMS and paraneoplastic cerebellar degeneration (with anti-P/Q–type VGCC autoantibodies) (Vincent, 2002), and with autoimmune autonomic neuropathy (with antineuronal nicotinic receptor autoantibodies) (Vernino et al., 1998). Some patients with paraneoplastic cerebellar degeneration or paraneoplastic ataxia/opsoclonus produce autoantibodies to ionotropic glutamate receptors (GluR1, GluR4, and GluR6); in these particular cases, the syndromes were associated with transitional cell carcinoma of the bladder and breast cancer (Gahring et al., 1995). It is not yet known whether these tumors express glutamate receptors.

Detection of Functional Autoantibodies

Assay Characteristics

By definition, functional autoantibodies alter the activity of another molecule and must therefore recognize conformational epitopes, sometimes even activity-dependent epitopes, *in vivo*. Consequently, functional autoantibodies can be difficult to detect by standard immunochemical approaches in which molecules may be denatured, reduced or fixed, or short peptides used. This is compounded by the low serum levels of these autoantibodies and the requirement for high serum concentration, leading to an increase in background binding and low signal-to-noise ratios (McLachlan and Rapoport, 1996). Functional autoantibody detection ideally requires the use of sensitive physiologic assays, as described below. Exceptions to this are the three well-studied neurologic disorders in which highly sensitive immunoprecipitation assays with radioiodinated toxins are used diagnostically: myasthenia gravis (α-bungarotoxin-labeled nicotinic receptors), LEMS (ω-conotoxin MVIIC-labeled P/Q-type calcium channels), and neuromyotonia (α-dendrotoxin-labeled VGKCs) [reviewed in Vincent (2002)]. However, it is not certain that autoantibodies detected using these assays are capable of producing a functional effect, since binding to intracellular versus extracellular regions of the antigen is not distinguished. Nevertheless, there is a generally good correlation between disease severity and titer for individual patients with these methods (Newsom-Davis et al., 1978; Shillito et al., 1995; Motomura et al., 1997).

Flow Cytometry

Alternative methods such as flow cytometry are needed to demonstrate binding of autoantibody to extracellular epitopes (Nguyen et al., 2000), but antigens are frequently not expressed at high enough density in native cell lines to enable sufficiently sensitive analysis; transfected cell lines are an alternative (see below; Vincent, 2002), although receptors and channels are not necessarily expressed in their native form in such systems.

Limitations of Conventional Assays

For most other diseases in which functional autoantibodies have been detected, functional assays have proved the most sensitive and specific. Assays used successfully to demonstrate functional effects of autoantibodies can employ isolated cells through to tissues and whole organs undergoing physiologic behaviors (see below). Functional assays are time consuming and not readily automated. A number of investigators have thus attempted to detect functional autoantibodies using simpler techniques, including peptide-based enzyme-linked immunosorbent assays (ELISAs).

However, these may not detect autoantibodies that recognize complex conformational epitopes. The most commonly studied autoantibodies in this regard are to G-protein-coupled (metabotropic) receptors; numerous studies have used linear peptides corresponding to the second extracellular loop of the receptor in ELISAs to detect the autoantibodies. However, in the majority of studies, the methods were not sufficiently stringent to rule out nonspecific binding of antibodies to peptides. Jahns et al. (1999) reported that when significant reactivity with a peptide was defined as a signal above the mean and two standard deviations of healthy subjects, a large number of false-positive anti-β_1-adrenoceptor autoantibodies were detected in patients with dilated cardiomyopathy when the peptide ELISA results were not corrected for nonspecific binding. Correcting for nonspecific binding reduced the apparent prevalence of the autoantibodies from 51% to 34%. However, only a subgroup of these autoantibodies, 26%, also recognized native receptor in cell membranes and had a functional effect on β_1-adrenoceptor activity (Jahns et al., 1999). Similarly, reports of autoantibodies binding to the second extracellular loop peptide from 5-hydroxytryptamine-4 (5-HT_4) receptors in Sjögren syndrome and systemic lupus erythematosus (SLE) (Eftekhari et al., 2000) could not be confirmed (Buyon, 1996; Cavill et al., 2002a). Binding of autoantibodies to second extracellular loop peptides from M3- and M4-muscarinic receptors (Bacman et al., 1996; 1998) could also not be confirmed using ELISA (Cavill et al., 2002b). Furthermore, autoantibodies to M3- or M4-muscarinic receptors could not be detected using immunoblot, immunoprecipitation or immunofluorescence techniques (Gordon et al., 2001; Cavill et al., 2002b) when appropriate controls were included, but were detectable in functional assays on colon or bladder smooth muscle (Waterman et al., 2000; Goldblatt et al., 2002). Studies detecting autoantibodies to second extracellular loop peptides from M2-muscarinic receptors in Chagas disease (Goin et al., 1997) and idiopathic dilated myopathy (Fu et al., 1993) also need to be repeated using more stringent techniques, since the autoantibody prevalence rates of 39% and 53%, respectively, are likely to include false positives.

The limitations of traditional immunochemical approaches have no doubt hampered the discovery of new functional autoantibodies. Future studies are most likely to be successful if patient immunoglobulin is tested in functional assays or by passive transfer techniques. Recently, for example, autoantibodies that activate L-type VGCCs and disrupt intestinal motor activity have been detected in patients with type 1 diabetes (Jackson et al., 2004); these autoantibodies were not detected when immunoblots of neuronal or pancreatic islet tissues were screened with patient sera or when tissue sections were screened immunohistochemically. Antibodies to glutamate receptors similarly could not be detected using Western blot or immunohistochemical approaches but could be detected immunocytochemically using human embryonic kidney (HEK) cells transfected with cDNA for the glutamate receptors, or electrophysiologically using fetal mouse cortical neurons in culture (Gahring et al., 1995).

Effects on Cell, Tissue, and Organ Function *In Vitro*

Cardiomyocytes

Functional autoantibodies have been proposed to contribute to a number of cardiovascular disorders, including dilated cardiomyopathy, Chagas disease, congenital heart block, pre-eclampsia, and hypertension. Isolated cultured neonatal rat myocytes have proved useful to demonstrate functional effects of autoantibodies in these diseases on the spontaneous rate of myocyte beating. Cardiomyocytes express M2-muscarinic receptors, β_1-adrenoceptors, α_1-adrenoceptors, and angiotensin 1 (AT1) receptors, among others. Autoantibodies from patients with dilated cardiomyopathy or Chagas disease have an agonist effect at M2-muscarinic receptors and slow the rate of myocyte beating (Wallukat et al., 1999c, Chiale et al., 2001) in an atropine-sensitive manner. Similarly, affinity-purified rabbit anti-M2-muscarinic receptor antibodies have a negative chronotropic effect (Fu et al., 1995). Anti-M2-muscarinic receptor autoantibodies also increase phosphoinositol production (Chiale et al., 2001). Conversely, antibodies that have an agonist effect at β_1-adrenoceptors, also found in patients with dilated cardiomyopathy and Chagas disease, have a positive chronotropic effect and increase cAMP production in a propranolol-sensitive manner (Chiale et al., 2001). These biochemical findings have been repeated in Chinese hamster fibroblasts transfected with β_1-adrenoceptors (Jahns et al., 1999) and COS-7 cells transfected with β_1-adrenoceptors or with M2-muscarinic receptors (Chiale et al., 2001). A subset of patients with primary hypertension, particularly those who are refractory to standard pharmacotherapy (Liao et al., 2002), produce autoantibodies that act as agonists at the α_1-adrenoceptor (Luther et al., 1997); these autoantibodies have a positive chronotropic effect on cultured neonatal rat cardiomyocytes and the effect is reversed by the α_1-adrenoceptor antagonist, prazosin. Similarly, autoantibodies to the AT1 receptor have an agonist-like effect and increase the rate of spontaneous beating of neonatal rat cardiomyocytes; this effect is reversed by the AT1 receptor antagonist, losartan (Wallukat et al., 1999b).

Autoantibodies that modify ion channel function can be studied using patch clamp electrophysiology on single cells. Immunoglobulin from babies born with congenital heart block, or from their mothers, has been shown to reduce whole cell and single-channel L-type VGCC currents in

isolated ventricular cardiomyocytes from rabbit and human fetal hearts (Garcia et al., 1994; Boutjdir et al., 1997).

Single-cell studies have limitations; epitopes that are not exposed *in vivo* may be accessible to the autoantibody in isolated cells, and receptor and channel expression can be altered by cell culturing. Thus, effects of autoantibodies on single cells do not predict responses in intact, fresh tissue. Some investigators have therefore also studied the effects of autoantibodies on whole, beating heart preparations. Garcia et al. (1994) made electrocardiographic recordings from whole rabbit hearts, perfused using the Langendorff technique. Heart block occurred in one-third of hearts perfused with anti-Ro/La-containing IgG from SLE patients but not with anti-ribonucleoprotein-positive IgG or with healthy control IgG. Similarly, affinity-purified anti-Ro52 IgG from mothers of children with congenital heart block induced complete atrioventricular block in human fetal hearts, perfused using the Langendorff technique (Boutjdir et al., 1997). Serum and IgG from patients with Chagas' disease, cardiac arrhythmias, and anti-M2-muscarinic receptor autoantibodies reduced heart rate and induced atrioventricular conduction block in isolated perfused rabbit hearts in an atropine-sensitive manner (de Oliveira et al., 1997); these effects were prevented by preincubation of the patient's IgG with peptides corresponding to the second extracellular loop of the M2-muscarinic receptor (Masuda et al., 1998).

Smooth Muscle Myocytes

Autonomic dysfunction is common in autoantibody-mediated disorders, and smooth muscle-containing organs are frequently affected. A number of recent studies have thus investigated the effect of functional autoantibodies on neuronally- or agonist-evoked smooth muscle contraction in strips of tissue dissected from mouse intestine, bladder, and vas deferens. Autoantibodies from patients with primary and secondary Sjögren syndrome or with scleroderma specifically inhibit M3-muscarinic receptor-mediated contractions in mouse bladder and colon but do not affect contractions mediated by neurotransmitters at other receptors, including P_{2X}-purinoceptors in the bladder and vas deferens, neurokinin-1 and neurokinin-2 receptors in the colon, and α_1-adrenoceptors in the vas deferens (Waterman et al., 2000; Goldblatt et al., 2002; Ohlsson et al., 2002), thus also ruling out effects of the autoantibodies on common ion channels involved in mediating smooth muscle contraction, such as the L-type VGCC. Smooth muscle preparations have proved to be exquisitely sensitive to antibody-mediated effects (Cavill et al., 2004), and are currently the gold standard method for detecting autoantibodies to M3-muscarinic receptors in systemic rheumatic diseases. As discussed above, traditional immunochemical approaches have so far failed to demonstrate consistently or convincingly these autoantibodies.

While tissue strips have been used to detect some novel autoantibodies, more physiologic approaches have also been employed in which whole organs are studied while undergoing spontaneous activity *in vitro*. Migrating motor complexes (MMCs) are a form of intestinal motility that still occurs in segments of isolated colon maintained in physiologic solutions. This behavior involves the activity of many different classes of neurons in the colon in highly organized spatial and temporal patterns (Brierley et al., 2001), by contrast with the synchronous activation of all neurons, which occurs in response to maximal electrical stimulation of tissue strips (Waterman et al., 2000). Jackson et al. (2004) used this approach to identify autoantibodies in patients with type 1 diabetes and autonomic dysfunction that act as agonists at the dihydropyridine-binding site on smooth muscle L-type VGCCs and cause profound disruption of MMC activity. The disruption was mimicked by the dihydropyridine agonist, BayK 8644 and competitively inhibited by the dihydropyridine antagonist, nicardipine (Figure 17.2) (Jackson et al., 2004).

Skeletal Muscle Myocytes

The best described autoimmune neurologic disorders affect skeletal neuromuscular transmission, and the mouse phrenic nerve/hemidiaphragm preparation has become a standard method for testing the effect of functional autoantibodies at the neuromuscular junction. The preparation is usually set up for intracellular recording of electrical activity in the skeletal muscle fibers of the diaphragm, in which spontaneous and evoked transmitter release are measured, respectively, as miniature end-plate potentials (MEPPs) and end-plate potentials (EPPs). EPP amplitudes are decreased by anti-P/Q-type VGCC autoantibodies in LEMS (Lang et al., 1983; Prior et al., 1985), whereas sera from patients with amyotrophic lateral sclerosis (ALS), possibly containing antibodies that activate P/Q-type calcium channels (Llinas et al., 1993), cause an increase in MEPP frequency and in EPP amplitude (Uchitel et al., 1988; Appel et al., 1991; O'Shaughnessy et al., 1998). Autoantibodies to VGKCs in patients with neuromyotonia also cause an increase in EPP amplitude (Shillito et al., 1995). An initial increase in MEPP frequency, followed by a decrease and then absence of MEPPs, has been reported in response to sera containing anti-GQ1b ganglioside antibodies from patients with the Fisher syndrome (Roberts et al., 1994).

Neurons and Neuron-Like Cell Lines

A growing number of neurologic diseases appear to involve functional autoantibody-mediated effects (Vincent et al., 1999), many of which have been characterized using cultured neurons, neuronal-like cell lines, or brain slices.

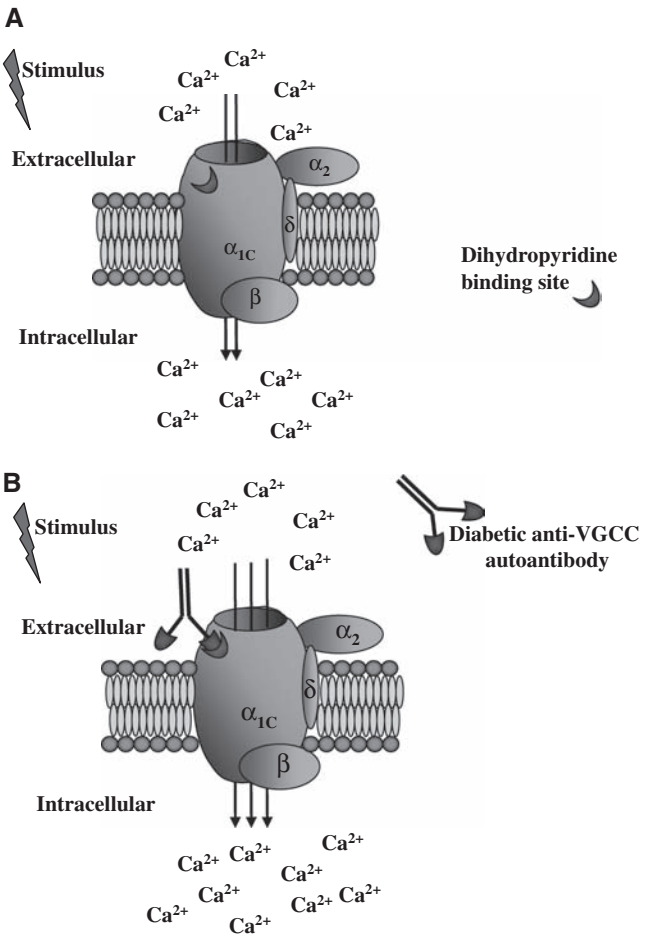


FIGURE 17.2 Schematic representation of the various subunits and their transmembrane organization in a smooth muscle L-type voltage-gated calcium channel (VGCC). In the closed conformation, intracellular entry of ions through the pore formed by the α_{1C} subunit is restricted. *A*, Following membrane depolarization, the channel opens and Ca^{2+} enters the cell. *B*, In type 1 diabetes, binding of autoantibodies to the α_{1C} subunit mimics the action of dihydropridine agonists and increases channel opening, causing enhanced calcium influx and altering calcium-mediated physiologic processes.

Adapted from Striessnig (1999).

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS), and autoantibodies to both ionotropic and metabotropic subtypes of glutamate receptor have been described in neurologic disorders. Autoantibodies to the ionotropic GluR3 receptor occur in various forms of epilepsy, including Rasmussen encephalitis (RE) (Wiendl et al., 2001; Mantegazza et al., 2002). Antibodies from RE patients activate GluR3 receptors (Twyman et al., 1995). Rabbit and murine antibodies to the GluR3B peptide similarly activate the receptor ion channel and cause death of cultured fetal mouse cortical neurons and of mouse somatosensory cortical neurons in slices, mimicking the excitotoxic effect of excess glutamate (Twyman et al., 1995; Levite et al., 1999). Excitotoxic neuronal cell death is also produced by agonistic anti-NR2 glutamate receptor autoantibodies from patients with neuropsychiatric lupus (DeGiorgio et al., 2001). Antibodies to GluR5 receptors from a subset of patients with paraneoplastic neurologic syndromes increase glutamate-evoked currents in cultured cortical neurons (Gahring et al., 1995). Metabotropic GluR1 receptors are inhibited by autoantibodies from some patients with cerebellar ataxia. Autoantibodies from these patients block induction of long-term depression in cultured cerebellar Purkinje neurons, a form of synaptic plasticity that may be associated with cerebellar motor learning behavior and is dependent on mGluR1 receptors, and also inhibit the basal activity of Purkinje cells in slices of mouse cerebellum (Coesmans et al., 2003).

Anti-VGCC autoantibodies occur in both LEMS and ALS. In LEMS, anti-VGCC autoantibodies cross-link and cause internalization of the channels (Peers et al., 1993), which over a period of hours, inhibits calcium flux and calcium currents in small cell lung carcinoma cells (Roberts et al., 1985; Viglione et al., 1993; 1995; Meriney et al., 1996), the rat neuroblastoma × glioma cell line (Peers et al., 1990), adrenal chromaffin cells (Viglione et al., 1992), and IMR32 neuroblastoma cells (Grassi et al., 1994). These effects are predominantly due to cross-linking of the P/Q-type VGCC (Motomura et al., 1997). The effect of ALS IgG is controversial. In general, the autoantibodies appear to enhance neurotransmitter release, and this has been demonstrated for norepinephrine (noradrenaline) release from rat brain cortical synaptosomes (Grassi et al., 1999), glutamate release from cultured rat hippocampal neurons (Andjus et al., 1997), and acetylcholine release from motor neurons in mouse hemidiaphragm (O'Shaughnessy et al., 1998; Uchitel et al., 1988). Release of each of these transmitters is dependent on calcium influx through P/Q-type VGCCs (Andjus et al., 1996; Grassi et al., 1999; Fratantoni et al., 2000), and increased calcium currents through P/Q-type channels in cerebellar Purkinje cells has been reported (Llinas et al., 1993). Conversely, ALS IgG has also been shown to inhibit dopamine release from rat PC12 cells, a process dependent on L-type VGCCs (Offen et al., 1998), and to inhibit calcium currents through L-type VGCCs in isolated skeletal muscle fibers (Delbono et al., 1993). Others, however, have reported depression of calcium currents in isolated cerebellar granule cells (Zhainazarov et al., 1994), which express P/Q-type VGCCs, or no effect on calcium flux in rat cerebral cortical synaptosomes (Thomas and Dunn, 1997).

Neuroblastoma cells have also been used to demonstrate a functional effect of autoantibodies from patients with Sydenham chorea; these autoantibodies recognize lysoganglioside GM1 and indirectly activate calcium-calmodulin kinase II; preliminary evidence suggests they can also stimulate transmitter release, although the mechanisms are unknown (Kirvan et al., 2003).

Antigen-Transfected Cells

Although effects of functional autoantibodies have been demonstrated on native cell lines, recent studies have capitalized on the specificity of transfected cell lines, and their high expression levels of the putative autoantigen. The majority of studies has measured binding of autoantibodies, rather than their functional effects, however.

Pinto et al. (2002) demonstrated that VGCC autoantibodies from patients with LEMS inhibit calcium flux in HEK cells expressing the α_{1A} calcium channel subunit (present in P/Q-type VGCCs), but not in cells expressing α_{1B} or α_{1E} subunits (present in N- and R-type VGCCs, respectively). In Chinese hamster ovary (CHO) cells transfected with the mGluR1α, but not the related mGluR5 receptor, autoantibodies from patients with cerebellar ataxia blocked glutamate-evoked IP_3 formation (Sillevis Smitt et al., 2000).

Passive Transfer of Human Autoantibodies

Passive immunization of laboratory animals with human functional autoantibodies is a crucial step in proving the pathogenic role of autoantibodies in disease (Drachman, 2003; Vincent, 2004) and can demonstrate the importance of a functional autoantibody, even if the precise target of the autoantibody is unknown. Passive transfer with functional effects has been achieved for relatively few diseases (Table 17.3). Typically, mice or rats are injected with purified IgG or whole serum for a period of 2 or more days, up to several weeks; the animals are then killed and tissues removed for studies *in vitro*, as above, or measurements made *in vivo*. Injection of mice with IgG from patients with LEMS for 8 days, for example, reproduces the characteristic reduction in neurotransmitter release from somatic motor nerve terminals (Lang et al., 1987) and autonomic nerve terminals (Waterman et al., 1997). After injection of IgG for only 2 days, features of Sjögren syndrome, including sicca symptoms, and bladder and gastrointestinal dysfunction, can be reproduced in mice (Robinson et al., 1998; Fang et al., 2004). Daily injection of mice for 3 days to 2 weeks with whole serum or immunoglobulin from patients with ALS causes an increase in neurotransmitter release from the skeletal neuromuscular junction (Uchitel et al., 1992; O'Shaughnessy et al., 1998).

Presumed autoantibody-mediated disorders of the CNS present additional problems for passive transfer studies in that the autoantibodies need to cross the blood–brain barrier. In some CNS disorders, such as cerebellar ataxia, intrathecal synthesis of IgG has been demonstrated; in others, autoantibodies are detectable in the cerebrospinal fluid (CSF), although synthesized peripherally. The presence of intrathecal IgG has been modeled by injecting patient IgG-containing autoantibodies to mGluR1 receptors directly into the cerebellar subarachnoid space of normal mice, producing severe, reversible ataxia (Sillevis Smitt et al., 2000) and reducing compensatory eye movements (Coesmans et al., 2003). An alternative approach has been to inject CSF containing GluR3 autoantibodies into rats via the carotid artery, followed by a pulse of mannitol to open the blood–brain barrier; this protocol produced a movement disorder within 24 h (Solaro et al., 2001). Intracerebral injection into mice of affinity-purified autoantibodies and of CSF from patients with neuropsychiatric lupus and anti-NR2 receptor autoantibodies produced hippocampal cell death; whether or not this altered significantly CNS function is unknown (DeGiorgio et al., 2001). The presence or synthesis of autoantibodies in CSF has also been demonstrated for anti-VGCC autoantibodies in paraneoplastic cerebellar degeneration (Graus et al., 2002), anti-VGKC autoantibodies in limbic encephalitis (Pozo-Rosich et al., 2003; Vincent et al., 2004), in Morvan syndrome (Liguori et al., 2001), and lysoganglioside GM1 antibodies in Sydenham chorea (Kirvan et al., 2003). Passive transfer studies are needed to test whether these autoantibodies have a functional effect in the CNS.

Whereas studies *in vitro* can detect short-term effects of autoantibodies over minutes to hours, passive transfer *in vivo* is valuable because this enables the longer-term effects of autoantibodies to be studied, so reproducing more closely the clinical situation. Furthermore, long-term exposure to functional autoantibodies reveals additional compensatory effects that take longer to develop and are not detectable in short-term studies *in vitro* (see below).

In general, functional autoantibodies appear to be present in human serum at very low concentrations, and the availability of sufficient serum for passive transfer studies has hampered progress. As an alternative, some investigators have raised artificial antibodies that recognize the same antigen as the human functional autoantibodies, and have used these in passive transfer-type studies. Nguyen et al. (2000) demonstrated that salivary secretion was inhibited 72 h after a single injection of monoclonal anti-rat M3-muscarinic receptor antibodies in nonobese diabetic (NOD).scid mice; injection of monoclonal anti-La antibodies had no effect (Nguyen et al., 2000), thus providing evidence for anti-M3-muscarinic receptor autoantibody-mediated secretory dysfunction in Sjögren syndrome. In a different disease, dilated cardiomyopathy, autoantibodies to cardiac troponin I may be pathogenic: administration of monoclonal anticardiac troponin I antibodies to normal BALB/c mice over 11 weeks impaired ventricular function, as assessed by a reduction in left ventricular ejection fraction and decreased systolic pressure development (Okazaki et al., 2003).

TABLE 17.3 Passive and active transfer studies

	Passive transfer studies		Active transfer studies	
Target	**Human autoantibodies**	**Monoclonal antibodies**	**Whole antigen**	**Peptide fragment**
β1-Adrenoceptor				Matsui et al. (1997; 1999), Iwata et al. (2001)
M2-Muscarinic receptor				Fu et al. (1996), Matsui et al. (1997; 1999)
M3-Muscarinic receptor	Fang, et al., 2004	Nguyen et al. (2000)		
TSH receptor			Marion et al. (1994)	Ohmori et al. (1992)
Metabotropic glutamate GluR1 receptor	Sillevis Smitt et al. (2000), Coesmans et al. (2003)			
Glutamate GluR3 receptor				Rogers et al. (1994), Levite and Hermelin (1999)
Neuronal nicotinic receptor			Lennon et al. (2003)	
Skeletal muscle nicotinic receptor	Toyka et al. (1997)		Patrick and Lindstrom (1973), Lennon et al. (1975)	Lindstrom et al. (1978)
P/Q-type VGCCs	LEMS: Fukunaga et al. (1983), Lang et al. (1987), Waterman et al. (1997) ALS: O'Shaughnessy et al. (1998)			
L-type VGCC (α_{1C} smooth muscle isoform)	Jackson et al. (2004)			
L-type VGCC (α_{1C} cardiac isoform)	Boutjdir et al. (1997)		Xiao et al. (2001b) (Ro52)	
VGKC	Sinha et al. (1991), Shillito et al. (1995)			
Cardiac troponin I		Okazaki et al. (2003)		

ALS, Amyotrophic lateral sclerosis, LEMS, Lambert–Eaton myasthenic syndrome; TSH, thyroid-stimulating hormone; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel.

Active Immunization with Antigen

Where passive immunization with patient IgG has been shown to reproduce pathophysiologic changes in animals, induction of experimental disease by antigen can provide additional evidence for the role of functional autoantibodies. However, the induction of a syndrome following immunization with a putative autoantigen is insufficient to prove a role for functional autoantibodies in the absence of evidence for their presence in patients and for a functional effect following passive transfer. A lupus-like syndrome can be produced in mice following immunization with a peptide fragment corresponding to the second extracellular loop of the 5-HT_4 receptor (Eftekhari et al., 2001). However, autoantibodies to the 5-HT_4 receptor could not be detected in patients with SLE or Sjögren syndrome when more stringent methods were used (Buyon et al., 2002; Cavill et al., 2002a). In the absence of other evidence, antibodies to the 5-HT_4 receptor are, therefore, at present an experimental curiosity and not of clinical relevance.

Glutamate Receptor 3

Active immunization with either whole antigen or a peptide fragment has been performed for a number of diseases in which functional autoantibodies play a role. Immunization of rabbits with GluR3 receptor peptides provided some of the first evidence that RE may be mediated by autoantibodies to these receptors. The rabbits developed seizures and histopathology of the brain reminiscent of that seen in patients with RE (Rogers et al., 1994). While mice immunized with GluR3B peptides also developed anti-GluR antibodies and significant inflammatory brain pathology, they did not show signs of epilepsy, suggesting that addi-

tional factors are required in some species for epilepsy to occur (Levite and Hermelin, 1999).

G-Protein-Coupled Receptors

Several investigators have reported that immunization of rabbits with second extracellular loop peptides from G-protein-coupled receptors results in production of functional autoantibodies to those receptors (Fu, 1995; 1999; Matsui et al., 1997; 1999; Iwata et al., 2001; Cavill et al., 2004). These procedures produce pathologic changes corresponding to those reported in patients (see below). Immunization of rabbits with peptides from β_1-adrenoceptors or M2-muscarinic receptors altered receptor expression and sensitivity, and resulted in dilated cardiomyopathy (Fu et al., 1996; Matsui et al., 1997; Iwata et al., 2001).

Ganglionic Nicotinic Acetylcholine Receptors

Autoantibodies to neuronal nicotinic receptors have been described in autoimmune autonomic neuropathies (Vernino et al., 1998; 2000; Balestra et al., 2000). Lennon et al. (2003) gave rabbits a single injection of a recombinant protein corresponding to the extracellular N-terminal domain of the α3 subunit present on neuronal nicotinic receptors in autonomic ganglia. The majority of rabbits produced antibodies to neuronal nicotinic receptors at serum levels of greater than 3 nM, and developed a dysautonomia syndrome reminiscent of that in patients, affecting sympathetic, parasympathetic, and enteric divisions of the autonomic nervous system. Specifically, the rabbits had gastrointestinal hypomotility, dilated pupils with impaired light response, ptosis, and urinary retention. These changes were evident within 20 days of inoculation, and the severity of the symptoms correlated with antibody titer (Lennon et al., 2003). Passive transfer of autonomic dysfunction to animals by the patient autoantibodies has recently been reported (Vernino et al., 2004).

Voltage-Gated Calcium Channels

VGCCs are targeted in several autoimmune conditions, including LEMS, ALS, type 1 diabetes, and congenital heart block; in each disease the epitopes appear to reside on a specific α_1 subunit. These are very large molecules (see Figure 17.1A), and it has not been possible to inject animals with whole subunits. Since the precise epitopes on the α_1 subunits are not yet known, there have been no obvious peptide sequences to use in active immunization studies. To model LEMS, Komai et al. (1999) chose to inject rats with a peptide corresponding to the S5–S6 linker region from domain III of the α_{1A} subunit. This is an extracellular domain known to be very important for the function of the P/Q-type VGCC, although it had not previously been shown to be antigenic. The rats produced antibodies that bound P/Q-type VGCCs and showed moderate weakness and a reduction in quantal acetylcholine release at the skeletal neuromuscular junction (Komai et al., 1999). Autonomic function in the rats was not assessed.

Antibodies recognizing the α_{1C} subunit of L-type VGCCs alter cardiac function in neonatal lupus congenital heart block (Boutjdir, 2000). The antibodies responsible for this effect have been reported to be the anti-Ro antibodies, and there is some homology between the three-dimensional structures of the L-type VGCC and Ro52 (Qu et al., 2001). To produce an active immunization model of congenital heart block, Xiao et al. (2001b) therefore chose to inject female rabbits with Ro52, rather than with a peptide from the α_{1C} subunit of L-type VGCCs. Offspring born to the immunized mothers had varying degrees of heart block and a reduction in calcium channel density in the heart (Xiao et al., 2001b). Sera and purified IgG from the mothers inhibited calcium currents in oocytes expressing the α_{1C} subunit of L-type VGCCs (Xiao et al., 2001b). Immunization of pregnant mice with recombinant Ro52 also resulted in the production of anti-Ro autoantibodies that crossed the placenta and correlated with atrioventricular conduction block in the pup (Boutjdir et al., 1997). These data are consistent with anti-Ro52 autoantibodies cross-reacting with L-type VGCCs and blocking conduction in the atrioventricular node. This hypothesis remains controversial, however, since sera containing anti-Ro52 antibodies did not alter L-type VGCC-dependent contractile responses of the vas deferens, whose α_{1C} pore-forming subunit shares 96% sequence similarity with the cardiac α_{1C} subunit isoform (Ohlsson et al., 2002).

PATHOPHYSIOLOGIC MECHANISMS OF FUNCTIONAL AUTOANTIBODIES

The effects of functional autoantibodies can be considered to occur in three stages. First, is the primary pharmacologic effect of the autoantibody on its target, which occurs within minutes to hours, and is reversible with time. This may be followed by secondary, plastic changes that compensate for the primary effect of the autoantibody. These changes generally occur over a time course of days to weeks. Lastly, the autoantibody may trigger chronic degenerative changes that are irreversible. Immunotherapies need to be administered prior to this phase of the disease if they are to be effective.

Primary Pharmacologic Effects of Autoantibodies

Primary pharmacologic effects of the autoantibodies are those that have been studied in detail in isolated

cells, tissues, and organs *in vitro* (see above). Functional autoantibodies may act as agonists or antagonists at neurotransmitter receptors, as exemplified by agonistic anti-β_1-adrenoceptor autoantibodies in idiopathic dilated cardiomyopathy and antagonistic anti-M3-muscarinic receptor autoantibodies in Sjögren syndrome. The effects of these autoantibodies are detectable within 30 min (Jahns et al., 1999; Waterman et al., 2000), and mimic the effect of synthetic agonists and antagonists to the receptors by interfering competitively or noncompetitively with ligand binding.

Agonists at G-protein-coupled receptors usually trigger receptor internalization (Edwardson and Szekeres, 1999) via β-arrestin- and clathrin-dependent or -independent mechanisms (Ferguson, 2001). Similarly, autoantibodies with agonist-type effects can cause receptor sequestration. Autoantibodies to M2-muscarinic receptors in Chagas disease cause receptor desensitization and internalization (Leiros et al., 1997) and anti-β_1-adrenoceptor autoantibodies in dilated cardiomyopathy cause receptor sequestration and prevent receptor recycling (Limas et al., 1991). Not all findings are consistent with this pattern, however. Other investigators have reported that autoantibodies to M2-muscarinic receptors (Wallukat et al., 1999a), to β_1-adrenoceptors (Magnusson et al., 1994) in dilated cardiomyopathy, and to α_1-adrenoceptors in hypertension (Luther et al., 1997) act as nondesensitizing agonists and do not cause receptor internalization (Wallukat et al., 1999a). At least some anti-M2-muscarinic receptor and anti-β_1-adrenoceptor antibodies act as only partial agonists, since they cannot evoke a full agonist response (Goin et al., 1994b; Perez Leiros et al., 1994; Staudt et al., 2001). This may account for the apparent lack of receptor internalization since there is a correlation between intrinsic activity of an agonist and its ability to cause receptor internalization (Edwardson and Szekeres, 1999). However, the reasons for the differences between full and partial agonist activity of autoantibodies and between those causing nondesensitizing agonist effects (Wallukat et al., 1999a) versus receptor desensitization and sequestration (Limas et al., 1991) remain to be explained.

Some functional autoantibodies do not produce a direct pharmacologic blockade, but instead inhibit channel or receptor function by causing cross-linking and internalization of the target. Anti-VGCC autoantibodies in LEMS do not have any effect within 6 h, but inhibit transmitter release after a longer period of incubation (Peers et al., 1993; Houzen et al., 1998), corresponding with the time taken to cause internalization of the calcium channels. A reduction in calcium channel number in the brain also occurs in paraneoplastic cerebellar degeneration in association with LEMS (Fukuda et al., 2003).

The acute effects of functional autoantibodies sometimes require bivalent antibodies. This is the case in LEMS, for example, suggesting that the autoantibodies cross-link the VGCCs and trigger their internalization (Peers et al., 1993). Monovalent autoantibodies derived from LEMS IgG do not have a functional effect (Peers et al., 1993). Similarly, a monoclonal antibody to the M2-muscarinic receptor, which mimics autoantibodies from patients with idiopathic dilated cardiomyopathy, only altered the rate of cardiomyocyte beating in its bivalent form (Elies et al., 1998). By contrast, monovalent F(ab) fragments of antibodies to M3-muscarinic receptors in Sjögren syndrome and scleroderma behave similarly to bivalent autoantibodies, and inhibit muscarinic receptor-mediated smooth muscle contraction (Cavill et al., 2003). Functional autoantibodies in myasthenia gravis and the Fisher syndrome also do not require antibody bivalency to exert their pathogenic effects (Sterz et al., 1986; Buchwald et al., 1998). A more complex situation may exist in some disorders involving autoimmunity to G-protein-coupled cardiovascular receptors. A monoclonal antibody to the β_2-adrenoceptor that mimics human autoantibodies has an agonist effect on rat cardiomyocytes; however, monovalent fragments of this antibody have an antagonist effect (Mijares et al., 2000). β_2-Adrenoceptors, like other G-protein-coupled receptors, are thought to exist in an inactive monomeric form and an active dimeric form (Hebert et al., 1996). The bivalent antibody may thus induce the formation of an active dimer, enabling signal transduction and producing an agonist-like effect. The monovalent antibody, however, may cause a conformational change in the ligand-binding site, thereby inhibiting allosterically the binding of agonists (Mijares et al., 2000).

Secondary Effects

Studies of tissues obtained from patients with autoantibody-mediated disorders, and from animals passively immunized with IgG from patients or actively immunized with antigen, provide ample evidence for secondary changes in receptor and channel expression. These compensatory changes can result in the opposite clinical picture from that predicted on the basis only of the acute effect of the autoantibody.

While anti-M3-muscarinic receptors acutely inhibit cholinergic responses and cause a functional denervation (Waterman et al., 2000; Goldblatt et al., 2002), long-term exposure to these antibodies is associated with an increase in muscarinic receptor number. M3-muscarinic receptor expression is increased in salivary gland biopsies of patients with Sjögren syndrome (Beroukas et al., 2002) and in mice passively immunized with IgG from a patient (Fang et al., 2004). These changes are pathophysiologically significant, resulting in cholinergic hyperresponsiveness and a characteristic range of symptoms. Early in Sjögren syndrome, prior to irreversible destruction of the salivary glands, sialorrhea occurs (Mignogna et al., 2003). In the passive transfer model of Sjögren syndrome, bladder smooth muscle shows hyper-

responsiveness to cholinergic stimulation, resulting in bladder detrusor instability (Fang et al., 2004), a feature also reported in patients (Leppilahti et al., 2003; Walker et al., 2003). Bronchial and gastrointestinal smooth muscle hyperresponsiveness also occur in patients with Sjögren syndrome (Tsianos et al., 1985; Gudbjornsson et al., 1991; La Corte et al., 1991; Ludviksdottir et al., 2000; Rosztoczy et al., 2001; Hocevar et al., 2003). The molecular mechanisms causing the upregulation of muscarinic receptors in Sjögren syndrome are unknown, but their manipulation offers potential therapeutic targets for the future.

Changes also occur in cardiovascular receptor expression and function in cardiomyopathy, and β-adrenoceptors have been the most thoroughly studied. Iwata et al. (2001) found a reduction in β_1-adrenoceptor expression and receptor sensitivity in the hearts of rabbits immunized for 6 months with β_1-adrenoceptor peptides [however, see Matsui et al. (1997)]. Incubation of isolated neonatal rat cardiomyocytes with anti-β_1-adrenoceptor autoantibodies for 72 h also caused a decrease in β_1-adrenoceptor expression (Podlowski et al., 1998). These findings correspond with clinical data of decreased adrenoceptor number and receptor sensitivity in patients with dilated cardiomyopathy (Bristow et al., 1982; Denniss et al., 1989). The receptor changes in rabbits and isolated cardiomyocytes could be prevented by treatment with the β_1-selective antagonist bisoprolol, which has inverse agonist activity (Podlowski et al., 1998; Iwata et al., 2001). Similarly, the decreased sensitivity to catecholamines and β-adrenoceptor density in human heart can be reversed using the related drug, metoprolol (Heilbrunn et al., 1989). Ventricular ectopy in patients with ventricular arrhythmias and anti-β_1-adrenoceptor antibodies can be suppressed using the β-adrenoceptor antagonist, nadolol (Chiale et al., 2001). Functional anti-β_1-adrenoceptor autoantibodies are found only in a subpopulation of patients with dilated cardiomyopathy (Jahns et al., 1999), so that other mechanisms such as elevated norepinephrine levels must contribute to altered receptor sensitivity and density in the remainder of patients.

Autoantibodies that stimulate M2-muscarinic receptors also occur in some forms of cardiomyopathy, particularly those associated with sinus node dysfunction (Chiale et al., 2001). Muscarinic receptors are upregulated in the human heart in dilated cardiomyopathy (Le Guludec et al., 1997), but it is not known what proportion of these patients have anti-M2-muscarinic receptor autoantibodies. In a model based on active immunization of rabbits, upregulation of M2-muscarinic receptors occurred after 6–12 months of immunization with peptides corresponding to the second extracellular loops of the receptor (Fu et al., 1996; Matsui et al., 1997). The response to cholinergic stimulation of isolated cardiomyocytes from the actively immunized animals was unfortunately not measured, so it is not clear whether there was any change in receptor sensitivity. Nevertheless, preliminary studies show that the muscarinic receptor antagonist, atropine, totally or partly corrects the cardiac electrophysiologic abnormalities in patients with sinus node dysfunction and anti-M2-muscarinic receptor autoantibodies (Chiale et al., 2001).

Alteration in receptor or channel expression is not always restricted to the specific target of the autoantibody, but may involve related receptors or ion channels. In LEMS, the initial downregulation in P/Q-type VGCCs is followed by an upregulation of N-type calcium channels in autonomic nerve terminals (Waterman et al., 1997) and of L-type calcium channels in motor nerve terminals (Xu et al., 1998; Flink and Atchison, 2002). A similar upregulation of L-type calcium channels at motor nerve terminals has been reported following passive transfer of IgG from patients with ALS (Fratantoni et al., 2000). These changes result in skeletal neuromuscular transmission becoming sensitive to dihydropyridine-type blockers. It is not clear whether similar changes occur *in vivo*; if they do, extra care is warranted in prescribing calcium channel blockers to these patients.

Chronic Degenerative Changes

Long-term studies of actively and passively immunized animals indicate that functional autoantibodies can cause chronic degenerative changes that are irreversible. Such changes are particularly evident in disorders of the CNS, in which neuronal death occurs.

Examination of a postmortem brain from a patient with paraneoplastic cerebellar ataxia and autoantibodies that block mGluR1 receptors showed a two-thirds reduction in the number of cerebellar Purkinje cells in the absence of an inflammatory cell infiltrate, consistent with autoantibody-mediated damage (Coesmans et al., 2003). The mechanism of this damage is unknown, but it is interesting to note that mGluR1 receptor antagonists also cause degeneration of Purkinje cells (Catania et al., 2001).

Autoantibodies that activate ionotropic glutamate receptors have been implicated in causing neuronal death through excitotoxic mechanisms, i.e., by overactivation of the glutamate receptor. Antibodies raised against GluR3 receptor peptides caused apoptosis of cultured rat hippocampal neurons in a complement-independent manner (Levite et al., 1999). Cell death was prevented by coadministration of the glutamate/AMPA receptor antagonist, CNQX, or of the GluR3 receptor peptide (Levite et al., 1999). Antibodies to the NR2 subunit of the *N*-methyl-D-aspartate (NMDA) receptor from patients with SLE are also able to kill neurons within 2 days of intracerebral injection into mice; hippocampal cell death was prevented by coadministration of the NMDA receptor antagonist, MK-801 (DeGiorgio et al., 2001). Serum autoantibodies and CSF from a patient with SLE and progressive cognitive decline also caused apoptosis of cultured human fetal neurons (DeGiorgio et al., 2001).

Apoptotic cell death was complement independent, since it also occurred when $F(ab)'_2$ fragments of the autoantibodies were used (DeGiorgio et al., 2001). In some neurologic disorders, complement-mediated mechanisms may also contribute to neuronal death. Thus, anti-GluR3 autoantibodies can trigger complement-mediated cell death; IgG from rabbits immunized with GluR3 peptides caused complement-dependent death of cultured cortical neurons, and GluR3 antibodies and complement were detected in the neocortex and hippocampus of the immunized rabbits (He et al., 1998). IgG and complement deposition also occur in the cerebral cortex of a subset of patients with RE (He et al., 1998; Whitney et al., 1999).

Functional autoantibodies that activate VGCCs are able to induce neuronal cell death by excitotoxic mechanisms. Immunoglobulin from patients with ALS caused complement-independent death of cultured motor neurons within 2 days, and this was prevented by the addition of toxins that block P/Q-type VGCCs (Smith et al., 1994). Furthermore, injection into mice of IgG from a patient with ALS over 2 weeks caused axonal degeneration and denervation of skeletal muscle fibers, which was evident 2–10 weeks after the treatment (Uchitel et al., 1992).

Whereas cell death is an important degenerative change in autoantibody-mediated neurologic disorders, fibrosis is common in diseases affecting non-neuronal tissues. Idiopathic dilated cardiomyopathy is characterized by dilation of the ventricles and thinning of the walls. These findings have been replicated in active immunization of models of dilated cardiomyopathy, using either M2-muscarinic receptor or β_1-adrenoceptor peptides as antigens over 12 months (Matsui et al., 1997). At the light microscopic level, multifocal degeneration and necrosis of myocardiocytes was evident, together with inflammatory cell infiltrates (Matsui et al., 1997). Another study found myocardial hypertrophy and interstitial fibrosis after 6 months' immunization of rabbits with β_1-adrenoceptor peptides (Iwata et al., 2001). Similarly, Fu et al. (1996) noted ultrastructural changes, such as mitochondrial swelling and disruption of cisternae, in rabbit heart after 6 months' immunization with M2-muscarinic receptor peptides.

CONCLUDING REMARKS

The discovery of functional autoantibodies has provided new insights into potential pathophysiologic mechanisms in numerous autoimmune diseases. Importantly, early detection of these autoantibodies and their removal by immunotherapy (plasma exchange, intravenous immunoglobulin) offers an additional approach to treatment that may halt disease progression and prevent the chronic irreversible changes that are typical of autoimmune disease.

There is likely to be a large family of functional autoantibodies. The earliest functional autoantibodies detected in the neurologic diseases myasthenia gravis, LEMS, and neuromyotonia, have their parallel in naturally occurring genetic disorders affecting the skeletal muscle nicotinic receptor (congenital myasthenia), P/Q-type VGCCs (spinocerebellar ataxia), and VGKCs (episodic ataxia/myokymia). It seems more than reasonable to assume that autoimmune equivalents will also be detected for diseases affecting other ion channels or receptors. Indeed, hundreds of G-protein-coupled receptors have now been discovered and sequenced, and these may represent vulnerable targets for as yet unidentified functional autoantibodies. The physiologic approaches and techniques described in this chapter should facilitate the discovery of these and other autoantibodies that have remained undetected using standard immunochemical approaches.

References

Adams, D.D. and Purves, H.D. (1956). The assessment of thyroid function by tracer tests with radioactive iodine. *N. Z. Med. J. 55*, 36–41.

Almon, R.R., Andrew, C.G., and Appel, S.H. (1974). Serum globulin in myasthenia gravis: inhibition of alpha-bungarotoxin binding to acetylcholine receptors. *Science 186*, 55–57.

Andjus, P.R., Khiroug, L., Nistri, A., and Cherubini, E. (1996). ALS IgGs suppress $[Ca^{2+}]i$ rise through P/Q-type calcium channels in central neurones in culture. *Neuroreport 7*, 1914–1916.

Andjus, P.R., Stevic-Marinkovic, Z., and Cherubini, E. (1997). Immunoglobulins from motoneurone disease patients enhance glutamate release from rat hippocampal neurones in culture. *J. Physiol. 504*, 103–112.

Andonopoulos, A.P., Christodoulou, J., Ballas, C., Bounas, A., and Alexopoulos, D. (1998). Autonomic cardiovascular neuropathy in Sjögren's syndrome. A controlled study. *J. Rheumatol. 25*, 2385–2388.

Andrews, P.I., Dichter, M.A., Berkovic, S.F., Newton, M.R., and McNamara, J.O. (1996). Plasmapheresis in Rasmussen's encephalitis. *Neurology 46*, 242–246.

Appel, S.H., Almon, R.R., and Levy, N. (1975). Acetylcholine receptor antibodies in myasthenia gravis. *N. Engl. J. Med. 293*, 760–761.

Appel, S.H., Engelhardt, J.I., Garcia, J., and Stefani, E. (1991). Immunoglobulins from animal models of motor neuron disease and from human amyotrophic lateral sclerosis patients passively transfer physiological abnormalities to the neuromuscular junction. *Proc. Natl. Acad. Sci. USA 88*, 647–651.

Atger, M., Misrahi, M., Young, J., Jolivet, A., Orgiazzi, J., Schaison, G., and Milgrom, E. (1999). Autoantibodies interacting with purified native thyrotropin receptor. *Eur. J. Biochem. 265*, 1022–1031.

Bacman, S., Perez Leiros, C., Sterin-Borda, L., Hubscher, O., Arana, R., and Borda, E. (1998). Autoantibodies against lacrimal gland M3 muscarinic acetylcholine receptors in patients with primary Sjögren's syndrome. *Invest. Ophthalmol. Vis. Sci. 39*, 151–156.

Bacman, S., Sterin-Borda, L., Camusso, J.J., Arana, R., Hubscher, O., and Borda, E. (1996). Circulating antibodies against rat parotid gland M3 muscarinic receptors in primary Sjögren's syndrome. *Clin. Exp. Immunol. 104*, 454–459.

Balestra, B., Moretti, M., Longhi, R., Mantegazza, R., Clementi, F., and Gotti, C. (2000). Antibodies against neuronal nicotinic receptor subtypes in neurological disorders. *J. Neuroimmunol. 102*, 89–97.

Barber, P.A., Anderson, N.E., and Vincent, A. (2000). Morvan's syndrome associated with voltage-gated K^+ channel antibodies. *Neurology 54*, 771–772.

Benatar, M. (2000). Neurological potassium channelopathies. *Q. J. Med. 93*, 787–797.

Beroukas, D., Goodfellow, R., Hiscock, J., Jonsson, R., Gordon, T.P., and Waterman, S.A. (2002). Up-regulation of M3-muscarinic receptors in labial salivary gland acini in primary Sjögren's syndrome. *Lab. Invest. 82*, 203–210.

Borda, E., Leiros, C.P., Bacman, S., Berra, A., and Sterin-Borda, L. (1999). Sjögren autoantibodies modify neonatal cardiac function via M1 muscarinic acetylcholine receptor activation. *Int. J. Cardiol. 70*, 23–32.

Boutjdir, M. (2000). Molecular and ionic basis of congenital complete heart block. *Trends Cardiovasc. Med. 10*, 114–122.

Boutjdir, M., Chen, L., Zhang, Z.H., Tseng, C.E., 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.

Brierley, S.M., Nichols, K., Grasby, D.J., and Waterman, S.A. (2001). Neural mechanisms underlying migrating motor complex formation in mouse isolated colon. *Br. J. Pharmacol.132*, 507–517.

Bristow, M.R., Ginsburg, R., Minobe, W., Cubicciotti, R.S., Sageman, W.S., Lurie, K., Billingham, M.E., Harrison, D.C., and Stinson, E.B. (1982). Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N. Engl. J. Med. 307*, 205–211.

Buchwald, B., Ahangari, R., Weishaupt, A., and Toyka, K.V. (2002). Intravenous immunoglobulins neutralize blocking antibodies in Guillain-Barré syndrome. *Ann. Neurol. 51*, 673–680.

Buchwald, B., Weishaupt, A., Toyka, K.V., and Dudel, J. (1998). Pre- and postsynaptic blockade of neuromuscular transmission by Miller-Fisher syndrome IgG at mouse motor nerve terminals. *Eur. J. Neurosci. 10*, 281–290.

Buckley, C., Oger, J., Clover, L., Tuzun, E., Carpenter, K., Jackson, M., and Vincent, A. (2001). Potassium channel antibodies in two patients with reversible limbic encephalitis. *Ann. Neurol. 50*, 73–78.

Buyon, J.P. (1996). Neonatal lupus. *Curr. Opin. Rheumatol. 8*, 485–490.

Buyon, J.P., Clancy, R., Di Donato, F., Miranda-Carus, M.E., Askanase, A.D., Garcia, J., Qu, Y., Hu, K., Yue, Y., Chan, E.K., and Boutjdir, M. (2002). Cardiac 5-HT(4) serotoninergic receptors, 52kD SSA/Ro and autoimmune-associated congenital heart block. *J. Autoimmun. 19*, 79–86.

Catania, M.V., Bellomo, M., Di Giorgi-Gerevini, V., Seminara, G., Giuffrida, R., Romeo, R., De Blasi, A., and Nicoletti, F. (2001). Endogenous activation of group-I metabotropic glutamate receptors is required for differentiation and survival of cerebellar Purkinje cells. *J. Neurosci. 21*, 7664–7673.

Cavill, D., Waterman, S., and Gordon, T.P. (2002a). 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.

Cavill, D., Waterman, S.A., and Gordon, T.P. (2002b). Failure to detect antibodies to extracellular loop peptides of the muscarinic M3 receptor in primary Sjögren's syndrome. *J. Rheumatol. 29*, 1342–1344.

Cavill, D., Waterman, S.A., and Gordon, T.P. (2003). Antiidiotypic antibodies neutralize autoantibodies that inhibit cholinergic neurotransmission. *Arthritis Rheum. 48*, 3597–3602.

Cavill, D., Waterman, S.A., and Gordon, T.P. (2004). Antibodies raised against the second extracellular loop of the human muscarinic M3 receptor mimic functional autoantibodies in Sjögren's syndrome. *Scand. J. Immunol. 59*, 261–266.

Chiale, P.A., Ferrari, I., Mahler, E., Vallazza, M.A., Elizari, M.V., Rosenbaum, M.B., and Levin, M.J. (2001). Differential profile and biochemical effects of antiautonomic membrane receptor antibodies in ventricular arrhythmias and sinus node dysfunction. *Circulation 103*, 1765–1771.

Christ, T., Wettwer, E., Dobrev, D., Adolph, E., Knaut, M., Wallukat, G., and Ravens, U. (2001). Autoantibodies against the beta1 adrenoceptor from patients with dilated cardiomyopathy prolong action potential duration and enhance contractility in isolated cardiomyocytes. *J. Mol. Cell. Cardiol. 33*, 1515–1525.

Clouston, P.D., Saper, C.B., Arbizu, T., Johnston, I., Lang, B., Newsom-Davis, J., and Posner, J.B. (1992). Paraneoplastic cerebellar degeneration. III. Cerebellar degeneration, cancer, and the Lambert-Eaton myasthenic syndrome. *Neurology 42*, 1944–1950.

Coesmans, M., Smitt, P.A., Linden, D.J., Shigemoto, R., Hirano, T., Yamakawa, Y., van Alphen, A.M., Luo, C., van der Geest, J.N., Kros, J.M., Gaillard, C.A., Frens, M.A., and de Zeeuw, C.I. (2003). Mechanisms underlying cerebellar motor deficits due to mGluR1-autoantibodies. *Ann. Neurol. 53*, 325–336.

Dalakas, M.C., Stein, D.P., Otero, C., Sekul, E., Cupler, E.J., and McCrosky, S. (1994). Effect of high-dose intravenous immunoglobulin on amyotrophic lateral sclerosis and multifocal motor neuropathy. *Arch. Neurol. 51*, 861–864.

de Oliveira, S.F., Pedrosa, R.C., Nascimento, J.H., Campos de Carvalho, A.C., and Masuda, M.O. (1997). Sera from chronic chagasic patients with complex cardiac arrhythmias depress electrogenesis and conduction in isolated rabbit hearts. *Circulation 96*, 2031–2037.

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. *Nat. Med. 7*, 1189–1193.

Delbono, O., Magnelli, V., Sawada, T., Smith, R.G., Appel, S.H., and Stefani, E. (1993). Fab fragments from amyotrophic lateral sclerosis IgG affect calcium channels of skeletal muscle. *Am. J. Physiol. 264*, C537–543.

Denniss, A.R., Marsh, J.D., Quigg, R.J., Gordon, J.B., and Colucci, W.S. (1989). Beta-adrenergic receptor number and adenylate cyclase function in denervated transplanted and cardiomyopathic human hearts. *Circulation 79*, 1028–1034.

Dingledine, R., Borges, K., Bowie, D., and Traynelis, S.F. (1999). The glutamate receptor ion channels. *Pharmacol. Rev. 51*, 7–61.

Drachman, D.B. (2003). Autonomic "myasthenia": the case for an autoimmune pathogenesis. *J. Clin. Invest. 111*, 797–799.

Edwardson, J.M. and Szekeres, P.G. (1999). Endocytosis and recycling of muscarinic receptors. *Life Sci. 64*, 487–494.

Eftekhari, P., Roegel, J.C., Lezoualc'h, F., Fischmeister, R., Imbs, J.L., and Hoebeke, J. (2001). Induction of neonatal lupus in pups of mice immunized with synthetic peptides derived from amino acid sequences of the serotoninergic 5-HT4 receptor. *Eur. J. Immunol. 31*, 573–579.

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-HT4 serotoninergic receptor could explain neonatal lupus congenital heart block. *Eur. J. Immunol. 30*, 2782–2790.

Elies, R., Fu, L.X., Eftekhari, P., Wallukat, G., Schulze, W., Granier, C., Hjalmarson, A., and Hoebeke, J. (1998). Immunochemical and functional characterization of an agonist-like monoclonal antibody against the M2 acetylcholine receptor. *Eur. J. Biochem. 251*, 659–666.

Evoli, A., Tonali, P.A., Padua, L., Monaco, M.L., Scuderi, F., Batocchi, A.P., Marino, M., and Bartoccioni, E. (2003). Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. *Brain 126*, 2304–2311.

Fang, W., Jackson, M.W., Maughan, V., Cavill, D., Smith, A.J., Waterman, S.A., and Gordon T.P. (2004). Passive transfer of Sjögren's syndrome IgG produces the pathophysiology of overactive bladder. *Arthritis Rheum. 50*, 3637–3645.

Ferguson, S.S. (2001). Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol. Rev. 53*, 1–24

Fletcher, C.F., Tottene, A., Lennon, V.A., Wilson, S.M., Dubel, S.J., Paylor, R., Hosford, D.A., Tessarollo,L., McEnery, M.W., Pietrobon, D., Copeland, N.G., and Jenkins, N.A. (2001). Dystonia and cerebellar atrophy in Cacna1a null mice lacking P/Q calcium channel activity. *FASEB J. 15*, 1288–1290.

Flink, M.T. and Atchison, W.D. (2002). Passive transfer of Lambert-Eaton syndrome to mice induces dihydropyridine sensitivity of neuromuscular transmission. *J. Physiol. 543*, 567–576.

Fratantoni, S.A., Weisz, G., Pardal, A.M., Reisin, R.C., and Uchitel, O.D. (2000). Amyotrophic lateral sclerosis IgG-treated neuromuscular junctions develop sensitivity to L-type calcium channel blocker. *Muscle Nerve 23*, 543–550.

Fu, L.X., Magnusson, Y., Bergh, C.H., Liljeqvist, J.A., Waagstein, F., Hjalmarson, A., and Hoebeke, J. (1993). Localization of a functional autoimmune epitope on the muscarinic acetylcholine receptor-2 in patients with idiopathic dilated cardiomyopathy. *J. Clin. Invest. 91*, 1964–1968.

Fu, M.L. (1995). Anti-peptide antibodies against an autoimmune epitope on human muscarinic receptor mimic functional autoantibodies against the same epitope in patients with idiopathic dilated cardiomyopathy. *Eur. Heart. J. 16* (Suppl O), 89–91.

Fu, M.L., Herlitz, H., Schulze, W., Wallukat, G., Micke, P., Eftekhari, P., Sjogren, K.G., Hjalmarson, A., Muller-Esterl, W., and Hoebeke, J. (2000). Autoantibodies against the angiotensin receptor (AT1) in patients with hypertension. *J. Hypertens. 18*, 945–953.

Fu, M.L., Leung, P.S., Wallukat, G., Bergstrom, G., Fu, H., Schulze, W., and Herlitz, H. (1999). Agonist-like activity of antibodies to angiotensin II receptor subtype 1 (AT1) from rats immunized with AT1 receptor peptide. *Blood Press. 8*, 317–324.

Fu, M.L., Schulze, W., Wallukat, G., Hjalmarson, A., and Hoebeke, J. (1995). Functional epitope analysis of the second extracellular loop of the human heart muscarinic acetylcholine receptor. *J. Mol. Cell. Cardiol. 27*, 427–436

Fu, M.L., Schulze, W., Wallukat, G., Hjalmarson, A., and Hoebeke, J. (1996). A synthetic peptide corresponding to the second extracellular loop of the human M2 acetylcholine receptor induces pharmacological and morphological changes in cardiomyocytes by active immunization after 6 months in rabbits. *Clin. Immunol. Immunopathol. 78*, 203–207.

Fukuda, T., Motomura, M., Nakao, Y., Shiraishi, H., Yoshimura, T., Iwanaga, K., Tsujihata, M., and Eguchi, K. (2003). Reduction of P/Q-type calcium channels in the postmortem cerebellum of paraneoplastic cerebellar degeneration with Lambert-Eaton myasthenic syndrome. *Ann. Neurol. 53*, 21–28.

Fukunaga, H., Engel, A.G., Lang, B., Newsom-Davis, J., and Vincent, A. (1983). Passive transfer of Lambert-Eaton myasthenic syndrome with IgG from man to mouse depletes the presynaptic membrane active zones. *Proc. Natl. Acad. Sci. USA 80*, 7636–7640.

Fukuoka, T., Engel, A.G., Lang, B., Newsom-Davis, J., Prior, C., and Wray, D.W. (1987). Lambert-Eaton myasthenic syndrome: I. Early morphological effects of IgG on the presynaptic membrane active zones. *Ann. Neurol. 22*, 193–199.

Gahring, L.C., Rogers, S.W., and Twyman, R.E. (1997). Autoantibodies to glutamate receptor subunit GluR2 in nonfamilial olivopontocerebellar degeneration. *Neurology 48*, 494–500.

Gahring, L.C., Twyman, R.E., Greenlee, J.E. and Roger, S.W. (1995). Autoantibodies to neuronal glutamate receptors in patients with paraneoplastic neurodegenerative syndrome enhance receptor activation. *Mol. Med. 1*, 245–253

Garcia, S., Nascimento, J.H., Bonfa, E., Levy, R., Oliveira, S.F., Tavares, A.V., and de Carvalho, 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.

Goin, J.C., Borda, E., Leiros, C.P., Storino, R., and Sterin-Borda, L. (1994a). Identification of antibodies with muscarinic cholinergic activity in human Chagas' disease: pathological implications. *J. Auton. Nerv. Syst. 47*, 45–52.

Goin, J.C., Leiros, C.P., Borda, E., and Sterin-Borda, L. (1997). Interaction of human chagasic IgG with the second extracellular loop of the human heart muscarinic acetylcholine receptor: functional and pathological implications. *FASEB J. 11*, 77–83.

Goin, J.C., Perez Leiros, C., Borda, E., and Sterin-Borda, L. (1994b). Modification of cholinergic-mediated cellular transmembrane signals by the interaction of human chagasic IgG with cardiac muscarinic receptors. *Neuroimmunomodulation 1*, 284–291.

Goldblatt, F., Gordon, T.P., and Waterman, S.A. (2002). Antibody-mediated gastrointestinal dysmotility in scleroderma. *Gastroenterology 123*, 1144–1150.

Gordon, T.P., Bolstad, A.I., Rischmueller, M., Jonsson, R., and Waterman, S.A. (2001). Autoantibodies in primary Sjogren's syndrome: new insights into mechanisms of autoantibody diversification and disease pathogenesis. *Autoimmunity 34*, 123–132.

Grassi, C., Magnelli, V., Carabelli, V., Sher, E., and Carbone, E. (1994). Inhibition of low- and high-threshold Ca^{2+} channels of human neuroblastoma IMR32 cells by Lambert-Eaton myasthenic syndrome (LEMS) IgGs. *Neurosci. Lett. 181*, 50–56.

Grassi, C., Martire, M., Altobelli, D., Azzena, G.B., and Preziosi, P. (1999). Characterization of Ca(2+)-channels responsible for K(+)-evoked [(3)H]norepinephrine release from rat brain cortex synaptosomes and their response to amyotrophic lateral sclerosis IgGs. *Exp. Neurol. 159*, 520–527.

Graus, F., Lang, B., Pozo-Rosich, P., Saiz, A., Casamitjana, R., and Vincent, A. (2002). P/Q type calcium-channel antibodies in paraneoplastic cerebellar degeneration with lung cancer. *Neurology 59*, 764–766.

Gudbjornsson, B., Hedenstrom, H., Stalenheim, G., and Hallgren, R. (1991). Bronchial hyperresponsiveness to methacholine in patients with primary Sjogren's syndrome. *Ann. Rheum. Dis. 50*, 36–40.

Hart, I.K., Maddison, P., Newsom-Davis, J., Vincent, A., and Mills, K.R. (2002). Phenotypic variants of autoimmune peripheral nerve hyperexcitability. *Brain 125*, 1887–1895.

Hart, I.K., Waters, C., Vincent, A., Newland, C., Beeson, D., Pongs, O., Morris, C., and Newsom-Davis, J. (1997). Autoantibodies detected to expressed K^+ channels are implicated in neuromyotonia. *Ann. Neurol. 41*, 238–246.

He, X.P., Patel, M.S., Whitney, K.D., Janumpalli, S., Tenner, A.J., and McNamara, J.O. (1998). Glutamate receptor GluR3 antibodies and death of cortical cells. *Neuron 20*, 153–163.

Hebert, T.E., Moffett, S., Morello, J.P., Loisel, T.P., Bichet, D.G., Barret, C., and Bouvier, M. (1996). A peptide derived from a beta2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. *J Biol. Chem. 271*, 16384–16392.

Heilbrunn, S.M., Shah, P., Bristow, M.R., Valantine, H.A., Ginsburg, R., and Fowler, M.B. (1989). Increased beta-receptor density and improved hemodynamic response to catecholamine stimulation during long-term metoprolol therapy in heart failure from dilated cardiomyopathy. *Circulation 79*, 483–490.

Hernandez, C.C., Barcellos, L.C., Gimenez, L.E., Cabarcas, R.A., Garcia, S., Pedrosa, R.C., Nascimento, J.H., Kurtenbach, E., Masuda, M.O., and Campos de Carvalho, A.C. (2003). Human chagasic IgGs bind to cardiac muscarinic receptors and impair L-type Ca^{2+} currents. *Cardiovasc. Res. 58*, 55–65.

Hocevar, A., Tomsic, M., Praprotnik, S., Hojnik, M., Kveder, T., and Rozman, B. (2003). Parasympathetic nervous system dysfunction in primary Sjogren's syndrome. *Ann. Rheum. Dis. 62*, 702–704.

Hoch, W., McConville, J., Helms, S., Newsom-Davis, J., Melms, A., and Vincent, A. (2001). Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat. Med. 7*, 365–368.

Hockerman, G.H., Peterson, B.Z., Johnson, B.D., and Catterall, W.A. (1997). Molecular determinants of drug binding and action on L-type calcium channels. *Annu. Rev. Pharmacol. Toxicol. 37*, 361–396.

Houzen, H., Hattori, Y., Kanno, M., Kikuchi, S., Tashiro, K., Motomura, M., Nakao, Y., and Nakamura, T. (1998). Functional evaluation of inhibition of autonomic transmitter release by autoantibody from Lambert-Eaton myasthenic syndrome. *Ann. Neurol. 43*, 677–680.

Hulme, E.C., Lu, Z.L., Saldanha, J.W., and Bee, M.S. (2003). Structure and activation of muscarinic acetylcholine receptors. *Biochem. Soc. Trans. 31*, 29–34.

Iwata, M., Yoshikawa, T., Baba, A., Anzai, T., Nakamura, I., Wainai, Y., Takahashi, T., and Ogawa, S. (2001). Autoimmunity against the second extracellular loop of beta(1)-adrenergic receptors induces beta-adrenergic receptor desensitization and myocardial hypertrophy *in vivo*. *Circ. Res. 88*, 578–586.

Jackson, M.W., Gordon, T.P., and Waterman, S.A. (2004). Disruption of intestinal motility by a calcium channel-stimulating autoantibody in type 1 diabetes. *Gastroenterology 126*, 819–828.

Jahns, R., Boivin, V., Siegmund, C., Inselmann, G., Lohse, M.J., and Boege, F. (1999). Autoantibodies activating human beta1-adrenergic receptors are associated with reduced cardiac function in chronic heart failure. *Circulation 99*, 649–654.

Jones, S.W. (1998). Overview of voltage-dependent calcium channels. *J. Bioenergetics Biomembranes 30*, 299–312.

Kazatchkine, M.D. and Kaveri, S. (2001). Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N. Engl. J. Med. 345*, 747–755.

Kelemen, J., Hedlund, W., Orlin, J.B., Berkman, E.M., and Munsat, T.L. (1983). Plasmapheresis with immunosuppression in amyotrophic lateral sclerosis. *Arch. Neurol. 40*, 752–753.

Kemp, E.H., Waterman, E.A., Hawes, B.E., O'Neill, K., Gottumukkala, R.V., Gawkrodger, D.J., Weetman, A.P., and Watson, P.F. (2002). The melanin-concentrating hormone receptor 1, a novel target of autoantibody responses in vitiligo. *J. Clin. Invest. 109*, 923–930.

Kimura, F., Smith, R.G., Delbono, O., Nyormoi, O., Schneider, T., Nastainczyk, W., Hofmann, F., Stefani, E., and Appel, S.H. (1994). Amyotrophic lateral sclerosis patient antibodies label Ca^{2+} channel alpha 1 subunit. *Ann. Neurol. 35*, 164–171.

Kirvan, C.A., Swedo, S.E., Heuser, J.S., and Cunningham, M.W. (2003). Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. *Nat. Med. 9*, 914–920.

Knowles, C.H., Lang, B., Clover, L., Scott, S.M., Gotti, C., Vincent, A., and Martin, J.E. (2002). A role for autoantibodies in some cases of acquired non-paraneoplastic gut dysmotility. *Scand. J. Gastroenterol. 37*, 166–170.

Komai, K., Iwasa, K., and Takamori, M. (1999). Calcium channel peptide can cause an autoimmune-mediated model of Lambert-Eaton myasthenic syndrome in rats. *J. Neurol. Sci. 166*, 126–130.

La Corte, R., Potena, A., Bajocchi, G., Fabbri, L., and Trotta, F. (1991). Increased bronchial responsiveness in primary Sjogren's syndrome. A sign of tracheobronchial involvement. *Clin. Exp. Rheumatol. 9*, 125–130.

Lang, B., Newsom-Davis, J., Peers, C., Prior, C., and Wray, D.W. (1987). The effect of myasthenic syndrome antibody on presynaptic calcium channels in the mouse. *J. Physiol. 390*, 257–270.

Lang, B., Newsom-Davis, J., Prior, C., and Wray, D. (1983). Antibodies to motor nerve terminals: an electrophysiological study of a human myasthenic syndrome transferred to mouse. *J. Physiol. 344*, 335–345.

Lang, B., Williams, B., Bermudez, I., Cross, H., Hughes, E., Wroe, S., Hart, Y., Vincent, A., and Palace, J. (2001). Autoantibodies in idiopathic forms of epilepsy (abstract). *J. Neuroimmunol. 118*, 145.

Le Guludec, D., Cohen-Solal, A., Delforge, J., Delahaye, N., Syrota, A., and Merlet, P. (1997). Increased myocardial muscarinic receptor density in idiopathic dilated cardiomyopathy: an *in vivo* PET study. *Circulation 96*, 3416–3422.

Lee, E.K., Maselli, R.A., Ellis, W.G., and Agius, M.A. (1998). Morvan's fibrillary chorea: a paraneoplastic manifestation of thymoma. *J. Neurol. Neurosurg. Psychiatry 65*, 857–862.

Leiros, C.P., Sterin-Borda, L., Borda, E.S., Goin, J.C., and Hosey, M.M. (1997). Desensitization and sequestration of human m2 muscarinic acetylcholine receptors by autoantibodies from patients with Chagas' disease. *J. Biol. Chem. 272*, 12989–12993.

Lennon, V.A., Ermilov, L.G., Szurszewski, J.H., and Vernino, S. (2003). Immunization with neuronal nicotinic acetylcholine receptor induces neurological autoimmune disease. *J. Clin. Invest. 111*, 907–913.

Lennon, V.A., Lindstrom, J.M., and Seybold, M.E. (1975). Experimental autoimmune myasthenia: A model of myasthenia gravis in rats and guinea pigs. *J. Exp. Med. 141*, 1365–1375.

Leppilahti, M., Tammela, T.L., Huhtala, H., Kiilholma, P., Leppilahti, K., and Auvinen, A. (2003). Interstitial cystitis-like urinary symptoms among patients with Sjogren's syndrome: a population-based study in Finland. *Am. J. Med. 115*, 62–65.

Lessof, M.H., Crawford, H.J., and Wood, R.M. (1959). Antibody to thyroglobulin in patients with thyroid disease. *Lancet 2*, 1172–1173.

Levite, M., Fleidervish, I.A., Schwarz, A., Pelled, D., and Futerman, A.H. (1999). Autoantibodies to the glutamate receptor kill neurons via activation of the receptor ion channel. *J. Autoimmun. 13*, 61–72.

Levite, M. and Hermelin, A. (1999). Autoimmunity to the glutamate receptor in mice—a model for Rasmussen's encephalitis? *J. Autoimmun. 13*, 73–82.

Leys, K., Lang, B., Vincent, A., and Newsom-Davis, J. (1989). Calcium channel autoantibodies in Lambert-Eaton myasthenic syndrome. *Lancet 2*, 1107.

Liao, Y.H., Wei, Y.M., Wang, M., Wang, Z.H., Yuan, H.T., and Cheng, L.X. (2002). Autoantibodies against AT1-receptor and alpha1-adrenergic receptor in patients with hypertension. *Hypertens. Res. 25*, 641–646.

Liguori, R., Vincent, A., Clover, L., Avoni, P., Plazzi, G., Cortelli, P., Baruzzi, A., Carey, T., Gambetti, P., Lugaresi, E., and Montagna, P. (2001). Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. *Brain 124*, 2417–2426.

Limas, C.J., Goldenberg, I.F., and Limas, C. (1991). Effect of antireceptor antibodies in dilated cardiomyopathy on the cycling of cardiac beta receptors. *Am. Heart J. 122*, 108–114.

Lindstrom, J., Einarson, B., and Merlie, J. (1978). Immunization of rats with polypeptide chains from torpedo acetylcholine receptor causes an autoimmune response to receptors in rat muscle. *Proc. Natl. Acad. Sci. USA 75*, 769–773.

Lindstrom, J.M., Seybold, M.E., Lennon, V.A., Whittingham, S., and Duane, D.D. (1976). Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates and diagnostic value. *Neurology 26*, 1054–1059.

Llinas, R., Sugimori, M., Cherksey, B.D., Smith, R.G., Delbono, O., Stefani, E., and Appel, S. (1993). IgG from amyotrophic lateral sclerosis patients increases current through P-type calcium channels in mammalian cerebellar Purkinje cells and in isolated channel protein in lipid bilayer. *Proc. Natl. Acad. Sci. USA 90*, 11743–11747.

Ludviksdottir, D., Janson, C., Bjornsson, E., Stalenheim, G., Boman, G., Hedenstrom, H., Venge, P., Gudbjornsson, B., and Valtysdottir, S. (2000). Different airway responsiveness profiles in atopic asthma, nonatopic asthma, and Sjogren's syndrome. BHR Study Group. Bronchial hyperresponsiveness. *Allergy 55*, 259–265.

Luther, H.P., Homuth, V., and Wallukat, G. (1997). Alpha 1-adrenergic receptor antibodies in patients with primary hypertension. *Hypertension 29*, 678–682.

Madrid, A., Gil-Peralta, A., Gil-Neciga, E., Gonzalez, J.R., and Jarrin, S. (1996). Morvan's fibrillary chorea: remission after plasmapheresis. *J. Neurol. 243*, 350–353.

Magnusson, Y., Wallukat, G., Waagstein, F., Hjalmarson, A., and Hoebeke, J. (1994). Autoimmunity in idiopathic dilated cardiomyopathy. Characterization of antibodies against the beta 1-adrenoceptor with positive chronotropic effect. *Circulation 89*, 2760–2767.

Mantegazza, R., Bernasconi, P., Baggi, F., Spreafico, R., Ragona, F., Antozzi, C., Bernardi, G., Granata, T., and The Italian Rasmussen's Encephalitis Study Group (2002). Antibodies against GluR3 peptides are not specific for Rasmussen's encephalitis but are also present in epilepsy patients with severe, early onset disease and intractable seizures. *J. Neuroimmunol. 131*, 179–185.

Marion, S., Braun, J.M., Ropars, A., Kohn, L.D., and Charreire, J. (1994). Induction of autoimmunity by immunization of mice with human thyrotropin receptor. *Cell. Immunol. 158*, 329–341.

Mason, W.P., Graus, F., Lang, B., Honnorat, J., Delattre, J.Y., Valldeoriola, F., Antoine, J.C., Rosenblum, M.K., Rosenfeld, M.R., Newsom-Davis, J., Posner, J.B., and Dalmau, J. (1997). Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. *Brain 120*, 1279–1300.

Masuda, M.O., Levin, M., De Oliveira, S.F., Dos Santos Costa, P.C., Bergami, P.L., Dos Santos Almeida, N.A., Pedrosa, R.C., Ferrari, I., Hoebeke, J., and Campos de Carvalho, A.C. (1998). Functionally active cardiac antibodies in chronic Chagas' disease are specifically blocked by *Trypanosoma cruzi* antigens. *FASEB J. 12*, 1551–1558.

Matsui, S., Fu, M.L., Hayase, M., Katsuda, S., Yamaguchi, N., Teraoka, K., Kurihara, T., and Takekoshi, N. (1999). Active immunization of combined beta1-adrenoceptor and M2-muscarinic receptor peptides induces cardiac hypertrophy in rabbits. *J. Card. Failure 5*, 246–254.

Matsui, S., Fu, M.L., Katsuda, S., Hayase, M., Yamaguchi, N., Teraoka, K., Kurihara, T., Takekoshi, N., Murakami, E., Hoebeke, J., and Hjalmarson, A. (1997). Peptides derived from cardiovascular G-protein-coupled receptors induce morphological cardiomyopathic changes in immunized rabbits. *J. Mol. Cell. Cardiol. 29*, 641–655.

Matsui, S., Fu, M.L., Shimizu, M., Fukuoka, T., Teraoka, K., Takekoshi, N., Murakami, E., and Hjalmarson, A. (1995). Dilated cardiomyopathy defines serum autoantibodies against G-protein-coupled cardiovascular receptors. *Autoimmunity 21*, 85–88.

McCombe, P.A., Wilson, R., and Prentice, R.L. (1992). Anti-ganglioside antibodies in peripheral neuropathy. *Clin. Exp. Neurol. 29*, 182–188.

McLachlan, S.M. and Rapoport, B. (1996). Monoclonal human autoantibodies to the TSH receptor — The holy grail and why are we looking for it? *J. Clin. Endocrinol. Metab. 81*, 3152–3154.

Meriney, S.D., Hulsizer, S.C., Lennon, V.A., and Grinnell, A.D. (1996). Lambert-Eaton myasthenic syndrome immunoglobulins react with multiple types of calcium channels in small-cell lung carcinoma. *Ann. Neurol. 40*, 739–749.

Meucci, N., Nobile-Orazio, E., and Scarlato, G. (1996). Intravenous immunoglobulin therapy in amyotrophic lateral sclerosis. *J. Neurol. 243*, 117–120

Mignogna, M.D., Fedele, S., Lo Russo, L., and Lo Muzio, L. (2003). Sialorrhoea as early oral clinical manifestation of primary Sjogren's syndrome? *Rheumatology (Oxford) 42*, 1113–1114.

Mijares, A., Lebesgue, D., Wallukat, G., and Hoebeke, J. (2000). From agonist to antagonist: Fab fragments of an agonist-like monoclonal anti-beta(2)-adrenoceptor antibody behave as antagonists. *Mol. Pharmacol. 58*, 373–379.

Motomura, M. (1999). The Lambert-Eaton myasthenic syndrome: a study of 100 Japanese cases. *Rinsho Shinkeigaku 39*, 1237–1239.

Motomura, M., Lang, B., Johnston, I., Palace, J., Vincent, A., and Newsom-Davis, J. (1997). Incidence of serum anti-P/Q-type and anti-N-type calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. *J. Neurol. Sci. 147*, 35–42.

Muth, J.N., Varadi, G., and Schwartz, A. (2001). Use of transgenic mice to study voltage-dependent Ca^{2+} channels. *Trends Pharmacol. Sci. 22*, 526–532.

Newsom-Davis, J. (1997a). Autoantibody-mediated channelopathies at the neuromuscular junction. *Prog. Clin. Neurosci. 3*, 337–346.

Newsom-Davis, J. (1997b). Autoimmune neuromyotonia (Isaacs' syndrome): an antibody-mediated potassium channelopathy. *Ann. N. Y. Acad. Sci. 835*, 111–119.

Newsom-Davis, J., Pinching, A.J., Vincent, A., and Wilson, S.G. (1978). Function of circulating antibody to acetylcholine receptor in myasthenia gravis: investigation by plasma exchange. *Neurology 28*, 266–272.

Nguyen, K.H., Brayer, J., Cha, S., Diggs, S., Yasunari, U., Hilal, G., Peck, A.B., and Humphreys-Beher, M.G. (2000). Evidence for antimuscarinic acetylcholine receptor antibody-mediated secretory dysfunction in NOD mice. *Arthritis Rheum. 43*, 2297–2306.

Offen, D., Halevi, S., Orion, D., Mosberg, R., Stern-Goldberg, H., Melamed, E., and Atlas, D. (1998). Antibodies from ALS patients inhibit dopamine release mediated by L-type calcium channels. *Neurology 51*, 1100–1103.

Ohlsson, M., Gordon, T.P., and Waterman, S.A. (2002). Role of anti-calcium channel and anti-receptor autoantibodies in autonomic dysfunction in Sjogren's syndrome. *J. Neuroimmunol. 127*, 127–133.

Ohmori, M., Endo, T., Ikeda, M., and Onaya, T. (1992). Immunization with human thyrotrophin receptor peptide induces an increase in thyroid hormone in rabbits. *J. Endocrinol. 135*, 479–484.

Okazaki, T., Tanaka, Y., Nishio, R., Mitsuiye, T., Mizoguchi, A., Wang, J., Ishida, M., Hiai, H., Matsumori, A., Minato, N., and Honjo, T. (2003). Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med. 9*, 1477–1483.

O'Neill, J.H., Murray, N.M., and Newsom-Davis, J. (1988). The Lambert-Eaton myasthenic syndrome. A review of 50 cases. *Brain 111*, 577–596.

O'Shaughnessy, T.J., Yan, H., Kim, J., Middlekauff, E.H., Lee, K.W., Phillips, L.H., and Kim, Y.I. (1998). Amyotrophic lateral sclerosis: serum factors enhance spontaneous and evoked transmitter release at the neuromuscular junction. *Muscle Nerve 21*, 81–90.

Pan, Z., Anderson, C.J., and Stafford, H.A. (1998). Anti-idiotypic antibodies prevent the serologic detection of antiribosomal P autoantibodies in healthy adults. *J. Clin. Invest. 102*, 215–222.

Patrick, J. and Lindstrom, J. (1973). Autoimmune response to acetylcholine receptor. *Science 180*, 871–872.

Peers, C., Johnston, I., Lang, B., and Wray, D. (1993). Cross-linking of presynaptic calcium channels: a mechanism of action for Lambert-Eaton myasthenic syndrome antibodies at the mouse neuromuscular junction. *Neurosci. Lett. 153*, 45–48.

Peers, C., Lang, B., Newsom-Davis, J., and Wray, D.W. (1990). Selective action of myasthenic syndrome antibodies on calcium channels in a rodent neuroblastoma × glioma cell line. *J. Physiol. 421*, 293–308.

Perez Leiros, C., Goren, N., Sterin-Borda, L., Lustig, L., and Borda, E. (1994). Alterations in cardiac muscarinic acetylcholine receptors in mice with autoimmune myocarditis and association with circulating muscarinic receptor-related autoantibodies. *Clin. Auton. Res. 4*, 249–255.

Perez Leiros, C., Sterin-Borda, L., Hubscher, O., Arana, R., and Borda, E.S. (1999). Activation of nitric oxide signaling through muscarinic receptors in submandibular glands by primary Sjogren syndrome antibodies. *Clin. Immunol. 90*, 190–195.

Pinto, A., Iwasa, K., Newland, C., Newsom-Davis, J., and Lang, B. (2002). The action of Lambert-Eaton myasthenic syndrome immunoglobulin G

on cloned human voltage-gated calcium channels. *Muscle Nerve 25*, 715–724.

Podlowski, S., Luther, H.P., Morwinski, R., Muller, J., and Wallukat, G. (1998). Agonistic anti-beta1-adrenergic receptor autoantibodies from cardiomyopathy patients reduce the beta1-adrenergic receptor expression in neonatal rat cardiomyocytes. *Circulation 98*, 2470–2476.

Pozo-Rosich, P., Clover, L., Saiz, A., Vincent, A., and Graus, F. (2003). Voltage-gated potassium channel antibodies in limbic encephalitis. *Ann. Neurol. 54*, 530–533.

Press, R., Mata, S., Lolli, F., Zhu, J., Andersson, T., and Link, H. (2001). Temporal profile of anti-ganglioside antibodies and their relation to clinical parameters and treatment in Guillain-Barré syndrome. *J. Neurol. Sci. 190*, 41–47.

Prior, C., Lang, B., Wray, D., and Newsom-Davis, J. (1985). Action of Lambert-Eaton myasthenic syndrome IgG at mouse motor nerve terminals. *Ann. Neurol. 17*, 587–592.

Qu, Y., Xiao, G.Q., Chen, L., and Boutjdir, M. (2001). Autoantibodies from mothers of children with congenital heart block downregulate cardiac L-type Ca channels. *J. Mol. Cell. Cardiol. 33*, 1153–1163.

Roberts, A., Perera, S., Lang, B., Vincent, A., and Newsom-Davis, J. (1985). Paraneoplastic myasthenic syndrome IgG inhibits $^{45}Ca^{2+}$ flux in a human small cell carcinoma line. *Nature 317*, 737–739.

Roberts, M., Willison, H., Vincent, A., and Newsom-Davis, J. (1994). Serum factor in Miller-Fisher variant of Guillain-Barré syndrome and neurotransmitter release. *Lancet 343*, 454–455.

Robinson, C.P., Brayer, J., Yamachika, S., Esch, T.R., Peck, A.B., Stewart, C.A., Peen, E., Jonsson, R., and Humphreys-Beher, M.G. (1998). Transfer of human serum IgG to nonobese diabetic Igmu null mice reveals a role for autoantibodies in the loss of secretory function of exocrine tissues in Sjogren's syndrome. *Proc. Natl. Acad. Sci. USA 95*, 7538–7543.

Rogers, S.W., Andrews, P.I., Gahring, L.C., Whisenand, T., Cauley, K., Crain, B., Hughes, T.E., Heinemann, S.F., and McNamara, J.O. (1994). Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science 265*, 648–651.

Rosztoczy, A., Kovacs, L., Wittmann, T., Lonovics, J., and Pokorny, G. (2001). Manometric assessment of impaired esophageal motor function in primary Sjogren's syndrome. *Clin. Exp. Rheumatol. 19*, 147–152.

Sanders, D.B., El-Salem, K., Massey, J.M., McConville, J., and Vincent, A. (2003). Clinical aspects of MuSK antibody positive seronegative MG. *Neurology 60*, 1978–1980.

Schott, J.M., Harkness, K., Barnes, J., della Rocchetta, A.I., Vincent, A., and Rossor, M.N. (2003). Amnesia, cerebral atrophy, and autoimmunity. *Lancet 361*, 1266.

Schwerer, B. (2002). Antibodies against gangliosides: a link between preceding infection and immunopathogenesis of Guillain-Barré syndrome. *Microbes Infect. 4*, 373–384.

Shillito, P., Molenaar, P.C., Vincent, A., Leys, K., Zheng, W., van den Berg, R.J., Plomp, J.J., Van Kempen, G.T.H., Chauplannaz, G., Wintzen, A.R., van Dijk, J.G., and Newsom-Davis, J. (1995). Acquired neuromyotonia: Evidence for autoantibodies directed against K^+ channels of peripheral nerves. *Ann. Neurol. 38*, 714–722.

Sillevis Smitt, P., Kinoshita, A., De Leeuw, B., Moll, W., Coesmans, M., Jaarsma, D., Henzen-Logmans, S., Vecht, C., De Zeeuw, C., Sekiyama, N., Nakanishi, S., and Shigemoto, R. (2000). Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. *N. Engl. J. Med. 342*, 21–27.

Sinha, S., Newsom-Davis, J., Mills, K., Byrne, N., Lang, B., and Vincent, A. (1991). Autoimmune aetiology for acquired neuromyotonia (Isaacs' syndrome). *Lancet 338*, 75–77.

Smith, C.U.M. (1996). *Elements of Molecular Neurobiology*. Chichester: John Wiley.

Smith, R.G., Alexianu, M.E., Crawford, G., Nyormoi, O., Stefani, E., and Appel, S.H. (1994). Cytotoxicity of immunoglobulins from amyotrophic lateral sclerosis patients on a hybrid motoneuron cell line. *Proc. Natl. Acad. Sci. USA 91*, 3393–3397.

Solaro, C., Mantegazza, R., Capello, E., Cilli, M., Cardellino, U., Giunti, D., Roccatagliata, L., Giometto, B., Mancardi, G.L., and Uccelli, A. (2001). Cerebral GvHD in a patient with Anti-GluR3 antibodies: a passive transfer study (abstract). *J. Neuroimmunol. 118*, 147.

Staudt, A., Mobini, R., Fu, M., Grosse, Y., Stangl, V., Stangl, K., Thiele, A., Baumann, G., and Felix, S.B. (2001). Beta(1)-adrenoceptor antibodies induce positive inotropic response in isolated cardiomyocytes. *Eur. J. Pharmacol. 423*, 115–119.

Sterin-Borda, L. and Borda, E. (2000). Role of neurotransmitter autoantibodies in the pathogenesis of chagasic peripheral dysautonomia. *Ann. N. Y. Acad. Sci. 917*, 273–280.

Sterz, R., Hohlfeld, R., Rajki, K., Kaul, M., Heininger, K., and Peper, K. (1986). Effector mechanisms in myasthenia gravis: end-plate function after passive transfer of IgG, Fab, and $F(ab')_2$ hybrid molecules. *Muscle Nerve 9*, 306–312.

Striessnig, J. (1999). Pharmacology, structure and function of cardiac L-type Ca^{2+} channels. *Cell Physiol. Biochem. 9*, 242–269.

Sutton, I. and Winer, J.B. (2002). The immunopathogenesis of paraneoplastic neurological syndromes. *Clin Sci (Lond.) 102*, 475–486.

Swedo, S.E. (1994). Sydenham's chorea. A model for childhood autoimmune neuropsychiatric disorders. *JAMA 272*, 1788–1791.

Thomas, M.M. and Dunn, S.M. (1997). Amyotrophic lateral sclerosis immunoglobulins are ineffective in altering calcium influx through presynaptic voltage-sensitive calcium channels. *Neurol. Res. 19*, 129–134.

Toyka, K.V., Drachman, D.B., Griffin, D.E., Pestronk, A., Winkelstein, J.A., Fishbeck, K.H. and Kao, I. (1977). Myasthenia gravis. Study of humoral immune mechanisms by passive transfer to mice. *N. Engl. J. Med. 296*, 125–131.

Tsianos, E.B., Chiras, C.D., Drosos, A.A. and Moutsopoulos, H.M. (1985). Oesophageal dysfunction in patients with primary Sjögren's syndrome. *Ann. Rheum. Dis. 44*, 610–613.

Twyman, R.E., Gahring, L.C., Spiess, J., and Rogers, S.W. (1995). Glutamate receptor antibodies activate a subset of receptors and reveal an agonist binding site. *Neuron 14*, 755–762.

Uchitel, O.D., Appel, S.H., Crawford, F., and Sczcupak, L. (1988). Immunoglobulins from amyotrophic lateral sclerosis patients enhance spontaneous transmitter release from motor-nerve terminals. *Proc. Natl. Acad. Sci. USA* 85, 7371–7374

Uchitel, O.D., Scornik, F., Protti, D.A., Fumberg, C.G., Alvarez, V., and Appel, S.H. (1992). Long-term neuromuscular dysfunction produced by passive transfer of amyotrophic lateral sclerosis immunoglobulins. *Neurology 42*, 2175–2180.

Vernino, S., Adamski, J., Kryzer, T.J., Fealey, R.D., and Lennon, V.A. (1998). Neuronal nicotinic ACh receptor antibody in subacute autonomic neuropathy and cancer-related syndromes. *Neurology 50*, 1806–1813.

Vernino, S., Cheshire, W.P., and Lennon, V.A. (2001). Myasthenia gravis with autoimmune autonomic neuropathy. *Auton. Neurosci. 88*, 187–192.

Vernino, S. and Lennon, V.A. (2002). Ion channel and striational antibodies define a continuum of autoimmune neuromuscular hyperexcitability. *Muscle Nerve 26*, 702–707.

Vernino, S., Low, P.A., Fealey, R.D., Stewart, J.D., Farrugia, G., and Lennon, V.A. (2000). Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N. Engl. J. Med. 343*, 847–855.

Vernino, S., Ermilov, L.G., Sha, L., Szurszewski, J.H., Low, P.A., and Lennon V.A. (2004). Passive transfer of autoimmune autonomic neuropathy to mice. *J. Neurosci. 24*, 7037–7042.

Viglione, M.P., Blandino, J.K., Kim, S.J., and Kim, Y.I. (1993). Effects of Lambert-Eaton syndrome serum and IgG on calcium and sodium cur-

rents in small-cell lung cancer cells. *Ann. N. Y. Acad. Sci. 681*, 418–421.

Viglione, M.P., Creutz, C., and Kim, Y.I. (1992). Lambert-Eaton syndrome: antigen–antibody interaction and calcium current inhibition in chromaffin cells. *Muscle Nerve 15*, 1325–1333.

Viglione, M.P., O'Shaughnessy, T.J., and Kim, Y.I. (1995). Inhibition of calcium currents and exocytosis by Lambert-Eaton syndrome antibodies in human lung cancer cells. *J. Physiol. (Lond.) 488*, 303–317.

Villani, F., Spreafico, R., Farina, L., Giovagnoli, A.R., Bernasconi, P., Granata, T., and Avanzini, G. (2001). Positive response to immunomodulatory therapy in an adult patient with Rasmussen's encephalitis. *Neurology 56*, 248–250.

Vincent, A. (2002). Measuring and evaluating the significance of autoantibodies in neurological disorders. *Clin. Appl. Immunol. Rev. 3*, 127–151.

Vincent, A. (2004). Encephalitis lethargica: part of a spectrum of post-streptococcal autoimmune diseases? *Brain 127*, 2–3.

Vincent, A., Buckley, C., Schott, J.M., Baker, I., Dewar, B.K., Detert, N., Clover, L., Parkinson, A., Bien, C.G., Omer, S., Lang, B., Rossor, M.N., and Palace, J. (2004). Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain 127*, 701–712.

Vincent, A., Lily, O., and Palace, J. (1999). Pathogenic autoantibodies to neuronal proteins in neurologica disorders. *J. Neuroimmunol. 100*, 169–180.

Walker, J., Gordon, T., Lester, S., Downie-Doyle, S., McEvoy, D., Pile, K., Waterman, S., and Rischmueller, M. (2003). Increased severity of lower urinary tract symptoms and daytime somnolence in primary Sjögren's syndrome. *J. Rheumatol. 30*, 2406–2412.

Wallukat, G., Fu, H.M., Matsui, S., Hjalmarson, A., and Fu, M.L. (1999a). Autoantibodies against M2 muscarinic receptors in patients with cardiomyopathy display non-desensitized agonist-like effects. *Life Sci. 64*, 465–469.

Wallukat, G., Homuth, V., Fischer, T., Lindschau, C., Horstkamp, B., Jupner, A., Baur, E., Nissen, E., Vetter, K., Neichel, D., Dudenhausen, J.W., Haller, H., and Luft, F.C. (1999b). Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J. Clin. Invest. 103*, 945–952.

Wallukat, G., Muller, J., Podlowski, S., Nissen, E., Morwinski, R., and Hetzer, R. (1999c). Agonist-like beta-adrenoceptor antibodies in heart failure. *Am. J. Cardiol. 83*, 75H–79H.

Wallukat, G., Wollenberger, A., Morwinski, R., and Pitschner, H.F. (1995). Anti-beta 1-adrenoceptor autoantibodies with chronotropic activity from the serum of patients with dilated cardiomyopathy: mapping of epitopes in the first and second extracellular loops. *J. Mol. Cell. Cardiol. 27*, 397–406.

Waterman, S.A., Gordon, T.P., and Rischmueller, M. (2000). Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjögren's syndrome. *Arthritis Rheum. 43*, 1647–1654.

Waterman, S.A., Lang, B., and Newsom-Davis, J. (1997). Effect of Lambert-Eaton myasthenic syndrome antibodies on autonomic neurons in the mouse. *Ann. Neurol. 42*, 147–156.

Whitney, K.D., Andrews, P.I., and McNamara, J.O. (1999). Immunoglobulin G and complement immunoreactivity in the cerebral cortex of patients with Rasmussen's encephalitis. *Neurology 53*, 699–708.

Wiendl, H., Bien, C.G., Bernasconi, P., Fleckenstein, B., Elger, C.E., Dichgans, J., Mantegazza, R., and Melms, A. (2001). GluR3 antibodies: prevalence in focal epilepsy but no specificity for Rasmussen's encephalitis. *Neurology 57*, 1511–1514.

Williams, G., Peshu, N., Newbold, C.I., Marsh, K., Newton, C.R.J.C., and Lang, B. (2001). Autoantibodies against ion channels in children who develop seizures following CNS infection (abstract). *J. Neuroimmunol. 118*, 148.

Wood, S.J. and Slater, C.R. (2001). Safety factor at the neuromuscular junction. *Prog. Neurobiol. 64*, 393–429.

Xiao, G.Q., Hu, K., and Boutjdir, M. (2001a). 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.

Xiao, G.Q., Qu, Y., Hu, K., and Boutjdir, M. (2001b). Down-regulation of L-type calcium channel in pups born to 52 kDa SSA/Ro immunized rabbits. *FASEB J. 15*, 1539–1545.

Xu, Y.F., Hewett, S.J., and Atchison, W.D. (1998). Passive transfer of Lambert-Eaton myasthenic syndrome induces dihydropyridine sensitivity of ICa in mouse motor nerve terminals. *J. Neurophysiol. 80*, 1056–1069.

Yuki, N., Ang, C.W., Koga, M., Jacobs, B.C., van Doorn, P.A., Hirata, K., and van der Meche, F.G. (2000). Clinical features and response to treatment in Guillain-Barré syndrome associated with antibodies to GM1b ganglioside. *Ann. Neurol. 47*, 314–321.

Yuki, N. and Miyagi, F. (1996). Possible mechanism of intravenous immunoglobulin treatment on anti-GM1 antibody-mediated neuropathies. *J. Neurol. Sci. 139*, 160–162.

Zeng, F.Y. and Wess, J. (1999). Identification and molecular characterization of m3 muscarinic receptor dimers. *J. Biol. Chem. 274*, 19487–19497.

Zhainazarov, A.B., Annunziata, P., Toneatto, S., Cherubini, E., and Nistri, A. (1994). Serum fractions from amyotrophic lateral sclerosis patients depress voltage-activated Ca^{2+} currents of rat cerebellar granule cells in culture. *Neurosci. Lett. 172*, 111–114.

CHAPTER

18

Adhesion Molecules and Chemoattractants in the Pathogenesis and Treatment of Autoimmune Diseases

CHARLES R. MACKAY*
**Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia*

ULRICH H. VON ANDRIAN†
†CBR Institute for Biomedical Research, Harvard Medical School, Boston, Massachusetts, USA

Cell migration is fundamental to inflammatory processes. The molecules that facilitate cell migration, such as adhesion molecules and chemoattractant receptors, also represent highly attractive targets for therapeutic intervention. Moreover, chemokine receptors and adhesion molecules have proved to be excellent markers for distinguishing functional subsets of T and B cells, since certain subsets need to be placed in the appropriate place to perform their immunologic roles. The discovery of numerous adhesion molecules and chemokine receptors over the past 20 years has enabled a more complete understanding of how specialized leukocyte subsets are channeled to appropriate microenvironments during an immune response. There is also an appreciation that, under resting conditions, lymphocyte migration is highly rationalized, with the existence of lymphoid versus non-lymphoid tissue migration streams, and tissue-specific migration pathways through the skin and gut. Different types of immune response evoke different subsets of leukocytes, which use different adhesion molecules and chemoattractant receptors. Thus type 1 inflammatory responses, which depend on type 1 helper T (Th1) cells and macrophages, differ from type 2 responses, which depend on Th2 cells and eosinophils, in the types of chemokines and chemokine receptors used. This chapter will concentrate on the adhesion, chemoattraction, and migration of leukocytes, particularly T cells, that are associated with autoimmune responses. Adhesion molecules and chemoattractant receptors are particularly promising targets for the development of new therapies to treat autoimmune diseases, so a brief synopsis of the most promising targets and the status of preclinical and clinical trials will be outlined.

MICROVASCULAR DETERMINANTS OF T-CELL RECRUITMENT

Specialized microvessels control T-cell migration from blood into tissues. In most microvascular beds, except the spleen, lung, and liver, postcapillary venules, but not arterioles or capillaries, bind leukocytes. Because intravascular leukocytes are subjected to extreme physical conditions (sheer stress), cells use specialized adhesion receptors, the selectins, which form stable bonds with counter-receptors in the vascular wall (Table 18.1) (Carlos and Harlan, 1994; Springer, 1994). Adhesion receptors on leukocytes and on vascular endothelial cells also function as tissue-specific recognition molecules. For example, the specialized high endothelial venules (HEVs) in lymph nodes and Peyer patches constitutively express *vascular addressins*, which

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 18.1 Selectins, integrins, and their ligands

Adhesion molecule	Distribution	Ligand(s), receptor(s)	Role in T-cell migration
Selectins			
L-selectin (CD62L)	Most leukocytes	PNAd, PSGL-1, MAdCAM-1, E-selectin, others	Homing to lymph nodes and Peyer patches
E-selectin (CD62E)	Endothelial cells	PSGL-1, ESL-1, CLA, sLe^{X+} glycol-proteins and -lipids	Memory/effector cell homing to skin and sites of inflammation
P-selectin (CD62P)	Endothelial cells, platelets	PSGL-1, CD24, PNAd	Memory/effector (Th1) cell homing to sites of inflammation; platelet-mediated interaction with $PNAd^+$ venules
Selectin ligands			
Sialyl-$Lewis^X$ (sCD15)	Myeloid cells; some memory (Th1) cells; HEVs; other cell types	All selectins	Function depends on presentation molecule
PSGL-1	All leukocytes	P-selectin; also binds L-/E-selectin	Effector cell homing to inflamed tissues
Peripheral node addressin (PNAd)	HEVs in LNs and inflamed tissues	L-selectin, P-selectin	Naïve/central memory T-cell homing to LNs
Cutaneous lymphocyte antigen (CLA)	Skin-homing T cells, DCs, granulocytes	E-selectin	Memory/effector T-cell homing to inflamed skin
β2 Integrins*			
αLβ2 (LFA-1, CD11a/CD18)	All leukocytes	ICAM-1, -2, -3, -4 and -5	Homing; inflammation; adhesion to APCs
αMβ2 (Mac-1, CD11b/CD18)	Myeloid cells, some activated T cells	ICAM-1, factor X, fibrinogen, $C3b_i$	Unknown
αXβ2 (p150/95, CD11c/CD18)	Dendritic cells	Fibrinogen, $C3b_i$	Unknown
αDβ2 (CD11d/CD18)	Monocytes, macrophages, eosinophils	VCAM-1, ICAM-1 and -3	Unknown
α4 Integrins*			
α4β1 (VLA-4)	Most leukocytes except neutrophils	VCAM-1, fibronectin, α4 integrin	Memory/effector cell homing to inflamed tissues, esp. lung
α4β7	Lymphocytes, NK, mast cells, basophils, monocytes	MAdCAM-1, fibronectin, weak binding to VCAM-1	Homing to gut and associated lymphoid tissues
Immunoglobulin superfamily			
ICAM-1 (CD54)	Most cell types	LFA-1, Mac-1, fibrinogen	Critical endothelial ligand for β2 integrins
ICAM-2 (CD102)	Endothelial cells, platelets	LFA-1	Unknown
VCAM-1 (CD106)	Endothelial cells, BM stroma, FDC, osteoblasts, mesothelium	α4β1, α4β7, αDβ2	Memory/effector cell homing to inflamed tissues
MAdCAM-1	HEVs in gut-associated lymphoid tissues; lamina propria	α4β7, L-selectin	T-cell homing to gut-associated lymphoid tissues

*The integrins are named according to the composition of their constituent α and β protein chains, which are each identified by a number or letter (e.g., α4β1 or αDβ2), but some integrins are often referred to by alternative names, e.g., LFA-1, shown in parenthesis.

APC, antigen-presenting cell; BM, bone marrow; CD, cluster of differentiation; CLA, cutaneous lymphocyte antigen; ESL-1, E-selectin ligand-1; FucT-VII, fucosyltransferase-VII; GlyCAM-1, glycosylation-dependent cell adhesion molecule-1; HEV, high endothelial venule; ICAM, intercellular cell adhesion molecule; IL-1, interleukin-1; LFA-1, leukocyte function-associated antigen-1; LPS, lipopolysaccharide; Mac-1, macrophage antigen-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; NK, natural killer cell; p150/95, protein with 150 kDa and 95 kDa subunits; PNAd, peripheral node addressin; PSGL-1, P-selectin glycoprotein ligand-1; sgp200, sialylated glycoprotein of 200 kD; sLe^X, sialyl-$Lewis^X$; Th, T-helper; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

support a considerable rate of traffic of "resting" lymphocytes, i.e., naïve T and B cells and some memory T cells, whereas endothelial cells elsewhere permit only minimal leukocyte binding unless they are exposed to inflammatory mediators. Thus, two vascular beds can be distinguished, which support fundamentally different types of leukocyte traffic: those in lymphoid organs, which recruit lymphocytes constitutively; and those in normal or inflamed tissues, which typically recruit effector leukocytes whose job is to combat pathogens (Figure 18.1).

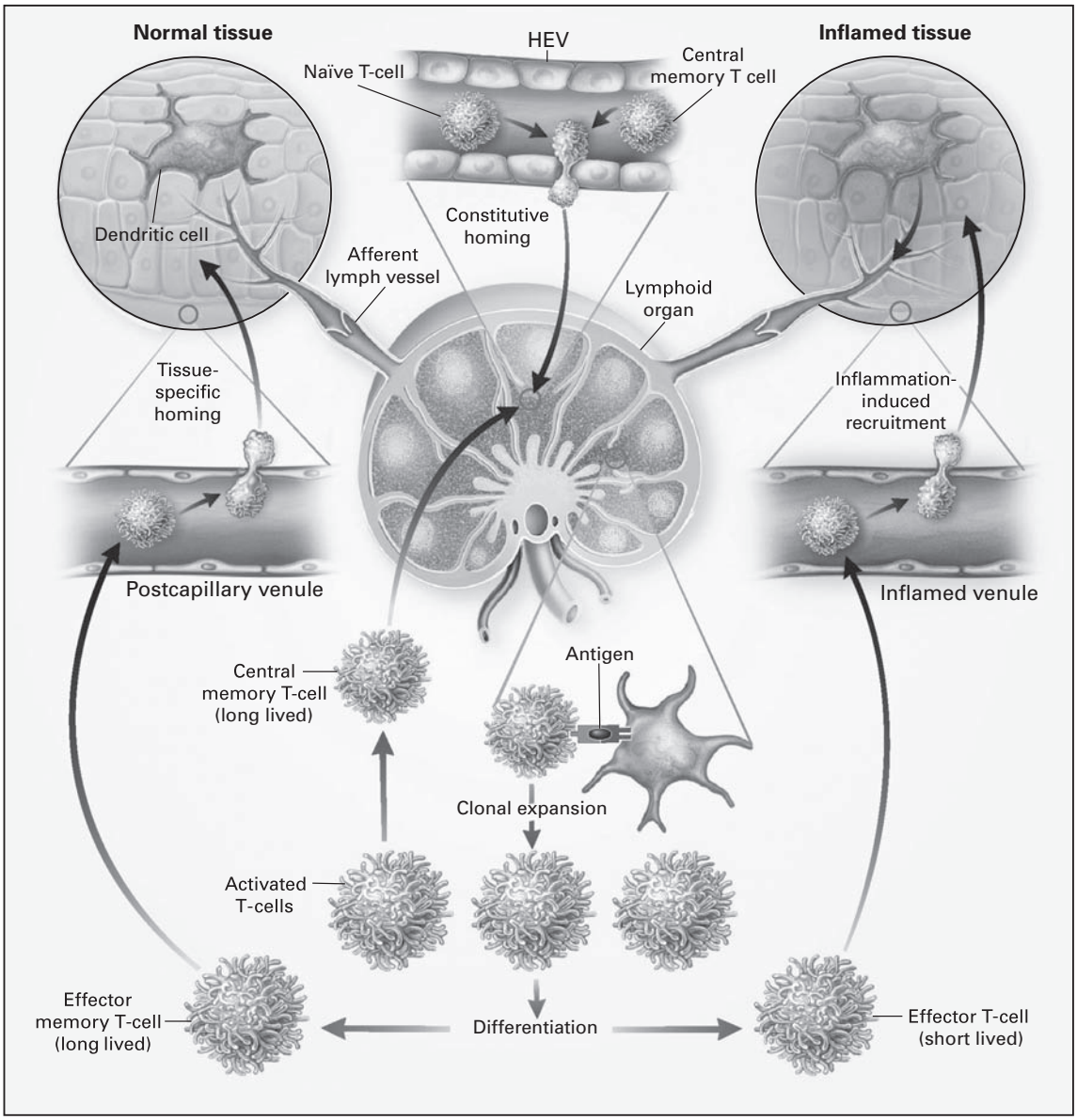


FIGURE 18.1 Migratory routes of T cells. Naïve T cells home continuously from the blood to lymph nodes and other secondary lymphoid tissues. Homing to lymph nodes occurs across high endothelial venules (HEVs), which express traffic molecules for constitutive lymphocyte recruitment. Lymph nodes are percolated by lymph fluid that is channeled to them from peripheral tissues, where dendritic cells (DCs) collect antigenic material. In inflamed tissues, DCs are mobilized to carry antigen to lymph nodes, where they stimulate antigen-specific T cells. Upon stimulation, T cells proliferate and differentiate into effector cells, which express receptors that enable them to migrate to sites of inflammation. While most effector cells are short lived, a few antigen-experienced cells survive for a long time. These memory cells are subdivided into two populations based on their migratory ability (Sallusto et al., 1999): one subset is termed *effector memory* T cells and is localized to peripheral tissues, and the other *central memory* T cells that express a similar repertoire of homing molecules as do naïve T cells migrate preferentially to lymphoid organs. The traffic signals that direct effector and memory cells to peripheral tissues are organ specific; for example, molecules required for migration to the skin are different from those to the gut; they are modulated by inflammatory mediators and are distinct for different T-cell subsets, e.g., Th1 and Th2 cells show differences in their responses to various chemoattractants.

Reproduced from von Andrian and Mackay (2000), with permission.

ADHESION MOLECULES

A central paradigm that underpins leukocyte extravasation is the multistep model of leukocyte binding to endothelium (Springer, 1994). Leukocytes must engage several sequential adhesion steps in order to leave the circulation (Figure 18.2). Initially, tethers are formed by adhesion receptors that are specialized to engage rapidly and with high tensile strength. The most important initiators of adhesion are the three selectins, expressed on leukocytes (L-selectin), endothelial cells (P- and E-selectin), and activated platelets (P-selectin) (Kansas, 1996). All selectins bind oligosaccharides related to sialyl-Lewis^X. The most relevant selectin-binding sugars are components of sialomucin-like glycoproteins (Vestweber and Blanks, 1999). Selectin-mediated binding of leukocytes to endothelium results in a characteristic rolling motion. To stop rolling, cells must engage additional (secondary) receptors (Lawrence and Springer, 1991; von Andrian et al., 1991), members of the integrin family, specifically LFA-1 (CD11a/CD18, $\alpha L\beta 2$), and the two $\alpha 4$ integrins, $\alpha 4\beta 1$ (VLA-4) and $\alpha 4\beta 7$. $\alpha 4$ Integrins can also mediate tethering and rolling, albeit less efficiently than selectins (Alon et al., 1995; Berlin et al., 1995).

CHEMOATTRACTANTS AND THEIR RECEPTORS

While selectins are constitutively active, integrins are activated by signals from chemoattractant receptors (Cyster, 1999; Kim and Broxmeyer, 1999). Just like adhesion molecules, chemoattractant receptors can be upregulated or lost as cells differentiate, allowing leukocytes to coordinate their migratory routes with their immunologic function. The most extensive family of chemoattractants, particularly for adaptive immune responses, are the chemokines (Table 18.2). While some chemokines trigger intravascular adhesion (Campbell et al., 1998), others direct leukocyte migration into and within extravascular spaces. Since lymphocytes must be positioned correctly to interact with other cells, the pattern of chemokine receptors and the type and distribution of chemokines in tissues critically influence immune responses (Cyster, 1999; Sallusto et al., 1999; Syrbe et al., 1999). Over 50 chemokines and 18 chemokine receptors have been identified (Kim and Broxmeyer, 1999; Zlotnik and Yoshie, 2000). It has been proposed that this multitude ensures robust recruitment of inflammatory cells, even if individual pathways are disabled by genetic defects or through subversion by pathogens (Mantovani, 1999). Also, the large number of chemokines may reflect the complex nature of the mammalian immune system, the numerous leukocyte cell types, and the distinct microenvironments wherein migration must be regulated. For instance, neutrophils may use different chemoattractant receptors sequentially, to travel from A to B to C, in a process termed *multistep navigation* (Foxman et al., 1997).

Chemokines are divided into four subfamilies based on the position of N-terminal cysteine residues. Chemokines are also classified as *inflammatory* or *lymphoid*. Inflammatory chemokines attract primarily neutrophils, monocytes, and other effector leukocytes. The major sources of these chemokines are activated endothelial cells, epithelial cells, and leukocytes, although virtually any cell has the potential to produce chemokines (and attract leukocytes) when stimulated by lipopolysaccharides (endotoxin) or inflammatory cytokines. Lymphoid chemokines are primarily produced in lymphoid tissues. They maintain constitutive leukocyte traffic and compartmentalization in the lymphoid tissue (Cyster, 1999; Jung and Littman, 1999). In addition to the chemokine receptors, the closely related "classical" chemoattractant receptors, such as C5aR, serve similar roles, particularly during inflammatory responses. The very large number of chemokines, and patterns of chemokine receptor expression on subsets of T cells, suggest that this system of chemoattractants developed to meet the elaborate demands of the adaptive immune system.

MULTISTEP ADHESION CASCADES

One consideration for drug development is how selective or broad-acting an antagonist should be. Chemokine and chemokine-receptor redundancy, and the expression of multiple receptors by cell types such as monocytes, questions whether a single receptor antagonist will always be effective. (Mayadas et al., 1993; Arbones et al., 1994; Bullard et al., 1996; Frenette et al., 1996; Wagner et al., 1996; Andrew et al., 1998; Berlin-Rufenach et al., 1999; Forster et al., 1999; Robinson et al., 1999; Stein et al., 2000; Warnock et al., 2000). Deficiencies in any one of the steps, i.e., selectin binding, chemoattractant signaling or integrin binding, can derail cell migration. The large number of leukocyte adhesion receptors, endothelial counter-receptor(s), chemokines, and chemokine receptors means that there are hundreds of possible three-step combinations, and thus great flexibility in the regulation of leukocyte migration (Butcher, 1991; Springer, 1994). Indeed, several multistep combinations occur uniquely in specialized tissues and serve to attract very distinct subsets of blood-borne leukocytes (Springer, 1994; Butcher and Picker, 1996; Robert and Kupper, 1999; Stein et al., 2000; Warnock et al., 2000). One consideration for drug development is, how selective do inhibitors of cell migration need to be? Apparent chemokine and chemokine receptor redundancy, for instance, multiple receptor expression on monocytes, questions whether a single receptor antagonist will always be effective. Nevertheless, studies *in vivo* using single-receptor antagonists, or with antibodies to individual

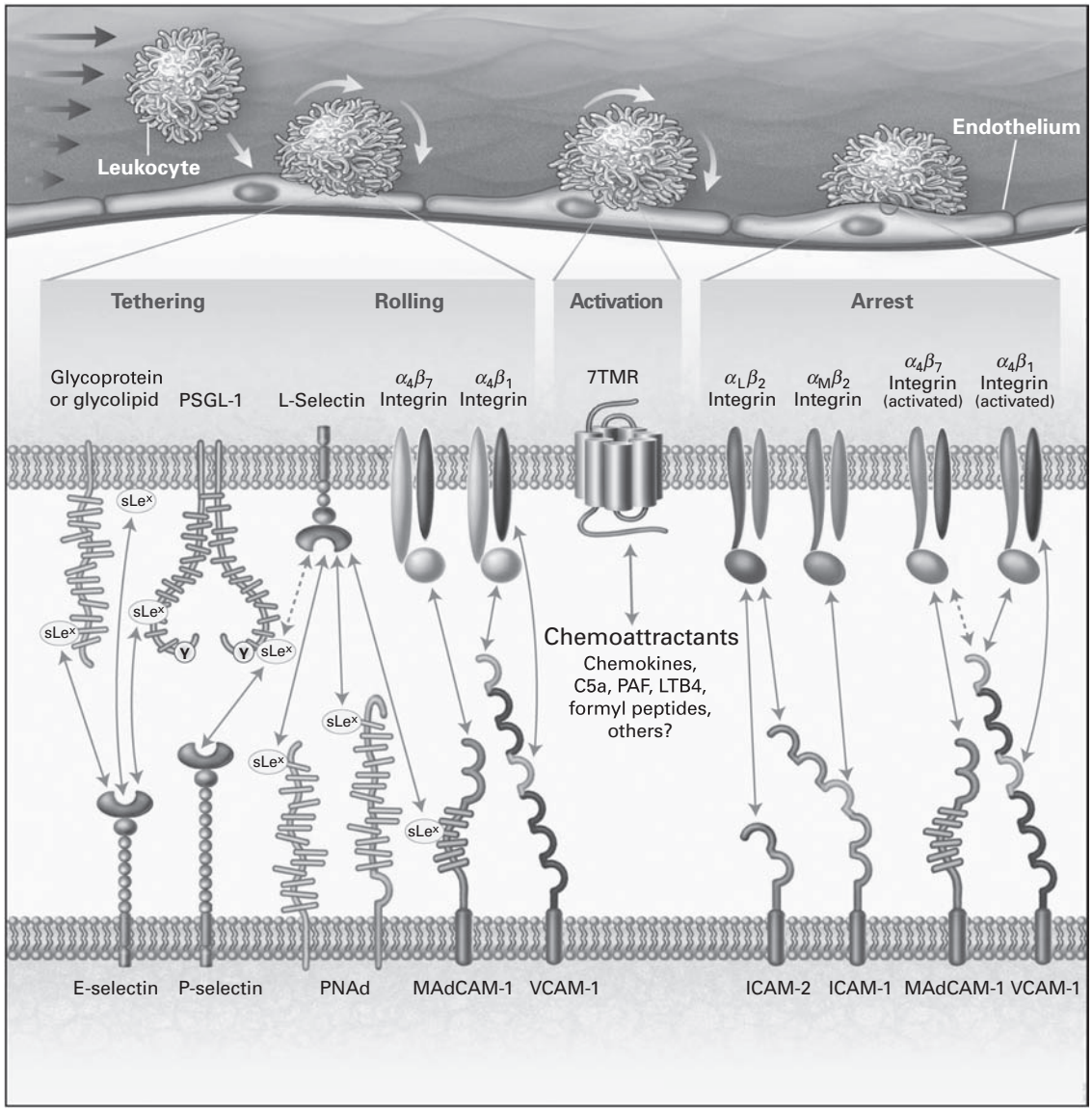


FIGURE 18.2 Essential molecular players in the multistep adhesion cascade. The top of this schematic diagram depicts the four distinct adhesion steps that leukocytes must undergo to accumulate in a blood vessel. Also shown are the predominant molecular determinants of each step with respect to leukocytes (middle of the diagram) and endothelial cells (bottom). A number of molecules can interact with more than one partner, symbolized by arrows. Leukocytes in the bloodstream (arrows at left symbolize the laminar flow profile) tether to endothelial cells and roll slowly downstream. Tethering is greatly facilitated by leukocyte receptors that occur at high density on the tips of microvillous surface protrusions (L-selectin, PSGL-1, and α4 integrins), whereas subsequent rolling is not influenced by the topography of adhesion receptors (Stein et al., 1999). The most efficient tethering molecules are L- and P-selectin. L-selectin recognizes sulfated sialyl-Lewis^X (sLe^X)-like sugars (PNAd) in high endothelial venules. It may also interact with other ligands on inflamed endothelial cells (not shown) and with PSGL-1 on adherent leukocytes (broken arrow). PSGL-1 binding to L- and P-selectin requires decoration with an sLe^X-like sugar in close vicinity to an N-terminal motif containing three tyrosines (Y) that must be sulfated. E-selectin can also interact with PSGL-1, but does not require sulfation and also recognizes other sLe^X-bearing glycoconjugates. E-selectin and the α4 integrins can tether some leukocytes, but their predominant function is to reduce rolling velocities. Rolling leukocytes respond to chemoattractants on endothelial cells because they express specific receptors with seven transmembrane domains (7TMR), which transmit intracellular signals through G proteins. The activating signal induces rapid activation of β2 and/or α4 integrins, which bind to endothelial immunoglobulin superfamily members. Note that α4 integrins can mediate activation-independent rolling interactions as well as firm arrest. However, the latter function requires integrin activation, symbolized by the open conformation of the integrin heterodimer. For a list of abbreviations, see Table 18.1.
Reproduced from von Andrian and Mackay (2000), with permission.

TABLE 18.2 Chemoattractant receptors and their ligands in leukocyte migration

Biologic activity	Chemoattractant receptor(s)	Predominant ligands*,†
Naïve T-cell migration		
To LN and Peyer patch	CCR7	CCL21 (SLC); CCL19 (Mip-3β)
Within lymphoid tissues	CXCR4	CXCL12 (SDF-1α)
Memory T-cell migration		
To lymphoid tissues	CCR7	CCL21 (SLC); CCL19 (Mip-3β)
To the skin	CCR4, CCR10	CCL17 (TARC); CCL22 (MDC-1) CCL27 (CTACK)
To the gut	CCR9	CCL25 (TECK)
To sites of inflammation	CCR2 CCR5	CCL2 (MCP-1) CCL5 (RANTES), CCL4 (MIP-1β)
Effector T-cell migration		
Th1 cells	CCR2 CCR5 CXCR3	CCL2 (MCP-1) CCL5, CCL4 (MIP-1β) CXCL9, 10, 11
Th2 cells	CCR3 CCR4 CCR8 CXCR4	CCL11 (Eotaxin) CCL17 (TARC); CCL22 (MDC-1) CCL1 (I-309) CXCL12 (SDF-1α)
B-cell migration	CCR7 CXCR4 CXCR5	CCL21 (SLC); CCL19 (Mip-3β) CXCL12 (SDF-1α) CXCL13 (BLC)
Dendritic cell migration		
To lymphoid tissues	CCR7	CCL21 (SLC); CCL19 (Mip-3β)
To normal skin	CCR6	CCL20 (MIP-3α)
To sites of inflammation	CCR1 CCR2 CCR5 CXCR1	CCL5, CCL3 CCL2 (MCP-1) CCL5, CCL4 (MIP-1β) CXCL8 (IL-8)
Monocyte recruitment	CCR1 CCR2 CCR5 CCR8 CXCR1 CX_3CR1 C5aR	CCL5, CCL3 CCL2 (MCP-1) CCL5, CCL4 (MIP-1β) CCL1 (I-309) CXCL8 (IL-8) C5a
Neutrophil recruitment	CXCR1 CXCR2 C5aR fmlpR	CXCL8 (IL-8) CXCL8 (IL-8); Groα, β, γ C5a fmlp
Eosinophil recruitment	CCR3 C5aR	CCL11 (Eotaxin) C5a

*The physiologic function of several chemokine receptors are multiple, since CCR9 promotes prothymocyte homing to the thymus, and T-cell migration to the gut; CXCR4 is widely expressed and appears to have multiple roles.

†An established nomenclature for chemokines (Zlotnik and Yoshie, 2000) has superseded older and in many cases duplicate names for a single chemokine. Frequently used alternative names are shown in parenthesis.

CCR, receptor for CC chemokine; CXCR, receptor for CXC chemokine; LN, lymph node, Th, helper T cell.

chemokine ligands, have proved very effective in animal models (Gong et al., 1997; Gonzalo et al., 1998; Ulbrich et al., 2003; Szekanecz and Koch, 2004). However, there has been considerable interest in targeting chemoattractant receptor signaling pathways, where numerous receptors would be affected: the phosphoinositide 3-kinases (PI3K) are a good example (Ward, 2004). Mice lacking one of the four isoforms, PI3Kγ, are viable, but their neutrophils and macrophages show impaired activation through chemoattractant receptors (Hirsch et al., 2000; Li et al., 2000), and poor chemotactic responses.

ORGANIZED LYMPHOID TISSUES: VENUES FOR NAÏVE T-CELL HOMING AND DENDRITIC CELL INTERACTIONS

The adhesion cascades that mediate homing of naïve T cells to lymph nodes and Peyer patches are now well understood (Bargatze et al., 1995; Warnock et al., 1998; Stein et al., 2000; Warnock et al., 2000). Circulating lymphocytes gain access to both organs by crossing HEVs (Marchesi and Gowans, 1964; Girard and Springer, 1995). A characteristic feature of HEVs in lymph nodes is expression of the peripheral node addressin (PNAd), whereas HEVs in Peyer patches express mucosal addressin cell adhesion molecule (MAdCAM)-1. L-Selectin binds both of these addressins, but it sustains rolling only on PNAd in lymph nodes, whereas in Peyer patches additional binding of α4β7 integrin to MAdCAM-1 is required (Bargatze et al., 1995; Warnock et al., 1998). Cells expressing high levels of α4β7, such as gut-homing effector T cells, tether directly to MAdCAM-1, whereas naïve T cells first engage L-selectin (Bargatze et al., 1995; Kunkel et al., 1998). The chemokines and chemokine receptors important for lymphocyte entry to lymphoid tissues are CCL19 and CCL21 binding to CCR7. CCR7 is expressed at high levels by naïve T cells and central memory T cells, but not effector memory T cells. CCR7 and another chemokine receptor, CXCR4, are expressed in a reciprocal manner with inflammatory chemokine receptors, such as CCR5 (Bleul et al., 1997), which marks mainly effector memory T cells.

Naïve T cells are normally restricted in their migration to lymphoid tissues. It is here where naïve T cells and other cells necessary for a primary immune response must gather and interact. Dendritic cells (DCs) in lymphoid tissues are usually derived from monocytes, which enter tissues and differentiate to "immature" DCs (see Chapter 4). Both monocytes and immature DCs express receptors for inflammatory chemokines and other chemoattractants that are released during inflammatory responses (Dieu et al., 1998; Sallusto et al., 1998). A subset of DCs in skin, the Langerhans cells, also express CCR6, which may promote their constitutive migration through normal skin. Immature DCs patrol tissues and engulf microorganisms, dead cells, and cellular debris. Upon exposure to inflammatory stimuli, they travel to regional lymph nodes via afferent lymph vessels, lose their receptors for inflammatory chemokines, and upregulate receptors for lymphoid chemokines (Sallusto and Lanzavecchia, 1999). CCR7 expression allows maturing DCs to home to the T-cell area of lymph nodes. While in transit, DCs also alter their functional role to that of antigen presentation, and begin producing chemokines that attract subsets of T cells (Sallusto and Lanzavecchia, 1999).

The rationale for naïve T cells being restricted in their migration to lymphoid tissues is that the large-scale percolation of naïve lymphocytes, all with different antigen receptors, allows the very rare antigen-specific cell to encounter its cognate antigen. Moreover, lymphoid tissues are the venue for T-cell–DC interactions, T–B-cell interactions, including the germinal center (GC) reaction, and are also a place to which antigen drains and is captured and retained. It is also possible that the restriction of naïve T cells to lymphoid tissues facilitates immune tolerance, by restricting exposure of naïve T cells to peripheral self-antigens.

EFFECTOR T-CELL MIGRATION

Naïve T cells differentiate to effector cells in lymphoid organs (see Figure 18.1). However, the principal sites where effector cells are needed are peripheral tissues, where pathogens are encountered. Thus, effector cells upregulate receptors for inflammation-induced endothelial adhesion molecules and inflammatory chemoattractants (Butcher and Picker, 1996). However, different pathogens require different effector responses, e.g., by Th1 or Th2 cells, and Th1 and Th2 cells express distinct receptors and obey different traffic signals (Syrbe et al., 1999). Distinctive chemokine receptors on Th1 cells include CCR5 and CXCR3 (Bonecchi et al., 1998; Sallusto et al., 1998), which bind inflammatory chemokines (see Table 18.1). In rheumatoid arthritis and multiple sclerosis (both often thought of as Th1 related), virtually all infiltrating T cells express CCR5 and CXCR3 (Qin et al., 1998). People with a homozygous mutation that disrupts the CCR5 gene (Paxton and Kang, 1998) may also be less susceptible to some inflammatory disorders, including rheumatoid arthritis (Gomez-Reino et al., 1999). Adhesion molecules also play a role; Th1 cells express abundant selectin ligands. P- and E-selectin, which occur on inflamed endothelium, and their ligand, PSGL-1, are critical for Th1 cell migration to inflamed skin (Austrup et al., 1997; Borges et al., 1997) and peritoneum (Xie et al., 1999). Expression of fucosyltransferase-VII is necessary for cells to synthesize selectin ligands (Maly et al., 1996). This enzyme is induced by interleukin (IL)-12, which drives Th1 differentiation, whereas T-cell exposure to the Th2 cytokine

IL-4 downmodulates selectin ligand expression (Wagers et al., 1998; Lim et al., 1999).

Th2 cells also express distinctive chemoattractant receptors, including CRTh2 and CCR3 (Sallusto et al., 1997). Eotaxin, a ligand of CCR3, has been implicated in eosinophil recruitment into hyperreactive airways and is prominent in mucosal tissues undergoing allergic and antiparasitic responses (Jose et al., 1994). Eotaxin production is stimulated by Th2 cytokines, such as IL-4 or IL-13, and is absent from Th1-mediated lesions (Ponath et al., 1996). CCR3 is also expressed on basophils and mast cells, which presumably allows these allergy-related leukocytes to colocalize and interact at sites of allergic inflammation (Gutierrez-Ramos et al., 1999). Other chemoattractant receptors that are also preferentially, but not exclusively, expressed on Th2 cells include CCR4, CCR8 and CXCR4 (Sallusto et al., 1998).

HOMING TO NON-LYMPHOID TISSUES

Antigen-experienced T cells often display a bias in their tissue migration patterns, which enhances their chances for re-encountering antigen. T cells that respond to cutaneous pathogens in skin-draining lymph nodes migrate preferentially to the skin, whereas effector cells that arise in Peyer patches in response to enteroviral infections migrate preferentially to the gut (Butcher and Picker, 1996). Indeed, lymphocytes express different homing receptors when they respond to orally administered antigen, compared to when the same antigen is given parenterally (Kantele et al., 1999). The best understood tissue-selective homing pathways are in the skin and intestine (Butcher and Picker, 1996; Robert and Kupper, 1999), but other selective migration streams may well exist, such as to the lung, joints, and central nervous system (Salmi et al., 1992). Thus, tissue-selective migration streams offer the prospect of selectively inhibiting cell migration through a diseased tissue, such as the gut, and leaving other tissues unaffected. For instance, inhibitors of $\alpha4\beta7$ integrin have been trialed in patients with inflammatory bowel disease.

CLINICAL APPLICATIONS

Chemoattractant receptors and adhesion molecules are highly promising targets for new anti-inflammatory therapies (Carlos and Harlan, 1994; Strieter et al., 1996; Oppenheimer-Marks and Lipsky, 1998; Homey and Zlotnik, 1999; Mackay, 2001; von Andrian and Engelhardt, 2003; Szekanecz and Koch, 2004), and the development of antagonists has been pursued aggressively by the biotechnology and pharmaceutical industries (Table 18.3). Numerous antibodies, recombinant soluble adhesion molecules, receptor-blocking mutant chemokines, and small molecules are being tested for clinical application in multiple sclerosis, inflammatory bowel disease, arthritis, and psoriasis. In general, studies on experimental animals have validated various receptors or ligands in inflammatory disease models, including asthma, rheumatoid arthritis, multiple sclerosis, sepsis, and transplantation (Grant et al., 2002; Haskell et al., 2002; Miller et al., 2003; von Andrian and Engelhardt, 2003). Subversion of immune responses by pathogens also provides a pointer to the best anti-inflammatory strategies. A telling feature of many pathogens is their subversion of host responses through the chemokine system (Alcami, 2003). Chemoattractant receptors probably offer the most promise, because the seven-transmembrane structure of these receptors lends itself to inhibition with organic small molecules.

Potent small molecule antagonists have been developed for a number of the chemoattractant receptors (see Table 18.3), especially CCR1, CCR3, CCR5, CXCR3, and CXCR4 (Haringman et al., 2003; Szekanecz and Koch, 2004). Chemokine receptor antagonists have proved highly efficacious in animal models, particularly in models of rheumatoid arthritis [reviewed by Szekanecz and Koch (2004)]. A noteworthy example is a small molecule antagonist of both CCR5 and CXCR3, termed TAK-779, which inhibits ligand binding of these two Th1-type chemokine receptors (Gao et al., 2003), and also inhibits the development of arthritis by interfering with T-cell migration to joint lesions (Yang et al., 2002). Effector T cells in the synovium of patients with rheumatoid arthritis express high levels of CCR5 and CXCR3 (Qin et al., 1998). It is highly likely that some of these drugs will succeed in clinical trials and find utility in the treatment of certain autoimmune diseases. Interactions involving integrins or selectins are more difficult to inhibit with small molecules, but there have been promising results with antagonists of $\alpha4\beta1$–VCAM-1 and LFA-1–ICAM-1 interactions (Kelly et al., 1999; Lin et al., 1999). It is likely that cell-migration inhibitors will go far towards alleviating the disabling effects of autoimmune inflammation well before the basic nature of autoimmunity is finally understood.

FUTURE DIRECTIONS

The targeting of cell-migration pathways is an emerging approach to treat inflammatory autoimmune diseases, with promising disease applications being multiple sclerosis, rheumatoid arthritis, and psoriasis, but there are likely many others (see Chapter 76). Eventually, we must translate the wealth of data on trafficking molecules into a clearer understanding of their physiologic and pathologic relevance to human health and disease. This will require new experi-

TABLE 18.3 Adhesion molecules and chemoattractant receptors as targets for the treatment of inflammatory diseases

Target pathway/receptor	Involved in leukocyte or T-cell migration to these sites*	Potential clinical applications†	Comments, examples of drug development†
Adhesion pathways			
α4β1–VCAM-1	Numerous inflamed tissues, Th2 lesions	Asthma, MS, vasculitis	Promising phase II data in MS (Miller et al., 2003; von Andrian and Engelhardt, 2003)
α4β7–MAdCAM-1	Nonpulmonary mucosal tissues	IBD	Promising mouse data, disappointing phase II data using anti-α4β7 mAb
Mac-1–ICAM-1	Inflamed tissues?	Ischemia–reperfusion	Anti-ICAM mAb discontinued in phase III
LFA-1–ICAM-1	Most inflamed tissues, T-cell interactions with APCs	Numerous	Promising phase III data with efazulimab for psoriasis
Selectins–PSGL-1	Acute inflammation, Th1 lesions, esp. in the skin	Numerous	Disappointing phase II data, mixed data with various antagonists (Ulbrich et al., 2003)
Fucosyltransferase-VII	Acutely inflamed tissues, Th1 lesions	Numerous	Requires intracellular inhibitors, none described
Chemokine receptors/ligands			
CXCR1,2–numerous CXC chemokines	Some inflamed tissues, esp. skin	Psoriasis, RA, reperfusion injuries	Efficacy of antagonists not determined in humans
CCR1–CCL3, 5	Numerous inflamed tissues	RA, others	Promising phase Ib data (Haringman et al., 2003)
CCR2–CCL2 (MCP-1)	Numerous inflamed tissues	RA, MS	Promising animal data (Gong et al., 1997)
CCR3–CCL11 (Eotaxin)	Th2 lesions, allergic inflammation	Asthma, allergies	Promising phenotypes in knock-out mice, but possible redundancy issues
CCR4–CCL17, 22	Skin, Th2 lesions	Asthma, psoriasis, atopic dermatitis	Importance not established *in vivo*
CCR5–CCL3, 4, 5	Th1 lesions	RA, HIV infection	Promising small molecule antagonists (Baba et al., 1999; Gao et al., 2003)
CCR9–CCL25	Intestine	IBD	Importance not yet established *in vivo*
CXCR3–CXCL9, 10, 11	Th1 lesions	RA, MS, transplantation	Possible redundancy of Th1 chemokine pathways. Promising data with CXCR3 knock-out mice
SDF-1α–CXCR4	Numerous tissues	HIV infection	Critical for embryogenesis; inhibitors may alter hematopoiesis
Classical and other chemoattractant receptors			
C5aR–C5a	Neutrophil, mast cells	RA, sepsis, reperfusion injuries	Disappointing clinical trial data in RA
BLR–LTB4	Mast-cell initiated inflammatory lesions	RA, asthma	Potent small molecule drugs developed, effectiveness in autoimmune disease unclear
S1P receptors (esp. S1P1)	Lymphocyte egress from tissues	Numerous	S1P receptor agonist drugs, i.e., FTY720, effective in animal models of disease (Goetzl and Graler, 2004; Ward, 2004)

*Most of these molecular pathways have been targeted with either small molecule antagonists or blocking mAbs, and are currently in clinical trials for various indications. In addition, a substantial amount of animal experimentation has validated these pathways for various diseases.

†For reviews of adhesion molecule or chemoattractant receptor antagonists, and their use in preclinical and clinical trials, see Ulbrich et al. (2003) and Szekanecz and Koch (2004).

APC, antigen-presenting cell; CCR, receptor for CC chemokine; CXCR, receptor for CXC chemokine; HIV, human immune deficiency virus; IBD, inflammatory bowel disease; ICAM-1, intercellular cell adhesion molecule-1; IL-8, interleukin-8; LAD, leukocyte adhesion deficiency syndrome; LFA-1, leukocyte function-associated antigen-1; Mac-1, macrophage antigen-1; MAdCAM-1, mucosal addressin cell adhesion molecule-1; MS, multiple sclerosis; PSGL-1, P-selectin glycoprotein ligand-1; RA, rheumatoid arthritis; Th, T-helper; VCAM-1, vascular cell adhesion molecule-1.

mental and therapeutic entities, such as antibodies, recombinant proteins, small molecule inhibitors, screening assays and, not least, meaningful clinical trials. One pivotal question is whether to treat autoimmune diseases with broad inhibitors (the sledge hammer approach, using, for instance, an inhibitor of a common signaling pathway of chemoattractant receptors), or whether such diseases should be treated with much more specific inhibitors, for instance, inflammatory bowel disease with a CCR9 or α4β7 inhibitor? Time will tell.

Acknowledgments

CRM is supported by grants from the National Health and Medical Research Council of Australia and the CRC for asthma.

References

Alcami, A. (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nat. Rev. Immunol. 3*, 36–50.

Alon, R., Kassner, P.D., Carr, M.W., Finger, E.B., Hemler, M.E., and Springer, T.A. (1995). The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol. 128*, 1243–1253.

Andrew, D.P., Spellberg, J.P., Takimoto, H., Schmits, R., Mak, T.W., and Zukowski, M.M. (1998). Transendothelial migration and trafficking of leukocytes in LFA-1-deficient mice. *Eur. J. Immunol. 28*, 1959–1969.

Arbones, M.L., Ord, D.C., Ley, K., Ratech, H., Maynard-Curry, C., Otten, G., Capon, D.J., and Tedder, T.F. (1994). Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity 1*, 247–260.

Austrup, F., Vestweber, D., Borges, E., Lohning, M., Brauer, R., Herz, U., Renz, H., Hallman, R., Scheffold, A., Radbruch, A., and Hamann, A. (1997). P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflammed tissues. *Nature 385*, 81–83.

Baba, M., Nishimura, O., Kanzaki, N., Okamoto, M., Sawada, H., Iizawa, Y., Shiraishi, M., Aramaki, Y., Okonogi, K., Ogawa, Y., Meguro, K., and Fujino, M. (1999). A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl. Acad. Sci. USA 96*, 5698–5703.

Bargatze, R.F., Jutila, M.A., and Butcher, E.C. (1995). Distinct roles of L-selectin and integrins α4β7 and LFA-1 in lymphocyte homing to Peyer's patch-HEV *in situ*: The multistep model confirmed and refined. *Immunity 3*, 99–108.

Berlin, C., Bargatze, R.F., von Andrian, U.H., Szabo, M.C., Hasslen, S.R., Nelson, R.D., Berg, E.L., Erlandsen, S.L., and Butcher, E.C. (1995). α4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell 80*, 413–422.

Berlin-Rufenach, C., Otto, F., Mathies, M., Westermann, J., Owen, M.J., Hamann, A., and Hogg, N. (1999). Lymphocyte migration in lymphocyte function-associated antigen (LFA)-1-deficient mice. *J. Exp. Med. 189*, 1467–1478.

Bleul, C.C., Wu, L., Hoxie, J.A., Springer, T.A., and Mackay, C.R. (1997). The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc. Natl. Acad. Sci. USA 94*, 1925–1930.

Bonecchi, R., Bianchi, G., Bordignon, P.P., D'Ambrosio, D., Lang, R., Borsatti, A., Sozzani, S., Allavena, P., Gray, P.A., Mantovani, A., and Sinigaglia, F. (1998). Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J. Exp. Med. 187,* 129–134.

Borges, E., Tietz, W., Steegmaier, M., Moll, T., Hallmann, R., Hamann, A., and Vestweber, D. (1997). P-selectin glycoprotein ligand-1 (PSGL-1) on T helper 1 but not on T helper 2 cells binds to P-selectin and supports migration into inflamed skin. *J. Exp. Med. 185*, 573–578.

Bullard, D.C., Kunkel, E.J., Kubo, H., Hicks, M.J., Lorenzo, I., Doyle, N.A., Doerschuk, C.M., Ley, K., and Beaudet, A.L. (1996). Infectious susceptibility and severe deficiency of leukocyte rolling and recruitment in E-selectin and P-selectin double mutant mice. *J. Exp. Med. 183*, 2329–2336.

Butcher, E.C. (1991). Leukocyte-endothelial cell recognition: Three (or more) steps to specificity and diversity. *Cell 67*, 1033–1036.

Butcher, E.C. and Picker, L.J. (1996). Lymphocyte homing and homeostasis. *Science 272*, 60–66.

Campbell, J.J., Hedrick, J., Zlotnik, A., Siani, M.A., Thompson, D.A., and Butcher, E.C. (1998). Chemokines and the arrest of lymphoyctes rolling under flow conditions. *Science 279*, 381–384.

Carlos, T.M. and Harlan, J.M. (1994). Leukocyte-endothelial adhesion molecules. *Blood 84*, 2068–2101.

Cyster, J.G. (1999). Chemokines and cell migration in secondary lymphoid organs. *Science 286*, 2098–102.

Dieu, M.C., Vanbervliet, B., Vicari, A., Bridon, J.M., Oldham, E., Ait-Yahia, S., Briere, F., Zlotnik, A., Lebecque, S., and Caux, C. (1998). Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J. Exp. Med. 188*, 373–386.

Forster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Muller, I., Wolf, E., and Lipp, M. (1999). CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99, 23–33.

Foxman, E.F., Campbell, J.J., and Butcher, E.C. (1997). Multistep navigation and the combinatorial control of leukocyte chemotaxis. *J. Cell Biol. 139*, 1349–1360.

Frenette, P.S., Mayadas, T.N., Rayburn, H., Hynes, R.O., and Wagner, D.D. (1996). Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell 84*, 563–574.

Gao, P., Zhou, X.Y., Yashiro-Ohtani, Y., Yang, Y.F., Sugimoto, N., Ono, S., Nakanishi, T., Obika, S., Imanishi, T., Egawa, T., Nagasawa, T., Fujiwara, H., and Hamaoka, T. (2003). The unique target specificity of a nonpeptide chemokine receptor antagonist: selective blockade of two Th1 chemokine receptors CCR5 and CXCR3. *J. Leukoc. Biol. 73*, 273–280.

Girard, J.-P. and Springer, T.A. (1995). High endothelial venules (HEVs): Specialized endothelium for lymphocyte migration. *Immunol. Today 16*, 449–457.

Goetzl, E.J. and Graler, M.H. (2004). Sphingosine 1-phosphate and its type 1 G protein-coupled receptor: trophic support and functional regulation of T Lymphocytes. *J. Leukoc. Biol.* 76, 30–35.

Gomez-Reino, J.J., Pablos, J.L., Carreira, P.E., Santiago, B., Serrano, L., Vicario, J.L., Balsa, A., Figueroa, M., and de Juan, M.D. (1999). Association of rheumatoid arthritis with a functional chemokine receptor, CCR5. *Arthritis Rheum. 42*, 989–992.

Gong, J.-H., Ratkay, L.G., Waterfield, J.D., and Clark-Lewis, I. (1997). An antagonist of monocyte chemoattractant protein1 (MCP-1) inhibits arthritis in the MRL-*lpr* mouse model. *J. Exp. Med. 186*, 131–137.

Gonzalo, J.A., Lloyd, C.M., Wen, D., Albar, J.P., Wells, T.N., Proudfoot, A., Martinez, A.C., Dorf, M., Bjerke, T., Coyle, A.J., and Gutierrez-Ramos, J.C. (1998). The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. *J. Exp. Med. 188*, 157–167.

Grant, E.P., Picarella, D., Burwell, T., Delaney, T., Croci, A., Avitahl, N., Humbles, A.A., Gutierrez-Ramos, J.C., Briskin, M., Gerard, C., and Coyle, A.J. (2002). Essential role for the C5a receptor in regulating the effector phase of synovial infiltration and joint destruction in experimental arthritis. *J. Exp. Med. 196*, 1461–1471.

Gutierrez-Ramos, J.C., Lloyd, C., and Gonzalo, J.A. (1999). Eotaxin: from an eosinophilic chemokine to a major regulator of allergic reactions. *Immunol. Today 20*, 500–504.

Haringman, J.J., Kraan, M.C., Smeets, T.J., Zwinderman, K.H., and Tak, P.P. (2003). Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. *Ann. Rheum. Dis. 62*, 715–721.

Haskell, C.A., Ribeiro, S., and Horuk, R. (2002). Chemokines in transplant rejection. *Curr. Opin. Investig. Drugs 3*, 399–405.

Hirsch, E., Katanaev, V.L., Garlanda, C., Azzolino, O., Pirola, L., Silengo, L., Sozzani, S., Mantovani, A., Altruda, F., and Wymann, M.P. (2000). Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science 287*, 1049–1053.

Homey, B. and Zlotnik, A. (1999). Chemokines in allergy. *Curr. Opin. Immunol*. *11*, 626–634.

Jose, P.J., Griffiths-Johnson, D.A., Collins, P.D., Walsh, D.T., Moqbel, R., Totty, N.F., Truong, O., Hsuan, J.J., and Williams, T.J. (1994). Eotaxin: A potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J. Exp. Med. 179*, 881–887.

Jung, S. and Littman, D.R. (1999). Chemokine receptors in lymphoid organ homeostasis. *Curr. Opin. Immunol. 11*, 319–325.

Kansas, G.S. (1996). Selectins and their ligands: Current concepts and controversies. *Blood 88*, 3259–3287.

Kantele, A., Zivny, J., Hakkinen, M., Elson, C.O., and Mestecky, J. (1999). Differential homing commitments of antigen-specific T cells after oral or parenteral immunization in humans. *J. Immunol. 162*, 5173–5177.

Kelly, T.A., Jeanfavre, D.D., McNeil, D.W., Woska, J.R., Jr., Reilly, P.L., Mainolfi, E.A., Kishimoto, K.M., Nabozny, G.H., Zinter, R., Bormann, B.J., and Rothlein, R. (1999). Cutting edge: A small molecule antagonist of LFA-1-mediated cell adhesion. *J. Immunol. 163*, 5173–5177.

Kim, C.H. and Broxmeyer, H.E. (1999). Chemokines: Signal lamps for trafficking of T and B cells for development and effector function. *J. Leukoc. Biol. 65*, 6–15.

Kunkel, E.J., Ramos, C.L., Steeber, D.A., Muller, W., Wagner, N., Tedder, T.F., and Ley, K. (1998). The roles of L-selectin, beta 7 integrins, and P-selectin in leukocyte rolling and adhesion in high endothelial venules of Peyer's patches. *J. Immunol*. *161*, 2449–2456.

Lawrence, M.B. and Springer, T.A. (1991). Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell 65*, 859–873.

Li, Z., Jiang, H., Xie, W., Zhang, Z., Smrcka, A.V., and Wu, D. (2000). Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. *Science 287*, 1046–1049.

Lim, Y.C., Henault, L., Wagers, A.J., Kansas, G.S., Luscinskas, F.W., and Lichtman, A.H. (1999). Expression of functional selectin ligands on Th cells is differentially regulated by IL-12 and IL-4. *J. Immunol. 162*, 3193–3201.

Lin, K., Ateeq, H.S., Hsiung, S.H., Chong, L.T., Zimmerman, C.N., Castro, A., Lee, W.C., Hammond, C.E., Kalkunte, S., Chen, L.L., Pepinsky, R.B., Leone, D.R., Sprague, A.G., Abraham, W.M., Gill, A., Lobb, R.R., and Adams, S.P. (1999). Selective, tight-binding inhibitors of integrin alpha4beta1 that inhibit allergic airway responses. *J. Med. Chem.* 42, 920–934.

Mackay, C.R. (2001). Chemokines: immunology's high impact factors. *Nat. Immunol. 2*, 95–101.

Maly, P., Thall, A.D., Petryniak, B., Rogers, C.E., Smith, P.L., Marks, R.M., Kelly, R.J., Gersten, K.M., Cheng, G., Saunders, T.L., Camper, S.A., Camphausen, R.T., Sullivan, F.X., Isogai, Y., Hindsgaul, O., von Andrian, U.H., and Lowe, J.B. (1996). The $\alpha(1,3)$ fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell 86*, 643–653.

Mantovani, A. (1999). The chemokine system: redundancy for robust outputs. *Immunol. Today* 20, 254–257.

Marchesi, V.T. and Gowans, J.L. (1964). The migration of lymphocytes through the endothelium of venules in lymph nodes: an electron microscope study. *Proc. R. Soc.* B *159*, 283–290.

Mayadas, T.N., Johnson, R.C., Rayburn, H., Hynes, R.O., and Wagner, D.D. (1993). Leukocyte rolling and extravasation are severely compromised in P-selectin-deficient mice. *Cell 74*, 541–554.

Miller, D.H., Khan, O.A., Sheremata, W.A., Blumhardt, L.D., Rice, G., Libonati, M.A., Wilmer-Hulme, A., Dalton, C., Miszkiel, K., and O'Connor, P. (2003). A controlled trial of Natalizumab in relapsing multiple sclerosis. *N. Engl. J. Med.* 348, 15–23.

Oppenheimer-Marks, N. and Lipsky, P.E. (1998). Adhesion molecules in rheumatoid arthritis. *Springer Semin. Immunopathol.* 20, 95–114.

Paxton, W.A. and Kang, S. (1998). Chemokine receptor allelic polymorphisms: relationships to HIV resistance and disease progression. *Semin. Immunol*. *10*, 187–194.

Ponath, P.D., Qin, S., Ringler, D.J., Clark-Lewis, I., Wang, J., Kassam, N., Smith, H., Shi, X., Gonzalo, J.A., Newman, W., Gutierrez-Ramos, J.C., and Mackay, C.R. (1996). Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J. Clin. Invest. 97*, 604–612.

Qin, S., Rottman, J.B., Myers, P., Kassam, N., Weinblatt, M., Loetscher, M., Koch, A.E., Moser, B., and Mackay, C.R. (1998). The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J. Clin. Invest. 101*, 746–754.

Robert, C. and Kupper, T.S. (1999). Inflammatory skin diseases, T cells, and immune surveillance. *N. Engl. J. Med. 341*, 1817–1828.

Robinson, S.D., Frenette, P.S., Rayburn, H., Cummiskey, M., Ullman-Cullere, M., Wagner, D.D., and Hynes, R.O. (1999). Multiple, targeted deficiencies in selectins reveal a predominant role for P-selectin in leukocyte recruitment. *Proc. Natl. Acad. Sci. USA 96*, 11452–11457.

Sallusto, F. and Lanzavecchia, A. (1999). Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. *J. Exp. Med. 189*, 611–614.

Sallusto, F., Lanzavecchia, A., and Mackay, C.R. (1998). Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol. Today 19*, 568–574.

Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature 401*, 708–712.

Sallusto, F., Mackay, C.R., and Lanzavecchia, A. (1997). Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. *Science 277*, 2005–2007.

Sallusto, F., Lenig, D., Mackay, C.R., and Lanzavecchia, A. (1998). Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med. 187*, 875–883.

Sallusto, F., Palermo, B., Hoy, A., and Lanzavecchia, A. (1999). The role of chemokine receptors in directing traffic of naïve, type 1 and type 2 T cells. *Curr. Top. Microbiol. Immunol. 246*, 123–128.

Sallusto, F., Schaerli, P., Loetscher, P., Schaniel, C., Lenig, D., Mackay, C.R., Qin, S., and Lanzavecchia, A. (1998). Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol. 28*, 2760–2769.

Salmi, M., Granfors, K., Leirisalo-Repo, M., Hamalainen, M., MacDermott, R., Leino, R., Havia, T., and Jalkanen, S. (1992). Selective endothelial binding of interleukin-2-dependent human T-cell lines derived from different tissues. *Proc. Natl. Acad. Sci. USA 89*, 11436–11440.

Springer, T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: The multi-step paradigm. *Cell 76*, 301–314.

Stein, J.V., Cheng, G., Stockton, B.M., Fors, B.P., Butcher, E.C., and von Andrian, U.H. (1999). L-selectin-mediated leukocyte adhesion *in vivo*: Microvillous distribution determines tethering efficiency, but not rolling velocity. *J. Exp. Med. 189*, 37–50.

Stein, J.V., Rot, A., Luo, Y., Narasimhaswamy, M., Nakano, H., Gunn, M.D., Matsuzawa, A., Quackenbush, E.J., Dorf, M.E., and von Andrian, U.H. (2000). The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6Ckine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. *J. Exp. Med. 191*, 61–76.

Strieter, R.M., Standiford, T.J., Huffnagle, G.B., Colletti, L.M., Lukacs, N.W., and Kunkel, S.L. (1996). "The good, the bad, and the ugly." The role of chemokines in models of human disease. *J. Immunol. 156*, 3583–3586.

Syrbe, U., Siveke, J., and Hamann, A. (1999). Th1/Th2 subsets: distinct differences in homing and chemokine receptor expression? *Springer Semin. Immunopathol. 21*, 263–285.

Szekanecz, Z. and Koch, A.E. (2004). Therapeutic inhibition of leukocyte recruitment in inflammatory diseases. *Curr. Opin. Pharmacol.* 4, 423–428.

Ulbrich, H., Eriksson, E.E., and Lindbom, L. (2003). Leukocyte and endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatory disease. *Trends Pharmacol. Sci. 24*, 640–647.

Vestweber, D. and Blanks, J.E. (1999). Mechanisms that regulate the function of the selectins and their ligands. *Physiol. Rev. 79*, 181–213.

von Andrian, U.H., Chambers, J.D., McEvoy, L.M., Bargatze, R.F., Arfors, K.E., and Butcher, E.C. (1991). Two-step model of leukocyte-endothelial cell interaction in inflammation: Distinct roles for LECAM-1 and the leukocyte β_2 integrins *in vivo*. *Proc. Natl. Acad. Sci. USA 88*, 7538–7542.

von Andrian, U.H. and Engelhardt, B. (2003). Alpha4 integrins as therapeutic targets in autoimmune disease. *N. Engl. J. Med. 348*, 68–72.

von Andrian, U.H. and Mackay, C.R. (2000). T-cell function and migration. Two sides of the same coin. *N. Engl. J. Med. 343*, 1020–1034.

Wagers, A.J., Waters, C.M., Stoolman, L.M., and Kansas, G.S. (1998). Interleukin 12 and interleukin 4 control T cell adhesion to endothelial selectins through opposite effects on alpha1, 3-fucosyltransferase VII gene expression. *J. Exp. Med. 188*, 2225–2231.

Wagner, N., Lohler, J., Kunkel, E.J., Ley, K., Leung, E., Krissansen, G., Rajewsky, K., and Muller, W. (1996). Critical role for β7 integrins in formation of the gut-associated lymphoid tissue. *Nature 382*, 366–370.

Ward, S.G. (2004). Do phosphoinositide 3-kinases direct lymphocyte navigation? *Trends Immunol. 25*, 67–74.

Warnock, R.A., Askari, S., Butcher, E.C., and von Andrian, U.H. (1998). Molecular mechanisms of lymphocyte homing to peripheral lymph nodes. *J. Exp. Med. 187*, 205–216.

Warnock, R.A., Campbell, J.J., Dorf, M.E., Matsuzawa, A., McEvoy, L.M., and Butcher, E.C. (2000). The role of chemokines in the microenvironmental control of T versus B cell arrest in Peyer's patch high endothelial venules. *J. Exp. Med. 191*, 77–88.

Xie, H., Lim, Y.C., Luscinskas, F.W., and Lichtman, A.H. (1999). Acquisition of selectin binding and peripheral homing properties by CD4(+) and CD8(+) T cells. *J. Exp. Med. 189*, 1765–1776.

Yang, Y.F., Mukai, T., Gao, P., Yamaguchi, N., Ono, S., Iwaki, H., Obika, S., Imanishi, T., Tsujimura, T., Hamaoka, T., and Fujiwara, H. (2002). A non-peptide CCR5 antagonist inhibits collagen-induced arthritis by modulating T cell migration without affecting anti-collagen T cell responses. *Eur. J. Immunol.* 32, 2124–2132.

Zlotnik, A. and Yoshie, O. (2000). Chemokines: a new classification system and their role in immunity. *Immunity* 12, 121–127.

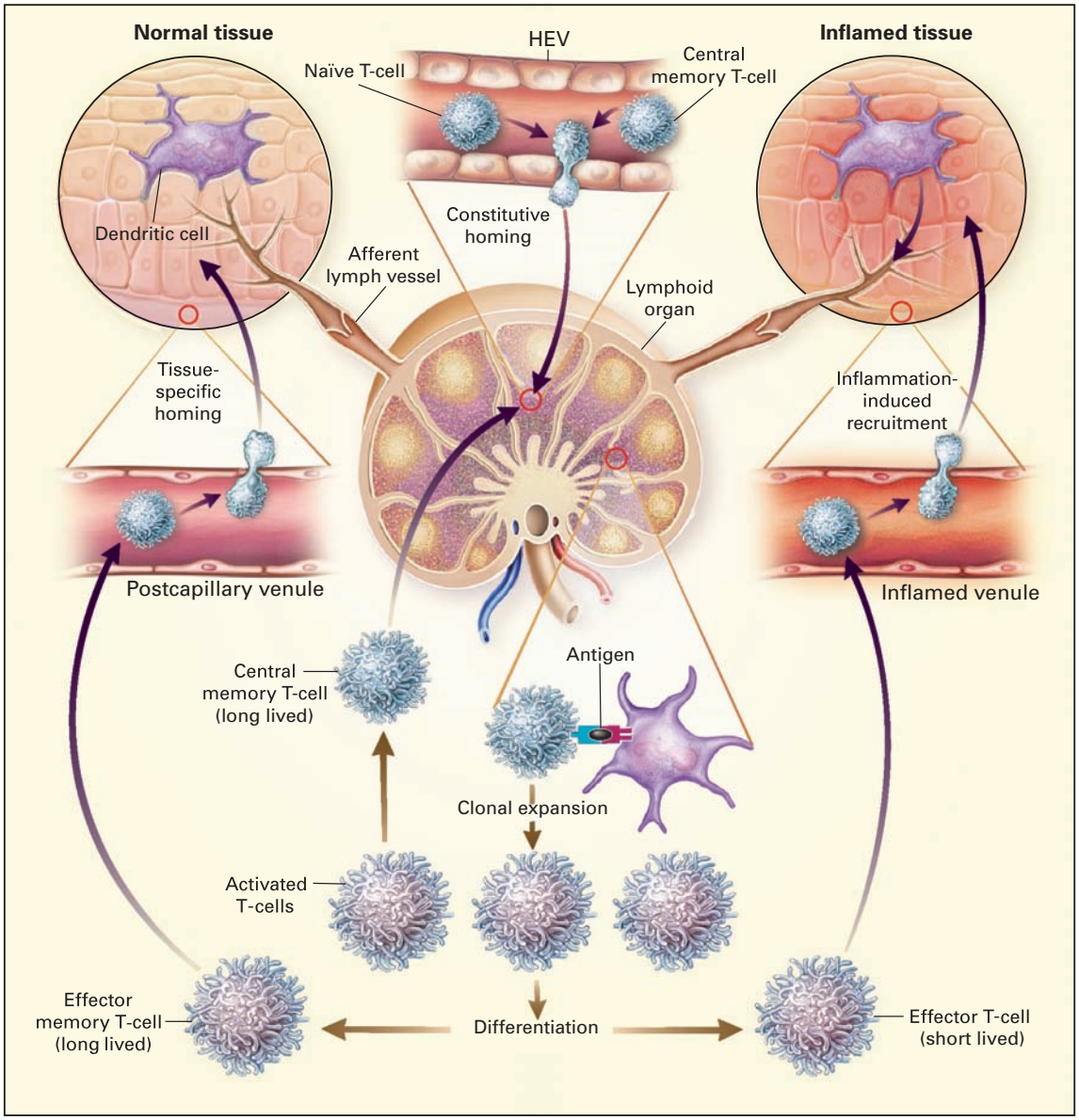


FIGURE 18.1 Migratory routes of T cells. Naïve T cells home continuously from the blood to lymph nodes and other secondary lymphoid tissues. Homing to lymph nodes occurs across high endothelial venules (HEVs), which express traffic molecules for constitutive lymphocyte recruitment. Lymph nodes are percolated by lymph fluid that is channeled to them from peripheral tissues, where dendritic cells (DCs) collect antigenic material. In inflamed tissues, DCs are mobilized to carry antigen to lymph nodes, where they stimulate antigen-specific T cells. Upon stimulation, T cells proliferate and differentiate into effector cells, which express receptors that enable them to migrate to sites of inflammation. While most effector cells are short lived, a few antigen-experienced cells survive for a long time. These memory cells are subdivided into two populations based on their migratory ability (Sallusto et al., 1999): one subset is termed *effector memory* T cells and is localized to peripheral tissues, and the other *central memory* T cells that express a similar repertoire of homing molecules as do naïve T cells migrate preferentially to lymphoid organs. The traffic signals that direct effector and memory cells to peripheral tissues are organ specific; for example, molecules required for migration to the skin are different from those to the gut; they are modulated by inflammatory mediators and are distinct for different T-cell subsets, e.g., Th1 and Th2 cells show differences in their responses to various chemoattractants.

Reproduced from von Andrian and Mackay (2000), with permission.

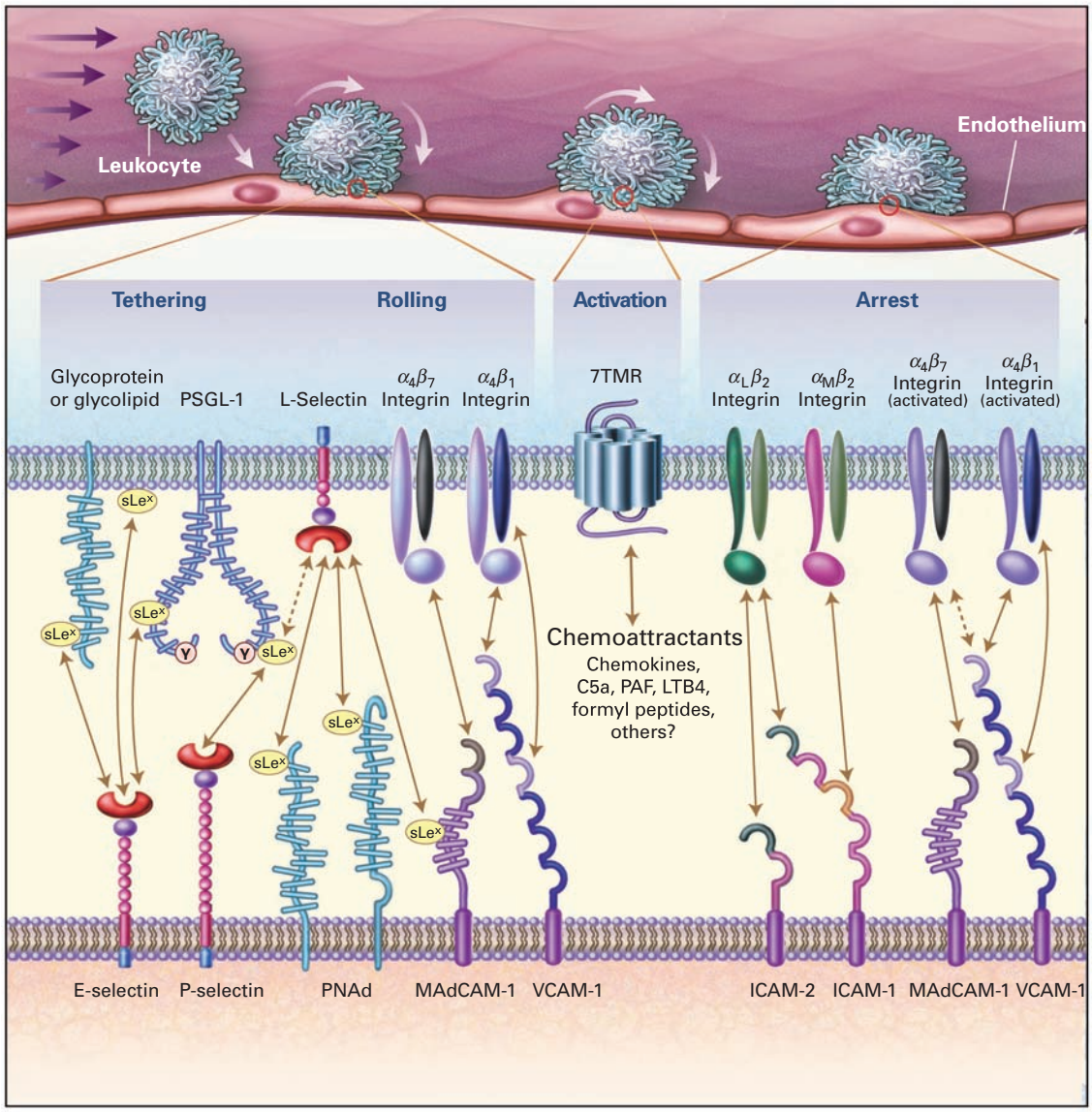


FIGURE 18.2 Essential molecular players in the multistep adhesion cascade. The top of this schematic diagram depicts the four distinct adhesion steps that leukocytes must undergo to accumulate in a blood vessel. Also shown are the predominant molecular determinants of each step with respect to leukocytes (middle of the diagram) and endothelial cells (bottom). A number of molecules can interact with more than one partner, symbolized by arrows. Leukocytes in the bloodstream (arrows at left symbolize the laminar flow profile) tether to endothelial cells and roll slowly downstream. Tethering is greatly facilitated by leukocyte receptors that occur at high density on the tips of microvillous surface protrusions (L-selectin, PSGL-1, and α4 integrins), whereas subsequent rolling is not influenced by the topography of adhesion receptors (Stein et al., 1999). The most efficient tethering molecules are L- and P-selectin. L-selectin recognizes sulfated sialyl-Lewis^X (sLe^X)-like sugars (PNAd) in high endothelial venules. It may also interact with other ligands on inflamed endothelial cells (not shown) and with PSGL-1 on adherent leukocytes (broken arrow). PSGL-1 binding to L- and P-selectin requires decoration with an sLe^X-like sugar in close vicinity to an N-terminal motif containing three tyrosines (Y) that must be sulfated. E-selectin can also interact with PSGL-1, but does not require sulfation and also recognizes other sLe^X-bearing glycoconjugates. E-selectin and the α4 integrins can tether some leukocytes, but their predominant function is to reduce rolling velocities. Rolling leukocytes respond to chemoattractants on endothelial cells because they express specific receptors with seven transmembrane domains (7TMR), which transmit intracellular signals through G proteins. The activating signal induces rapid activation of β2 and/or α4 integrins, which bind to endothelial immunoglobulin superfamily members. Note that α4 integrins can mediate activation-independent rolling interactions as well as firm arrest. However, the latter function requires integrin activation, symbolized by the open conformation of the integrin heterodimer. For a list of abbreviations, see Table 18.1.

Reproduced from von Andrian and Mackay (2000), with permission.

CHAPTER

19

Signaling Pathways in T and B Lymphocytes

ELIZABETH G. ARAUJO AND MARCUS R. CLARK

Department of Medicine, Section of Rheumatology, University of Chicago, Chicago, Illinois, USA

Most normal and aberrant immune responses require the coordinated activation of both the innate and adaptive immune responses. The innate immune system responds to patterns and contexts indicative of danger. The lack of specificity of the innate immune system allows it to respond quickly and broadly to infectious organisms. Phylogenically, the innate immune system is ancient. Indeed, the first descriptions of the primary receptors and signaling pathways of the innate immune system were made in invertebrates amenable to genetic dissection.

However, there are limitations to the innate immune system. It does not learn from previous immunologic challenges. The innate immune system is invariant and does not adapt to common pathogens in the environment. For the innate immune system, every war is always the first war. To circumvent these limitations, higher vertebrates have developed adaptive immunity. This system does not respond to broad conserved patterns but to specific non-self-antigenic epitopes restricted to the invading organism. The adaptive and innate immune systems are highly interdependent and rarely does one respond to an infection without the participation of the other. However, it is the adaptive immunity that allows the immune system to develop a memory of previous infections, and to respond quickly and effectively the next time a similar infection occurs. Exploitation of the adaptive immune system, through the use of vaccines, has been used with great success to rid humanity of many forms of pestilence. Unfortunately, aberrant adaptive immune responses are at the root of most autoimmune diseases.

In this chapter we will review the structure and signaling pathways regulated by two important receptors of the adaptive immune system, the T- and B-cell antigen receptors. We will also examine how signaling through important coreceptors determines cellular responses. By comparing and contrasting these two receptor systems, we hope to illustrate both common mechanisms of responding to antigen and important differences. Understanding how the antigen receptors function is of clear and direct importance to those interested in the pathogenesis and treatment of autoimmune diseases. As will be demonstrated, changes in how a lymphocyte responds to antigen can lead to the breaking of tolerance and the development of autoimmune diseases. Conversely, therapeutically targeting the signaling pathways activated by the antigen receptors and their coreceptors holds the promise of reinstating antigen-specific tolerance and inducing drug-free remissions in patients afflicted with autoimmunity.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

COMMON AND DIVERGENT THEMES IN RECEPTOR STRUCTURE

All the antigen recognition receptors of the immune system, including the B-cell receptor (BCR), the T-cell receptor (TCR), and most Fc receptors have a common general structure. Each contains an antigen recognition substructure noncovalently associated with a substructure required for both surface expression and for the activation of signaling pathways. In the case of lymphocytes, the antigen-recognition substructures are clonotypic; specific for one antigenic epitope. As covered in Chapter 2, specificity arises from recombination of the gene segments encoding the variable regions. The TCR antigen recognition substructure is comprised of two transmembrane polypeptide chains, named α and β (or γ and δ in a small subset of T cells), which recognize antigenic peptides presented in the context of either major histocompatibility complex (MHC) class I or class II molecules. The associated signaling substructure is comprised of four different invariant chains, CD3γ, δ, ε, and TCRζ_2. Regarding TCR stoichiometry, recent data have demonstrated that the majority of the complexes contain a single TCRαβ heterodimer associated with one CD3εγ, one CD3εδ, and one homodimer ξ_2 (Figure 19.1). There are three basic (positively charged) residues in the transmembrane region of the TCRαβ heterodimer and a pair of acidic (negatively charged) residues in each of the three signaling dimers. Each acidic residue pair binds a different basic TCRαβ residue to assemble a TCR in which TCRαβ is in the center surrounded by the signaling dimers (Call et al., 2002).

The first evidence that the CD3γ, δ, ε, and TCRζ_2 chains are responsible for signal transduction was demonstrated using a chimeric protein linking the extracellular and transmembrane domains of CD8 to the cytoplasmic domain of the ξ chain (Weiss and Littman, 1994). Similar experiments were used to examine the functional capacity of CD3ε. Subsequent genetic studies have demonstrated that the cytoplasmic tails of the various signaling subunits, especially TCRζ, are required for T-cell development (Shores and Love, 1997). From these data, it is clear that each chain can independently signal and mediate biologic functions. However, it is likely that each signaling chain of the TCR also contributes unique signaling functions to the receptor complex.

The BCR is also comprised of separate antigen recognition and signaling substructures. Antigen is recognized by membrane-bound immunoglobulin (mIg) which is identical in specificity to the secreted immunoglobulin potentially produced by each cell. The IgM and IgD isotypes have short cytosolic tails of only three amino acids, while other isotypes, such as IgG, have larger cytosolic tails that may influence signaling through the BCR (Wakabayashi et al., 2002). The signaling substructure of the BCR is composed of a single disulfide-linked heterodimer of Igα and Igβ noncovalently associated with mIg in a 1:1 stoichiometry (Wolfgang et al., 2000). Like the TCR, the transmembrane domains of mIg and Igα/Igβ have charged amino

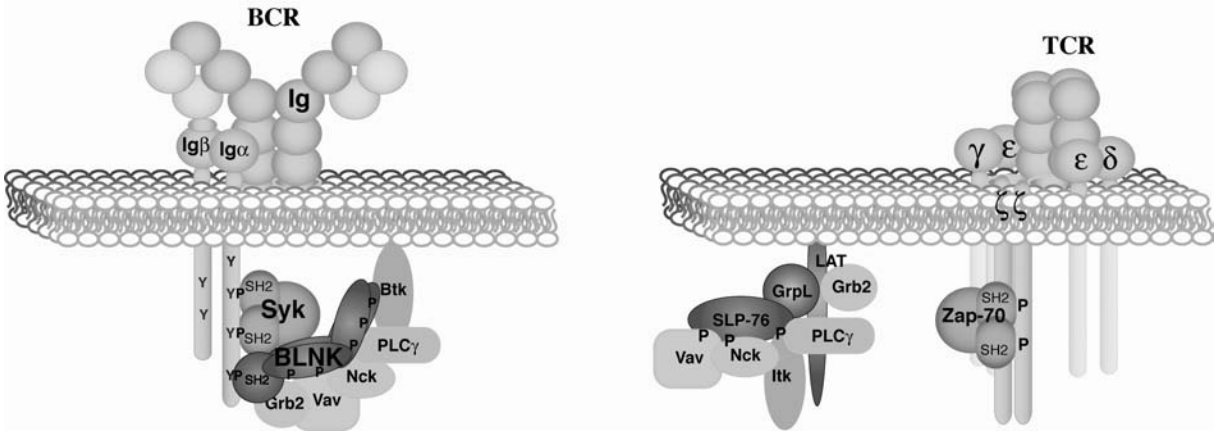


FIGURE 19.1 Schematic comparison of activated antigen receptor complexes. The antigen receptors are multimeric complexes containing separate antigen recognition and signaling substructures. Following engagement by ligand, the immunoreceptor tyrosine-based activation motif (ITAM) tyrosines in the signaling subunits of each receptor are phosphorylated, which serves to recruit either the tyrosine kinase ZAP-70 [T-cell receptor (TCR)] or Syk [B-cell receptor (BCR)]. Scaffolding molecules are then phosphorylated by these kinases. In the case of the TCR, the scaffold consists of LAT–GrpL–SLP-76 and with the BCR, BLNK. Other scaffolding molecules are likely to be involved, including LAB and BAM32. These phosphorylated scaffolds then assemble and coordinate the activation of signaling effectors that initiate downstream signaling pathways.

acids. Those in mIg have been demonstrated to be important for receptor assembly. However, the overall structure of the BCR complex is still unknown. The Igα/Igβ cytosolic tails have also been studied extensively both *in vitro* and *in vivo*. In general, the functions of Igα and Igβ appear to be more specialized than those of each TCR signaling chain. The bulk of evidence indicates that Igα is the major signaling chain of the complex (see below). Expression of both mIg and Igα are required for normal B cell development and for maintaining B cells in the periphery (Lam et al., 1997; Wang and Clark, 2003; Kraus et al., 2004). In contrast, Igβ is required for the maturation of peripheral B cells (Reichlin et al., 2001) and for proper BCR-mediated antigen processing and presentation to T cells (Clark et al., 2004).

RECOGNITION OF ANTIGEN

In contrast to their similarities in overall receptor structure, the TCR and BCR recognize antigen in very different ways. As described in Chapter 6, T cells recognize antigenic peptides processed by antigen-presenting cells (APCs) and presented in the context of either MHC class I or class II. The affinity of a typical TCR for MHC class II/peptide complex is low (Kd = 10^{-5}) and is a compilation of TCR binding to both MHC and peptide. Also, the TCR usually recognizes MHC concurrently with binding of the coreceptors CD4 (MHC class II restricted) or CD8 (MHC class I). Coreceptor engagement enhances binding affinity and, as discussed below, is integral to the initiation of signaling. In spite of such low affinities, productive engagement of a single MHC class II–peptide complex can elicit a detectable signaling response and as few as 10 engaged receptors can initiate full signaling (Irvine et al., 2002). The exquisite sensitivity of the TCR is probably due to several factors, including the ability of engaged TCRs to recruit nonengaged TCRs to the APC interface and the ability of a single MHC–peptide complex to engage multiple TCRs.

In contrast, the BCR usually recognizes native antigens that have not undergone processing. The initial affinities of antigen for receptor are often in the same range as that for the TCR. However, because of the unique ability of the BCR to undergo affinity maturation, affinities at the end of an acute immune response can be 10^{-9} or greater. Although the BCR can recognize monovalent antigens, such antigens are unlikely to be encountered routinely during normal immune responses and they do not lead to productive B-cell activation (see below). Normally, antigens are either presented to B cells directly by infectious agents, which provide repetitive epitope arrays, or by cells of the immune system, including dendritic cells (DCs) which capture antigenic complexes on their cell surface. The normal polyvalency of non-self-antigens induces BCR aggregation and the initiation of signaling pathways that ultimately result in the activation and differentiation of peripheral B cells.

INITIATION OF SIGNALING

Neither the TCR nor the BCR contain intrinsic catalytic activity. Rather, upon receptor engagement, tyrosine kinases are recruited by each complex and these become the primary enzymatic activities that mediate signal initiation. The mechanisms by which this occurs have been studied in great detail (Weiss and Littman, 1994; Campbell, 1999). Within the cytosolic tails of Igα, Igβ, TCRζ, and the CD3 complex are one or more conserved signaling motifs known as the immunoreceptor tyrosine-based activation motifs (ITAMs). Each ITAM contains a pair of YXXL (where X corresponds to a variable residue) spaced by six to eight variable amino acids. In the case of the TCR, the ITAM tyrosines become phosphorylated following coengagement of MHC and CD4/8. The cytosolic tail of the latter is bound to the Src-family tyrosine kinase (SFTK) Lck, which is the primary kinase responsible for phosphorylating the CD3 and TCRζ ITAMs. Another SFTK, Fyn, can phosphorylate the TCR ITAMs under some circumstances. The SFTKs have several important structural features. First, their N-terminal domains can be attached to various lipid moieties (referred to as acetylation) that anchor them to the plasma membrane where they can readily interact with the antigen receptors. Second, besides their enzymatic activity, they can interact with other molecules of the signaling cascade, via interactions through their Src homology-2 (SH2) and -3 (SH3) domains. SH2 domains bind phosphorylated tyrosines in specific peptide contexts while SH3 domains bind to proline-based motifs. Finally, the SFTKs have negative regulatory tyrosines in their C-terminal tails. Phosphorylation of this regulatory tyrosine by Csk (C-terminal Src kinase) provides a binding site for its own SH2 domain, maintaining the SFTKs in a closed and inactive conformation. Dephosphorylation of the C-terminal tyrosine by the transmembrane tyrosine phosphatase CD45 allows the SFTKs to adopt an open and active conformation (Hermiston et al., 2003) As discussed below, a dynamic balance of tyrosine kinases and phosphatases determines SFTK activity.

The regulatory tyrosine kinase Csk contains an N-terminal SH3 domain, an SH2 domain, and a catalytic kinase domain. In contrast to the SFTKs, it is not modified by lipids and therefore always resides in the cytosol. The Csk SH2 domain interacts with a transmembrane protein, known as Cbp/PAG (Csk-binding/phosphoprotein associated with glycosphingolipid-enriched microdomains), which resides in lipid rafts where it is constituitively phosphorylated

by SFTKs. In addition to this SFTK inhibitory loop, the SH3 domain of Csk binds to cytoplasmic protein tyrosine phosphatases (PEP and PTP-PEST), which in turn dephosphorylate the SFTK catalytic domain tyrosine. Hence, the coordinated activities of the tyrosine kinase Csk and the phosphatases PEP and PTP-PEST suppress SFTK activity within lipid rafts (Hermiston et al., 2002).

CD45 is a transmembrane receptor-like phosphatase expressed on all nucleated cells of hematopoietic origin. There are a large number of CD45 isoforms expressed on cells as a consequence of mRNA splicing of three exons that encode portions of its extracellular domain (see below). As with the majority of transmembrane phosphatases, CD45 contains two phosphatase homology domains in its cytosolic region, although it appears that only the catalytic activity of the proximal domain is required for TCR signaling (Desai et al., 1994).

T cells lacking CD45 fail to proliferate or produce cytokines in response to either antigen or TCR cross-linking. Furthermore, patients with mutations in the CD45 gene have severe combined immunodeficiency (Kung et al., 2000). It is thought that CD45 is required for normal lymphocyte development and peripheral function because it is the primary phosphatase that dephosphorylates the C-terminal negative regulatory site of the SKTKs (Mcfarland et al., 1993).

The phosphatase activity of CD45 is inhibited by the formation of homodimers. This occurs when the catalytic domain of one CD45 molecule binds to the highly conserved juxtamembrane region of another. Mutations in this latter inhibitory wedge prevent homodimerization and predispose to autoimmunity (Majeti et al., 2000). Homodimerization is also controlled by extracellular domain O-linked carbohydrates present on specific CD45 isoforms. The smallest isoform of CD45 (CD45RO), which is expressed on activated T cells, homodimerizes with the highest efficiency. In contrast, $CD45RA^+$, which is expressed on naïve T cells, homodimerizes poorly. By regulating homodimerization, the different CD45 isoforms determine basal CD45 phosphatase activity. This, in turn, allows for different TCR-signaling thresholds on different T-cell subsets (Xu and Weiss, 2002).

Once phosphorylated, the TCR ITAMs recruit the tyrosine kinase ZAP-70 (zeta-associated protein of 70 kDa). ZAP-70 has two N-terminal SH2 domains and a C-terminal tyrosine kinase domain. After both autophosphorylation and direct phosphorylation by Lck, ZAP-70 becomes the primary kinase responsible for the activation of downstream pathways. In humans, the absence of ZAP-70 is associated with a severe combined immunodeficiency syndrome in which $CD8^+$ T cells fail to develop and TCR signaling in $CD4^+$ T cells is completely defective (Chan et al., 1994; Elder et al., 1994). In mice, a spontaneous point mutation in ZAP-70 causes defects in thymic selection that lead to chronic autoimmune arthritis that resembles rheumatoid arthritis (Sakaguchi et al., 2003).

The overall organization of BCR signal initiation is similar; however, there are some important differences. Several members of the SFTKs are expressed in B cells, including Fyn, Blk, and Lyn, and they all are capable of phosphorylating the Igα/Igβ ITAM tyrosines. Unlike the TCR, there is no coreceptor that recruits these SFTKs to the receptor. It has been postulated that either the SFTKs are preassembled in the resting receptor (Cambier et al., 1994) or that movement of the BCR into specialized lipid microdomains in the plasma membrane surface (lipid rafts) provides access to the Src-family kinases. This latter possibility will be discussed below.

It is also controversial as to whether the Src-family kinases are even required for BCR ITAM phosphorylation (Saijo et al., 2003). Under some circumstances, this function can be performed by the ZAP-70 homolog in B cells, Syk (Rolli et al., 2002). Furthermore, the Src-family kinases may activate specific signaling pathways independently of Syk (Saijo et al., 2003). Regardless of the specifics, in both the TCR and BCR the coordinated activation of one or more SFTKs and ZAP-70/Syk are required to activate downstream signaling pathways.

PROPAGATION OF SIGNALING: ASSEMBLY OF SIGNALOSOMES

The recruitment of ZAP-70/Syk to the antigen receptors provides an activated kinase at the receptor complex. For these kinases to activate signaling cascades, proximal substrates must be brought into proximity. This is accomplished by a series of linker molecules that have been identified over the past several years. These molecules not only link enzyme to substrate but they assemble divergent signaling molecules together in arrays that ensure the efficient activation of complex and important signaling pathways (see Figure 19.1).

T-Cell Receptor

Once activated, ZAP-70 phosphorylates two important linkers for TCR signaling: LAT (linker for activation of T cells) and SLP-76 (SH2-domain containing leukocyte protein of 76 kDa) (Tomlinson et al., 2000). LAT is a 36–38 kDa protein expressed in T cells, natural killer (NK) cells, and mast cells. The amino acid sequence of this protein reveals 10 tyrosine residues in its cytosolic domain and two cysteine residues in its juxtamembrane region. These cysteine residues are acylated and target LAT to lipid rafts. The phosphorylation of the tyrosine residues creates binding sites for SH2-domain-containing proteins, including phospholipase Cγ1 (PLCγ1), Grb-2 (a regulator of Ras), Gads

(also referred to as GrpL, or Mona), and the p85 subunit of phosphoinositide 3-kinase (PI3K) (Zhang et al., 1998).

SLP-76 contains three tyrosine residues in the N-terminal region, a central proline-rich domain, and a SH2 domain in the C-terminus. Once phosphorylated, the three phosphotyrosines provide docking sites for SH2-domain-containing proteins, including Vav (a guanine-nucleotide-exchange factor for the small GTPase Rac), the adaptor Nck, and Itk, a Tec family tyrosine kinase that activates PLCγ1. The proline-rich region in SLP-76 constitutively binds the SH3-domain Gads, which also contains an SH2 that binds phospho-SLP76 (Law et al., 1999). In this way, Gads acts as a bridge to assemble SLP-76 and LAT into a single activation platform (Jordan et al., 2003).

B-Cell Receptor

The primary linker in B lymphocytes that couples the BCR to most distal signaling pathways is BLNK (B-cell linker protein, also termed SLP-65 or BASH) (Kurosaki and Tsukada, 2000). This 65 kDa protein, which is a direct substrate of Syk, contains a C-terminal SH2-domain, at least five tyrosines that are phosphorylated following BCR ligation, and multiple potential SH3-binding proline-rich motifs (Chiu et al., 2002). Structurally, BLNK is very similar to SLP-76. It binds to many of the same signaling molecules, including Vav, Nck, Btk (the only Tec kinase expressed in B cells), and Grb-2. Additionally, BLNK binds PLCγ2, a function attributed to LAT in T cells. Structurally and functionally, BLNK is a composite of SLP-76 and LAT. A LAT structural homolog has been described in B cells, LAB. However, it is unclear if it is required for normal PLCγ2 activation. Furthermore, it does not detectably interact with BLNK. Therefore, the available evidence indicates that BLNK is the primary linker involved in initial BCR signaling. However, it is not the only linker. In addition to LAB, BAM32 has been implicated in Erk activation and cytoskeletal reorganization (Han et al., 2003). GrpL is also expressed in B cells but apparently does not play a critical role in BCR signaling.

In contrast to the relationship of the TCR with its primary linkers, BLNK is directly recruited to the BCR complex (Kabak et al., 2002). Following BCR engagement, a unique conserved non-ITAM tyrosine in the cytosolic tail of Igα is phosphorylated, and this directly binds the SH2 domain of BLNK. BLNK is then rapidly phosphorylated and the next level of signaling molecules are recruited and activated.

ACTIVATION OF DOWNSTREAM SIGNALING

Following assembly of the signalsomes in T and B cells, there is a multitude of signaling processes that are activated which ultimately regulate transcriptional programs. We will concentrate on the signaling pathways that regulate two of the most important transcriptional factors, nuclear factor of activated T cells (NFAT) and nuclear factor of κ light chain in B cells (NF-κB) (Figure 19.2).

Intracellular Calcium and NFAT

One important molecule that participates in signal propagation is PLCγ (Kurosaki, 1999; Kurosaki et al., 2000). There are at least 10 different isoforms of phospholipase, with PLCγ1 being the predominant isoform in T cells and PLCγ2 being the predominant isoform in B cells. Both PLCγs have a similar structure with an N-terminal pleckstrin homology (PH) domain that binds phosphatidylinositides, followed by a catalytic domain, two tandem SH2-domains, an SH3-domain, and then a second catalytic domain. PLCγ is recruited to either BLNK or SLP76/LAT after phosphorylation by Syk or ZAP-70, respectively. Recruitment brings PLCγ into proximity with the plasma membrane, which contains the phosphatidylinositols that are its substrate. However, this is not sufficient for activation. Concurrent with PLCγ recruitment, a member of the Itk family of tyrosine kinases is also recruited to the linker complex. The Itk [interleukin (IL)-2 tyrosine kinase] family member then phosphorylates PLCγ at several sites, enhancing its intrinsic catalytic activity (Watanabe et al., 2001). In the case of T cells, Itk or Rlk can serve this function, while in B cells, the only Itk family member expressed is Btk (Bruton tyrosine kinase). Illustrating the singular importance of Btk, mutations in the molecule are associated with X-linked agammaglobulinemia (Tsukada et al., 1993).

Once activated, PLCγ cleaves phosphatidylinositol bisphosphate (PIP_2) into inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to specific receptors on specialized endoplasmic reticulum compartments, promoting release of calcium into the cytosol. Increased intracellular calcium activates a variety of signaling molecules, including calcineurin, a serine/threonine phosphatase. This enzyme dephosphorylates a transcription factor known as NFAT (nuclear factor of activated T cells) (Shibasaki et al., 1996), which is expressed in both T and B cells. Once dephosphorylated, NFAT is released from the cytosol and migrates to the nucleus, promoting the initiation of multiple genes either directly or in partnership with other transcriptional factors such as AP-1 (activator protein-1) (Hogan et al., 2003). Independently of calcineurin, intracellular calcium activates other transcriptional factors, including Nur77 and Nor1, which regulate the proapoptotic inhibitor Bim.

The NFAT pathway is important therapeutically because drugs such as cyclosporine A and tacrolimus are selective inhibitors of calcineurin (Shibasaki et al., 1997). They block the translocation of NFAT to the nucleus, preventing the transcription of genes required for lymphocyte survival.

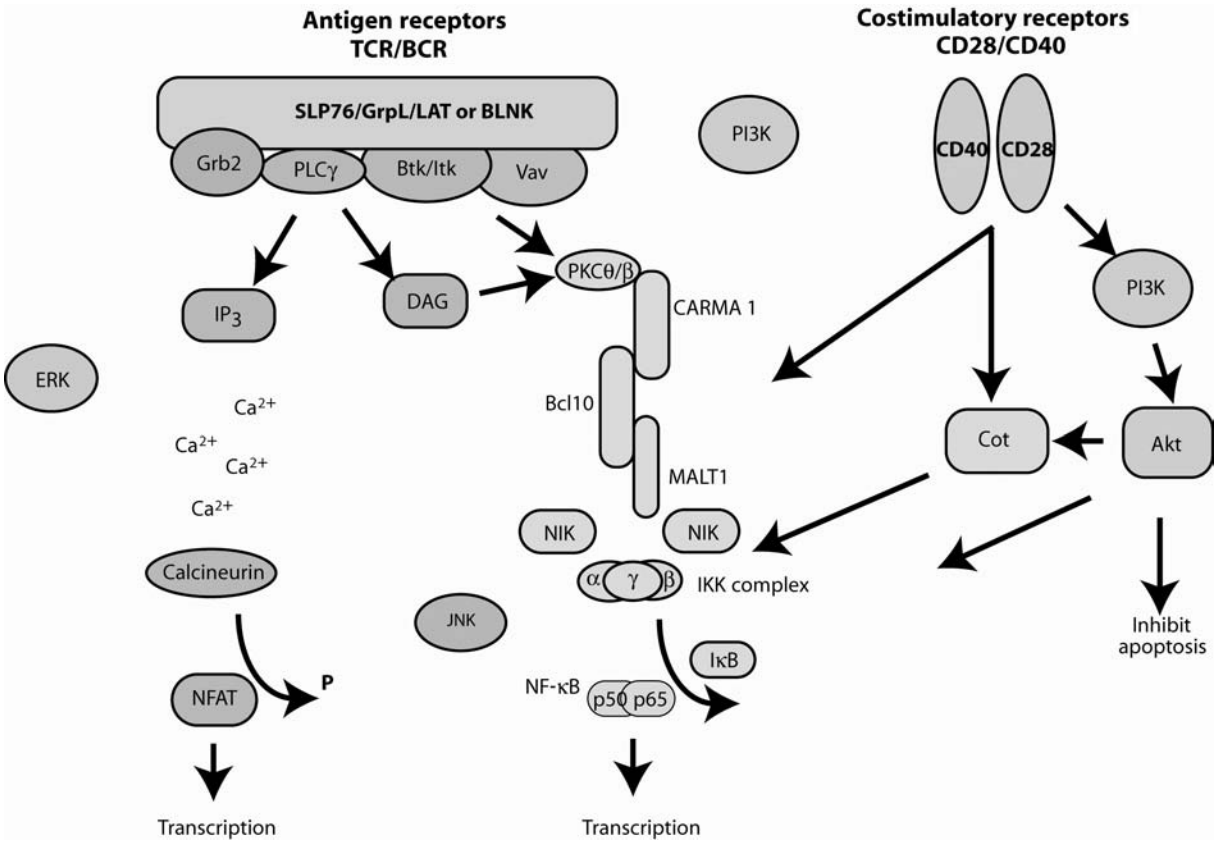


FIGURE 19.2 Signaling pathways activated by the antigen receptors and their primary coreceptors. Following antigen receptor aggregation, multiple molecules are organized on scaffolds that are in close proximity to the activated kinases associated with each receptor. The coordinated recruitment of PLCγ and Btk serves to activate PLCγ and generate free intracellular calcium and diacylglycerol (DAG). Full activation of PLCγ is also dependent upon the activation of phosphoinositide 3-kinase (PI3K) and Vav. Increases in intracellular calcium activate calcineurin, which dephosphorylates nuclear factor of activated T cells (NFAT), allowing it to translocate to the nucleus. ERK is downstream of both Grb2 and PLCγ. Both calcium and DAG contribute to the activation of multiple protein kinase Cs (PKCs), including PKCθ in T cells and PKCβ in B cells. These latter PKCs phosphorylate CARMA1, which leads to IKK activation, IκB degradation, and, finally, the activation of NF-κB. The activation of NF-κB through the T-cell receptor (TCR) is also dependent upon NIK, which can directly phosphorylate IKK. JNK is also downstream of CARMA1. Costimulation provides for the efficient activation of NF-κB through multiple pathways, including CARMA1, NIK (CD40), and, in the case of CD28, indirectly through Akt. Akt also activates multiple downstream pathways, including some which protect against apoptosis. This schematic drawing is a simplified representation of some of the signaling pathways activated by the indicated receptors and has been drawn to emphasize common signaling themes.

These drugs are used in the treatment of several autoimmune diseases and in transplantation. Although quite successful in suppressing the immune system, the use of these inhibitors could theoretically impede the induction of anergy (see below).

The other product that results from cleavage of phosphatidylinositol bisphosphate by PLCγ is DAG. DAG remains in the inner face of the membrane and together with elevated calcium levels activates the classical family of protein kinase Cs (PKC). Of these serine/threonine kinases, PKCθ in T cells and PKCβ in B cells have recently been implicated in the activation of the NF-κB (nuclear factor of kappa light chain in B cells) transcription factor family.

Activation of NF-κB

NF-κB is regulated by a wide variety of receptors of the immune system, including the TCR, BCR, tumor necrosis factor (TNF) receptor family members, and the Toll-like receptors (TLRs). NF-κB activation is central to the proinflammatory effect of these divergent receptors. Not surprisingly, NF-κB is highly activated in a variety of inflammatory diseases and is a major target for drug development in

autoimmune diseases and transplantation. Extensive gene-targeting experiments have demonstrated the importance of the NF-κB pathway in regulating the transcription of genes that control cellular proliferation and cell survival (Li and Verma, 2002).

There are five NF-κB subunits—NF-κB1 (p50), NF-κB2 (p52), RELA (p65), RELB, and c-REL. Active transcriptional complexes contain heterodimers of p50 or p52 paired with a REL transcription factor. NF-κB exists in the cytoplasm in its inactive form, associated with inhibitory proteins that are known as inhibitors of NF-κB (IκB α, β, ε) (Li and Verma, 2002). Upon activation through a variety of receptors, the IκB complex is phosphorylated, ubiquitinated, and degraded, leaving NF-κB free to migrate to the nucleus and initiate transcription. Optimal activation of NF-κB by the antigen receptors usually requires concurrent stimulation through the costimulatory receptors CD28 for T cells or, in the case of B cells, CD40.

Recently, there has been substantial progress in defining the signaling pathways linking the antigen receptors to IκB phosphorylation (Lucas et al., 2004). Following antigen-receptor ligation, PKCθ in T cells and PKCβ in B cells are recruited into proximity of the activated antigen receptors through mechanisms that are still unclear. These kinases recruit the lymphoid-restricted adaptor CARMA1 (CARD 11), which, in turn, leads to the sequential recruitment of the Bcl10 and MALT1 adaptors. This complex then directly regulates the serine-threonine kinase complex that phosphorylates IκB, IKK (IκB kinases). IKK is comprised of two catalytic subunits α and β, and a regulatory subunit, γ(NEMO). Bcl10 targets NEMO for ubiquitination, which is necessary for NF-κB activation (Zhou et al., 2004). In addition to the CARMA1–Bcl10–MALT1 complex, NIK (NF-κB inducing kinase), directly activates IKK and may be required for normal NF-κB activation through the TCR.

The CARMA1 scaffold may provide lymphocytes with a unique integration point for divergent signals that regulate NF-κB (Jun and Goodnow, 2003). For example, in Bcl-$10^{-/-}$ mice, NF-κB activation through the antigen and costimulatory receptors is impaired, but activation through TLR4 is normal. Similarly, point mutations have been introduced into CARMA1 that uncouple BCR and CD28, but not TCR or TLR4, from NF-κB activation. These observations have led to the idea that CARMA1–Bcl10–MALT1 may function to integrate multiple environmental inputs and translate them into a composite signal regulating NF-κB.

LIPID RAFTS AND FORMATION OF THE IMMUNOLOGIC SYNAPSE

The plasma membrane is not homogenous but contains organized subdomains rich in sphingolipid and cholesterol that are rich in acylated signaling molecules, such as the SFTKs, LAT, and Ras. These specialized microdomains are referred to as lipid rafts or glycolipid-enrichedmicrodomains (GEMs) (Dykstra et al., 2003). In resting cells, the antigen receptors are located outside the lipid rafts. However, following receptor ligation there is a rapid translocation of both the TCR and BCR into the lipid rafts. Despite intense investigation, the exact role of lipid rafts in receptor function is still controversial. In immature B cells the BCR does not segregate into lipid rafts and signaling is grossly intact. Therefore, lipid rafts do not appear necessary for signal initiation. However, the concentration of important signaling molecules in the lipid rafts in resting cells, and the recruitment of additional signaling effectors following receptor ligation, suggest that lipid rafts may contribute to signal propagation and amplification.

Concurrent with lipid raft coalescence, productive presentation of antigen to T cells is associated with the organization of the TCR and costimulatory molecules into supramolecular activation clusters (SMACs), which define the immunologic synapse on T cells. Classically, the mature immunologic synapse consists of a central SMAC (c-SMAC) containing engaged TCRs and a peripheral SMAC (p-SMAC) consisting of an encircling ring of adhesion molecules or integrins (Davis and Dustin, 2004). However, variations in this structure have been reported to occur in cytolytic T and NK-cells. As is the case with lipid rafts, there is still controversy as to what the SMACs do. Furthermore, their exact relationship to lipid rafts is not clear. However, formation of the SMAC requires active signaling and a reorganization of the actin cytoskeleton, while simple segregation to lipid rafts does not (Miletic et al., 2003). There is evidence to support a role for SMACs in enhancing signaling and for integrating positive and negative signals (Lee et al., 2003). Formation of the SMAC polarizes the cell, allowing important cytokines or cytotoxic granules to be secreted at the T-cell–APC interface. It has also been postulated that they play a role in the degradation of the receptor and signal termination (Lee et al., 2003). It is likely that SMAC formation helps commit a cell to a particular biologic response.

ROLE OF COSTIMULATION

Lymphocytes require two distinct signals to achieve full activation. The first is antigen specific and involves the TCR and BCR (signal 1). The second signal, also known as signal 2, is antigen independent and requires the participation of costimulatory receptors. In T cells, antigen receptor signaling without concurrent costimulation can result in anergy and clonal deletion. In B cells, a lack of costimulation does not necessarily lead to anergy but can result in developmental arrest and cell death.

Costimulatory receptors cannot activate lymphocytes on their own. Rather, they augment and complement signals provided by the antigen receptor. Below we describe the influence of two main costimulatory molecules in T and B lymphocyte activation and the downstream pathways that they regulate.

CD28

CD28 is a 44-kDa disulfide-linked glycoprotein expressed constitutively in the majority of $CD4^+$ and $CD8^+$ T cells. It binds to CD80 (B7.1) and CD86 (B7.2), expressed on the surface of APCs. The interaction of CD28 with its ligands promotes T-cell proliferation, IL-2 production, and survival of naïve T cells. The latter effect may be related to the fact that CD28 ligation upregulates the antiapoptotic Bcl-2 family member, Bcl-X_L (Alegre et al., 2001). Finally, CD28 costimulation decreases the threshold of T-cell activation, possibly by promoting the formation of the immunologic synapse. Much research has focused on the molecular mechanisms coupling CD28 ligation to its downstream events (Frauwirth and Thompson, 2002).

Like the TCR, CD28 lacks intrinsic kinase activity. The cytoplasmic tail contains four tyrosine residues that are phosphorylated by the SFTKs Lck and Fyn. One effector that is recruited to these phosphotyrosines is PI3K. Once activated, PI3K generates 3′ phosphorylated inositol lipids, which then recruit PH domain-containing proteins to the plasma membrane. One of the best characterized is Akt (previously known as protein kinase B or PKB), which is a serine/threonine kinase that activates NF-κB. The mechanisms that link Akt to NF-κB pathway are not completely understood but possibilities include the direct phosphorylation of IKKα by Akt, or indirect phosphorylation of the IKKs through the activation of intermediate kinases, such as Cot (a member of the mitogen-activated protein kinase family) (Kane and Weiss, 2003). Recruitment of Vav also occurs with CD28 costimulation, contributing to calcium mobilization, cytoskeletal remodeling, and transcriptional activation.

Blockade of the CD28 pathway holds great promise in the treatment of autoimmune diseases. CTLA4Ig is a soluble fusion protein that binds to CD80 (B7.1) and CD86 (B7.2) on APCs, acting as a competitive inhibitor of CD28. CTLA4Ig has been used successfully in animal models of diabetes and systemic lupus erythematosus (SLE). Furthermore, it was efficacious in preliminary clinical trials of psoriasis and rheumatoid arthritis (Dall'Era and Davis, 2004). Larger studies are ongoing in rheumatoid arthritis (See Chapter 76).

CD40

CD40 is a 48-kDa transmembrane glycoprotein expressed in B cells, DCs, macrophages, and epithelial cells. Its ligand is CD154, a membrane protein, expressed mainly on activated T cells. Engagement of CD40 promotes B-cell clonal expansion, germinal center formation, and isotype switching. Engagement of CD40 initiates signaling pathways that activate mitogen-activated protein kinases and the NF-κB pathway. This is accomplished through the recruitment of adapter proteins known as TRAFs (TNF receptor-associated factors) to the cytoplasmic tail of CD40 and the formation of a multimeric signaling complex that includes NIK. In DCs, the activation of the NF-κB pathway via CD40 is associated with upregulation of BCl-x and Bcl-2 (Quezada et al., 2004).

Anti-CD154 has been used to block the CD40/CD154 pathway in many autoimmune disease models with success. These animal models include, but are not limited to, autoimmune encephalomyelitis (EAE), collagen-induced arthritis, diabetes, SLE, and thyroiditis. As with CTLA4Ig, clinical trials will be needed to address the efficacy and safety of CD40 blockade in humans (Quezada et al., 2004) (See Chapter 76).

DETERMINING RECEPTOR THRESHOLD

The signaling threshold through the antigen receptors is dependent upon the presence and activity of coreceptors and regulatory molecules (Pritchard and Smith, 2003). This has been best defined for the BCR in which both positive and negative coreceptors have been defined. However, similar regulatory mechanisms have been recently defined in T cells (Chen, 2004).

A primary negative regulator of the BCR is CD22, which is constitutively associated with the resting BCR. Following BCR stimulation, the cytosolic tail of CD22 is phosphorylated at a tyrosine within a conserved motif known as the immmunoreceptor tyrosine-based inhibitiory motif (ITIM). The ITIMs are found within a wide range of receptors, including NK-cell receptors, PD1, FcγRIIb1, and others involved in the negative regulation of lymphocytes (Ravetch and Lanier, 2000). In the case of CD22, phosphorylation is mediated by the Src-family tyrosine Lyn. In contrast to the general redundancy of most SFTKs, only Lyn can phosphorylate the CD22 ITIM. The CD22 phospho-ITIM then recruits SHP-1, which is an SH2-domain-containing tyrosine phosphatase. SHP-1 has a broad specificity and appears to be able to dephosphorylate several proteins involved in proximal BCR signaling (Cornall et al., 1998; Zhang et al., 2000). The importance of CD22, SHP-1, and Lyn have been defined in genetically targeted and naturally occurring mutant mice (Yu et al., 2003). Deletion of CD22 results in hyperproliferative peripheral B cells and antibodies to dsDNA, while deletion of either Lyn or SHP-1 results in an autoimmune disease similar to SLE. The phenotype of mice

deficient in SHP-1 is the most severe, reflecting the negative regulatory role SHP-1 plays in a wide range of immune receptors, including the TCR.

Another negative regulatory receptor on B cells is FcγRIIb1. Like CD22 it has an ITIM. However, the receptor is not associated with the resting BCR and only becomes phosphorylated if the BCR and FcγRIIb1 are coengaged (Unkeless and Jin, 1997). This occurs when the BCR engages immune complexes or when anti-idiotypic antibodies are generated against the specificity of the BCR. These are negative regulatory loops that inhibit further antibody production. Coengagement of the BCR and FcγRIIb1 leads to the phosphorylation of the FcγRIIb1 ITIM and the recruitment of another negative regulatory molecule SHIP (SH2-containing inositol phosphatase) which dephosphorylates PIP3 (Ono et al., 1997). By counteracting the phosphokinase activity of PI-3 kinase, there is less membrane recruitment of molecules containing PH domains, such as PLCγ and Btk. Through this, and other mechanisms (Tamir et al., 2000), coligation of the BCR and FcγRIIb1 attenuates intracellular calcium responses and inhibits B-cell proliferation.

While CD22 and FcγRIIb1 effectively raise receptor threshold, coengagement of CD19 and the BCR dramatically lowers it (Carter and Barrington, 2004). CD19 is the signaling subunit of a receptor complex for the complement cleavage product C3d [complement receptor 2 (CR2) also containing CD21 and Tapa 81]. Coengagement of CR2 and the BCR by opsonized immune complexes can enhance *in vivo* B-cell–mediated immune responses by up to a 1000-fold (Carter and Fearon, 1992). Enhanced BCR responsiveness is dependent upon SFTK-mediated phosphorylation of the CD19 cytosolic tail, which serves to further amplify SFTK activation and recruit PI-3 kinase (Kurosaki, 2002). Deletion of CD19 results in defects in peripheral maturation and diminished immune responses (Wang and Brooks, 2002). Conversely, transgenic overexpression of CD19 induces SLE-like manifestations (Tuscano et al., 2003).

SIGNALING AND TOLERANCE

Depending upon the nature and context of the ligand, antigen-receptor engagement can result in cellular activation, cell death or tolerance. Each is an active process that arises from the activation of different signaling programs. The signaling pathways induced by activating ligands has been described above. In contrast, anergizing ligands induce only a subset of these signaling pathways and the biologic responses are fundamentally different. In tolerant T cells, the TCR is uncoupled from the ERK, JNK, and NF-κB pathways, while in tolerant B cells the BCR is uncoupled from JNK and NF-κB (Jun and Goodnow, 2003). However, in both cases low-magnitude calcium oscillations are sufficient to activate NFAT. In T cells, costimulation through CD28 can prevent the induction of anergy, while stimulation of B cells through either the lipopolysaccharide (LPS) receptor (TLR4) or CD40 prevents it. It is likely that engaging coreceptors or TLRs prevents anergy by activating NF-κB and JNK.

Recently, it has become apparent that the processes regulating the degradation of antigen receptors and their downstream signaling pathways are important for determining responses to antigen (Davis, 2004). Normal protein homeostasis is regulated by poly-ubiquitination. Ubiquitin is an 8-kDa molecular tag that targets proteins for degradation by the proteosome. Ubiquitination has been demonstrated to regulate TCR internalization and degradation, as well as the steady-state levels of ZAP-70, Syk, PI-3 kinase, the Src-family kinases, and BLNK. Ubiquitin-mediated degradation of IκB is required for normal activation of NF-κB. There are hundreds of ubiquitin ligases but two that have been demonstrated to be important in antigen-receptor function are C-cbl (Casitas B-lineage lymphoma) and Cbl-b (Zhang, 2004). C-cbl is predominantly expressed in developing lymphocytes, while Cbl-b predominates in the periphery. Consistent with their relative expression patterns, thymocytes from C-cbl$^{-/-}$ mice have enhanced ZAP-70 activation and positive selection. In contrast, Cbl-b$^{-/-}$ mice develop a lupus-like disease with hyperproliferative T and B cells. Cbl-b deficiency complements CD28 deficiency, suggesting that Cbl-b might be involved in responses to costimulation. Interestingly, Cbl-b targets PKCθ and is upregulated in anergic T cells. Furthermore, Cbl-b$^{-/-}$-deficient mice are resistant to anergy induction (Heissmeyer et al., 2004). These and other data indicate that targeted degradation of specific signaling molecules may be an important mechanism controlling antigen-receptor responsiveness.

SUMMARY

In this overview, we have provided an outline of what is known about several pathways that regulate lymphocyte activation and tolerance. The discussion is by no means complete. Several important signaling processes were not covered and we have simplified the signaling cascades of others. This was done to provide the reader with an overall concept of how antigen engagement is translated into cellular decisions. Furthermore, we have highlighted the signaling processes that may contribute to autoimmunity and those signaling pathways that hold the most promise for therapeutic intervention.

Acknowledgments

We would like to thank Marisa Alegre and Iannis Aifantis for reading the manuscript and helpful discussions. This work was supported by grants from the NIH (GM 52736) and the Arthritis Foundation.

References

Alegre, M.L., Frauwirth, K.A., and Thompson, C.B. (2001). Recent developments in the two-signal model of T cell activation. *Nat. Rev. Immunol. 1*, 220–228.

Call, M.E., Pyrdol, J., Wiedmann, M., and Wucherpfennig, K.W. (2002). The organizing principle in the formation of the T cell receptor-CD3 complex. *Cell 111*, 967–979.

Cambier, Pleiman C.M., and Clark, M.R. (1994). Signal transduction by the B cell antigen receptor and its coreceptors. *Ann. Rev. Immunol. 12*, 457–486.

Campbell, K.S. (1999). Signal transduction from the B cell antigen-receptor. *Curr. Opin. Immunol. 11*, 256–264.

Carter, R.H. and Barrington, R.A. (2004). Signaling by the CD19/CD21 complex on B cells. *Curr. Dir. Autoimmun. 7*, 4–32.

Carter, R.H., and Fearon, D.T. (1992). CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science 256*, 105–107.

Chan, A.C., Kadlecek, T.A., Elder, M.E., Fillipovich, A.H., Kuo, W.L., Iwashima, M., Parslow, T.G., and Weiss, A. (1994). ZAP-70 deficiency in an autosomal recessive form of severe combined immunodeficiency. *Science 264*, 1599–1601.

Chen, L. (2004). Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat. Rev. Immunol. 4*, 336–347.

Chiu, C.W., Dalton, M., Ishiai, M., Kurosaki, T., and Chan, A.C. (2002). BLNK: molecular scaffolding through 'cis'-mediated organization of signaling proteins. *EMBO J. 21*, 6461–6472.

Clark, M.R., Massenburg, D., Siemasko, K., Hou, P., and Zhang, M. (2004). B-cell antigen receptor signaling requirements for targeting antigen to the MHC class II presentation pathway. *Curr. Opin. Immunol. 16*, 382–387.

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*, 497–508.

Dall'Era, M. and Davis, J. (2004). CTLA4Ig: a novel inhibitor of costimulation. *Lupus 13*, 372–376.

Davis, D.M. (2004). Ubiquitin ligases and the immune response. *Ann. Rev. Immunol. 22*, 81–127.

Davis, D.M. and Dustin, M.L. (2004). What is the importance of the immunological synapse? *Trends Immunol. 25*, 323–327.

Desai, D., Sap, J., Silvennoinen, O., Schlessinger, J., and Weiss, A. (1994). The catalytic activity of the CD45 membrane-proximal phosphatase domain is required for TCR signaling and regulation. *EMBO J. 13*, 4002–4010.

Dykstra, M.L., Cherukuri, A., Sohn, H.W., Tzeng, S.-J., and Pierce, S.K. (2003). Location is everything: lipid rafts and immune cell signaling. *Ann. Rev. Immunol. 21*, 457–481.

Elder, M.E., Lin, D., Clever, J., Chan, A.C., Hope, T.J., Weiss, A., and Parslow, T.G. (1994). Human severe combined immunodeficiency due to a defect in ZAP-70, a T cell tyrosine kinase. *Science 264*, 1596–1599.

Frauwirth, K.A. and Thompson, C.B. (2002). Activation and inhibition of lymphocytes by costimulation. *J. Clin. Invest. 109*, 295–299.

Han, A., Saijo, K., Mecklenbrauker, I., Tarakhovsky, A., and Nussenzweig, M.C. (2003). Bam32 links the B cell receptor to Erk and Jnk and mediates B cell proliferation but not survival. *Immunity 19*, 621–632.

Heissmeyer, V., Macian, F., Im, S.H., Varma, R., Feske, S., Venuprasad, K., Gu, H., Liu, Y.C., Dustin, M.L., and Rao, A. (2004). Calcineurin imposes T cell unresponsiveness through target proteolysis of signaling proteins. *Nat. Immunol. 5*, 255–265.

Hermiston, M.L., Xu, Z., Majeti, R., and Weiss, A. (2002). Reciprocal regulation of lymphocyte activation by tyrosine kinases and phosphatases. *J. Clin. Invest. 109*, 9–14.

Hermiston, M.L., Xu, Z., and Weiss, A. (2003). CD45: a critical regulator of signaling thresholds in immune cells. *Annu. Rev. Immunol. 21*, 107–137.

Hogan, P.G., Chen, L.P., Nardone, J., and Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev. 17*, 2205–2232.

Irvine, D.J., Purbhoo, M., Krogsgaard, M., and Davis, M.M. (2002). Direct observation of ligand recognition by T cells. *Nature 419*, 845–849.

Jordan, M.S., Singer, A.L., and Koretzky, G. (2003). Adaptors as central mediators of signal transduction in immune cells. *Nat. Immunol. 4*, 110–116.

Jun, J.E., and Goodnow, C.C. (2003). Scaffolding of antigen receptors for immunogenic versus tolergenic signaling. *Nat. Immunol. 4*, 1057–1064.

Kabak, S., Skaggs, B.J., Gold, M.R., Affolter, M., West, K.L., Foster, M.S., Siemasko, K., Chan, A.C., Aebersold, R., and Clark, M.R. (2002). The direct recruitment of BLNK to immunoglobulin alpha couples the B-cell antigen receptor to distal signaling pathways. *Mol. Cell. Biol. 22*, 2524–2535.

Kane, L.P. and Weiss, A. (2003). The PI-3 kinase/Akt pathway and T cell activation: pleiotropic pathways downstream of PIP3. *Immunol. Rev. 192*, 7–20.

Kraus, M., Alimzhanov, M.D., Rajewsky, N., and Rajewky, K. (2004). Survival of resting mature B lymphocytes dependes on BCR signaling via the Ig-alpha/Ig-beta heterodimer. *Cell 117*, 787–800.

Kung, C., Pingel, J.T., Heikinheimo, M., Klemola, T., Varkila, K., Yoo, L.I., Vuopala, K., Poyhonen, M., Uhari, M., and Rogers, M. (2000). Mutations in the tyrosine phosphatase CD45 gene in a child with severe combined immundeficiency. *Nat. Med. 6*, 343–345.

Kurosaki, T. (1999). Genetic analysis of B cell antigen receptor signaling. *Annu. Rev. Immunol. 17*, 555–592.

Kurosaki, T. (2002). Regulation of B-cell signal transduction by adaptor proteins. *Nat. Rev. Immunol. 2*, 354–363.

Kurosaki, T., Maeda, A., Ishiai, M., Hashimoto, A., Inabe, K., and Takata, M. (2000). Regulation of the phospholipase C-gamma2 pathway in B cells. *Immunol. Rev. 176*, 19–29.

Kurosaki, T. and Tsukada, S. (2000). BLNK: Connecting Syk and Btk to calcium signals. *Immunity 12*, 1–5.

Lam, K.-P., Kuhn, R., and Rajewsky, K. (1997). *In vivo* ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell 90*, 1073–1083.

Law, C.L., Ewings, M.K., Chaudhary, P.M., Solow, S.A., Yun, T.J., Marshall, A.J., Hood, L., and Clark, E. (1999). GrpL, a Grb2-related adaptor protein, interacts with SLP-76 to regulate nuclear factor of activated T cell activation. *J. Exp. Med. 189*, 1243–1253.

Lee, K.H., Dinner, A.R., Tu, C., Campi, G., Raychaudhuri, S., Varma, R., Sims, T.N., Burack, W.R., Wu, H., and Wang, J. (2003). The immunological synapse balances T cell receptor signaling and degradation. *Science 302*, 1218–1222.

Li, Q. and Verma, I.M. (2002). NF-kappaB regulation in the immune system. *Nat. Rev. Immunol. 2*, 725–734.

Lucas, P.C., McAllister-Lucas, L.M., and Nunez, G. (2004). NF-kappaB signaling in lymphocytes: a new cast of characters. *J. Cell. Sci. 117*, 31–39.

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*, 1059–1070.

Mcfarland, E.D.C., Hurley, T.R., Pingel, J.T., Sefton, B.M., Shaw, A., and Thomas, M.L. (1993). Correlation between src family member regulation by the protein-tyrosine-phosphatase CD45 and transmembrane signaling through the T-cell receptor. *Proc. Natl. Acad. Sci. USA 90*, 1402–1406.

Miletic, A.V., Swat, M., Fujikawa, K., and Swat, W. (2003). Cytoskeletal remodeling in lymphocyte activation. *Curr. Opin. Immunol. 15*, 261–268.

Niiro, H., and Clark, E.A. (2002). Regulation of B-cell fate by antigen-receptor signals. *Nat. Rev. Immunol. 2*, 945–956.

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*, 293–301.

Pingel, J.T., and Thomas, M.L. (1989). Evidence that the leukocyte-common antigen is required for antigen-induced T lymphocyte proliferation. *Cell 58*, 1055–1065.

Pritchard, N.R. and Smith, K.G.C. (2003). B cell inhibitory receptors and autoimmunity. *Immunology 108*, 263–273.

Quezada, S.A., Jarvinen, L.Z., Lind, E.F., and Noelle, R.J. (2004). CD40/CD154 interactions at the interface of tolerance and immunity. *Annu. Rev. Immunol. 22*, 307–328.

Ravetch, J.V. and Lanier, L.L. (2000). Immune inhibitory receptors. *Science 290*, 84–89.

Reichlin, A., Hu, Y., Meffre, E., Nagaoka, H., Gong, S., Kraus, M., Rajewsky, K., and Nussenzweig, M.C. (2001). B cell development is arrested at the immature B cell stage in mice carrying a mutation in the cytoplasmic domain of immunoglobulin β. *J. Exp. Med. 193*, 13–23.

Rolli, W., Gallwitz, T., Wossning, T., Flemming, A., Schamel, W.W., Zurn, C., and Reth, M. (2002). Amplification of B cell antigen receptor signaling by Syk/ITAM positive feedback loop. *Mol. Cell 10*, 1057–1069.

Saijo, K., Schmedt, C., Su, I. H., Karasuyama, H., Lowell, C.A., Reth, M., Adachi, T., Patke, A., Santana, A., and Tarakhovsky, A. (2003). Essential role of Src-family protein tyrosine kinases in NF-kappaB activation during B cell development. *Nat. Immunol. 4*, 274–279.

Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., and Sakaguchi, S. (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature 426*, 454–460.

Shibasaki, F., Kondo, E., Akagi, T., and McKeon, F. (1997). Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2. *Nature 386*, 728–731.

Shibasaki, F., Price, E.R., Milan, D., and Mckeon, F. (1996). Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. *Nature 382*, 370–373.

Shores, E.W., and Love, P.E. (1997). TCR zeta chain in T cell development. *Curr. Opin. Immunol. 9*, 380–389.

Tamir, I., Stolpa, J.C., Helgason, C.D., Nakamura, K., Bruhns, P., Daeron, M., and Cambier, J.C. (2000). The RasGAP-binding protein p62dok is a mediator of inhibitory FcgammaRIIB signals in B cells. *Immunity 12*, 347–358.

Tomlinson, M.G., Lin, A., and Weiss, A. (2000). Lymphocytes with a complex: adapter proteins in antigen receptor signaling. *Immunol. Today 21*, 584–591.

Tsukada, S., Saffran, D.C., Rawlings, D.J., Parolini, O., Allen, R.C., Klisak, I., Sparkes, R.S., Kubagawa, H., Mohandas, T., and Quan, S. (1993). Deficient expression of a B-cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell 72*, 279–290.

Tuscano, J.M., Harris, G.S., and Tedder, T.F. (2003). B lymphocytes contribute to autoimmune disease pathogenesis: current trends and clinical implications. *Autoimmun. Rev. 2*, 101–108.

Unkeless, J.C. and Jin, J. (1997). Inhibitory receptors, ITIM sequences and phosphatases. *Curr. Opin. Immunol. 9*, 338–343.

Wakabayashi, C., Adachi, T., Wienands, J., and Tsubata, T. (2002). A distinct signaling pathway used by the IgG-containing B cell antigen receptor. *Science 298*, 2392–2395.

Wang, L.D. and Clark, M.R. (2003). B cell antigen receptor signaling in lymphocyte development. *Immunology 110*, 411–420.

Wang, Y. and Brooks, S.R. (2002). The physiologic role of CD19 cytoplasmic tyrosines. *Immunity 17*, 501–514.

Watanabe, D., Hashimoto, S., Ishiai, M., Matsushita, M., Baba, Y., Kishimoto, T., Kurosaki, T., and Tsukada, S. (2001). Four tyrosine residues in phospholipase C-gamma2, identified as Btk-dependent phosphorylation sites, are reuired for B cell antigen receptor-coupled calcium signaling. *J. Biol. Chem. 276*, 38595–38601.

Weiss, A. and Littman, D.R. (1994). Signal transduction by lymphocyte antigen receptors. *Cell 76*, 263–274.

Wolfgang, W.A., Reth, S., and Reth, M. (2000). Monomeric and oligomeric complexes of the B cell antigen receptor. *Immunity 13*, 5–14.

Xu, Z. and Weiss, A. (2002). Negative regulation of CD45 by differential homodimerization of the alternatively spliced isoforms. *Nat. Immunol. 3*, 764–771.

Yu, C.C., Mamchak, A.A., and DeFranco, A.L. (2003). Signaling mutations and autoimmunity. *Curr. Dir. Autoimmun. 6*, 61–88.

Zhang, J. (2004). Ubiquitin ligases in T cell activation and autoimmunity. *Clin. Immunol. 111*, 234–240.

Zhang, J., Somani, A.-K., and Siminovitch, K.A. (2000). Roles of the SHP-1 tyrosine phosphatase in the negative regulations of cell signalling. *Semin. Immunol. 12*, 361–378.

Zhang, W., Sloan-Lancaster, J., Kitchen, J., Trible, R.P., and Samelson, L.E. (1998). LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell 92*, 83–92.

Zhou, H., Wertz, I., O'Rourke, K., Ultsch, M., Seshagiri, S., Eby, M., Xiao, W., and Dixit, V.M. (2004). Bcl10 activates NF-kappaB pathway through ubiquitination of NEMO. *Nature 427*, 167–171.

CHAPTER

20

Genetics and Autoimmunity: HLA and MHC Genes

VEENA TANEJA AND CHELLA S. DAVID

Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

The human immune system has evolved over the centuries to combat onslaughts of invading microorganisms. The immune system operates via two ways—innate immunity, a nonspecific first line of defense after infection, and adaptive immunity, an acquired immunity against a specific entity. The main feature of the adaptive immunity is its diverse nature of immune response. The major players for adaptive immunity are the genes of the major histocompatibility complex (MHC). The MHC region encodes for polymorphic human leukocyte antigen (HLA) molecules that are critical for differentiating self from nonself. Mature T cells recognize foreign antigen when it is presented in the context of self-MHC. The loss of self-tolerance of the immune system against the body's own tissues/antigens leads to autoimmunity. The development of autoimmune diseases, such as rheumatoid arthritis (RA), type 1 diabetes (T1D), multiple sclerosis (MS) or myasthenia gravis (MG) is determined by both genetic and nongenetic factors. Among the genetics factors, the MHC region is known to be the most important. Within this region, certain HLA class II genes confer the strongest susceptibility to the majority of MHC-associated disorders.

MAJOR HISTOCOMPATIBILITY COMPLEX

Human MHC molecules are called HLAs, which are homologous to the H-2 of mice. The MHC region is located on the short arm of chromosome 6 in humans and is 3500 kb long. It encodes for two types of HLA, designated as class I and class II. HLA class I molecules contain one heavy chain of 44 kDa and one non-MHC-encoded nonpolymorphic β2 microglobulin of 12 kDa. The heavy chain consists of three extracellular domains of 90 residues each. The polymorphic residues in class I molecules are located on the α1 and α2 domains of the heavy chain. The products of class I genes include HLA-A, HLA-B, and HLA-C, and are expressed on all nucleated cells. Class I molecules can bind 8–10 amino acid-long peptides that have been processed endogenously.

HLA class II molecules are present as heterodimers on the cell surface, consisting of an α chain (32–34 kDa) and a β chain (29–32 kDa) each with two extracellular domains of about 90 amino acid residues (α1, α2 and β1, β2). The β chains of three class II genes, HLA-DR, DQ, and DP, are highly polymorphic, while the α chains are generally

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

nonpolymorphic. The class II molecules are expressed on antigen-presenting cells (APCs), like B lymphocytes, macrophages, dendritic cells (DCs), and endothelial and other organ-specific APCs. In general, class II molecules can accommodate peptides of up to 10–25 residues. In humans, DR, DQ, and DP are in linkage disequilibrium and are inherited en bloc. MHC genes are expressed codominantly in each individual. Crystal structures of DR and DQ have shown that the MHC molecule has a single peptide-binding cleft, which can accommodate a variety of peptides according to their charge, stability, and binding affinity.

MAJOR HISTOCOMPATIBILITY COMPLEX AND AUTOIMMUNITY

Susceptibility to many diseases with an autoimmune etiology is associated with HLA-DR and/or -DQ alleles. Table 20.1 gives some of the most significant associations between HLA and diseases. Despite over 25 years of investigation, the exact role of the HLA class II molecules in these disease processes is unknown. However, class I genes have not been associated with any autoimmune disease with the exception of HLA-B27, which is associated with a predisposition to a group of diseases collectively named "spondyloarthropathies" (see Chapter 33).

The major function of MHC genes is to clear infection. The cytotoxic T lymphocytes can lyse virus-infected targets only in the context of MHC class I molecules. During evolution, the HLA genes mutated to cope with the clearance of new pathogens. The migration of populations from Africa and Asia to other parts of the world exposed them to new microenvironments. The survival of such populations depended on clearing infections. This led to mutations of HLA genes and the generation of newer subtypes of a gene. The hypothesis we propose is that these mutations led to selection of subtypes of a gene with flexible antigen-binding sites so as to generate a strong immune response to the pathogens. An inverse quantitative correlation exists between the viral load in blood and T-cell responses to infection by the MHC. The HLA alleles that can generate efficient immune response are selected.

Unfortunately, even though these genes protect from infection, they can present autoantigens in the periphery or influence selection of autoreactive T cells in the thymus that can lead to autoimmunity. In the thymus, T cells are selected on the basis of their weak interaction with self-MHC molecules expressed in the thymus. Thus, T cells reactive with self-peptide that can bind with high affinity to MHC molecules are negatively selected in the thymus, while those with weak interactions with MHC may escape negative selection (Figure 20.1; see Chapters 5, 6 and 8). Such a mechanism may explain the selection of a T-cell repertoire that is protective or susceptible to autoreactivity. Elution of peptides from HLA class II molecules suggests that some of these peptides may indeed be derived from HLA molecules themselves (Chicz et al., 1994; Vogt et al., 1994). Thus HLA molecules not only function in the thymus by presenting peptides but also serve as donors of self-peptides. This intricate relationship between MHC, self-peptides, and the T-cell receptor (TCR) could determine the specificity of T cells in the periphery. Thus, in the periphery T cells can recognize non-self-antigens to clear infection, recognize self-antigens to cause autoimmunity, or can become tolerant/anergic. However, studies to resolve these issues in humans have been hampered by the lack of knowledge of "culprit"

TABLE 20.1 Association of various autoimmune diseases with HLA class II alleles and haplotypes

Disease	Alleles	Haplotypes
Rheumatoid arthritis	DRB1*04, DQB1*0302	DRB1*04\DQB1*0302 DRB1*04\DQB1*0302 DRB1*10\DQB1*0501
Type 1 diabetes	DRB1*03, DRB1*04 DQB1*0302	DRB1*04\DQB1*0302 DRB1*0301\DQB1*02
Multiple sclerosis	DRB1*15	DRB1*15\DQB1*0601
Celiac disease	DRB1*0301, DRB1*0701 DQB1*0302	DRB1*0301\DQB1*02 DRB1*0701\DQB1*02 DRB1*04\DQB1*0302
Graves' disease	DRB1*0301, DQB1*02	DRB1*0301\DQB1*02
Autoimmune thyroiditis	DRB1*0301	DRB1*0301\ DQB1*02
Myasthenia gravis	DRB1*03	DRB1*0301\ DQB1*02
Autoimmune adrenalitis	DRB1*0301	DRB1*0301\DQB1*0201

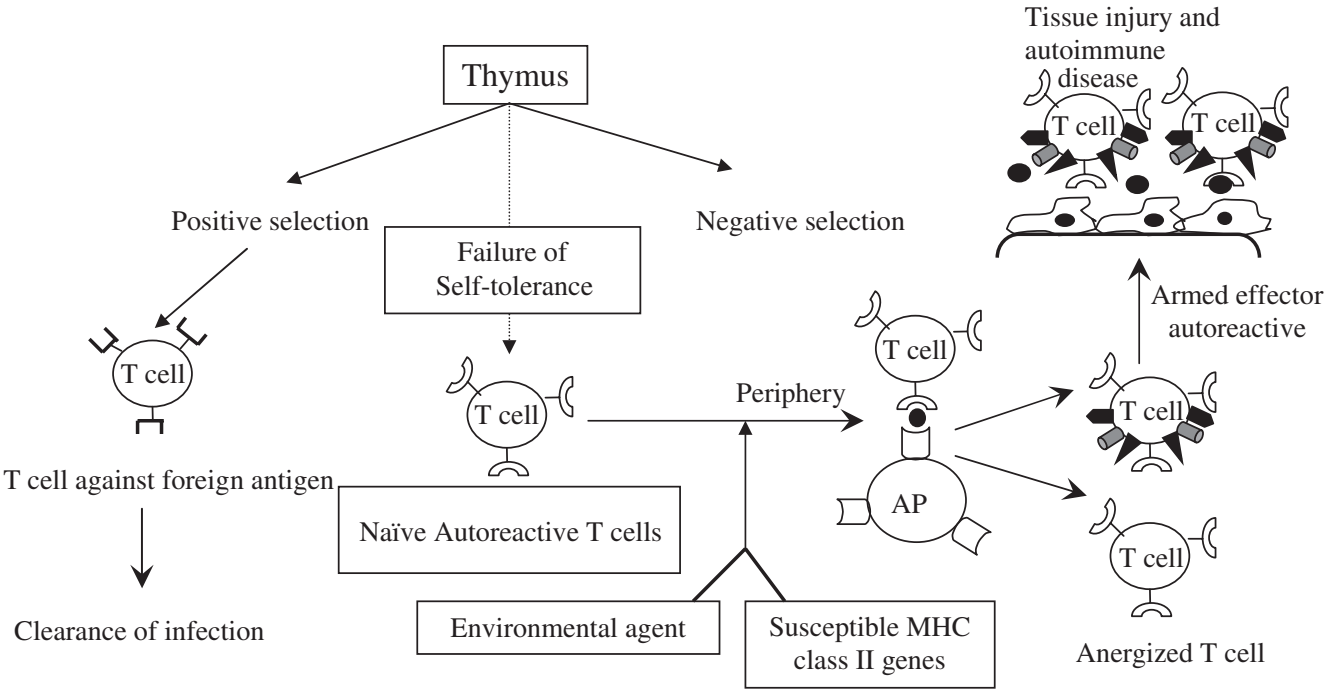


FIGURE 20.1 HLA molecules select the T-cell repertoire in the thymus by presenting peptides and also serving as donors of self-peptides. T cells are selected on the basis of their weak interaction with self-MHC molecules expressed in the thymus. Thus, T cells reactive with self-peptide binding with high affinity to MHC are negatively selected in the thymus, while those with weak interactions may escape negative selection. Such a mechanism may explain the selection of an autoreactive T-cell repertoire. In the periphery, positively selected T cells can recognize nonself antigens and clear infection. The autoreactive T cells that have escaped can recognize viral peptides that mimic self-antigen, or cryptic epitopes that are exposed during infection, causing inflammation and autoimmunity. APC, antigen-presenting cell.

autoantigens and difficulty in obtaining samples from affected organs. The other problem has been the linkage disequilibrium of HLA class II alleles, DR and DQ, which makes it difficult to interpret the association of a disease with a haplotype or specific allele.

HLA-B27

There are 24 HLA-B27 alleles (subtypes), which seem to have evolved from the most commonly observed subtype, B*2705 (Ball and Khan, 2001). Differences in exons 2 and 3, which encode the α1 and α2 domains of the B27 molecule distinguish the various subtypes. However, the natural polymorphism of this molecule influences both peptide specificity and its pathogenic role. Individuals carrying B27 are at lower risk of infection from the human immunodeficiency virus (HIV). B27 recognizes an immunodominant epitope in a conserved part of the capsid protein of HIV (McMichael and Klenerman, 2002). This leads to vigorous cytotoxic T lymphocyte (CTL) responses, resulting in elimination of the virus. Thus HLA-B27 carries out its natural function very effectively. Because of the superiority of the B27 molecule in providing viral immunity, the B27 gene had an advantageous selection during evolution. This could explain why B27 genes are found in almost all geographic regions with such high frequency, and also why so many subtypes of B27 were generated to eliminate new viral antigens that evolve.

HLA-B27 transgenic rats and mice have provided a wealth of information regarding the HLA-B27-linked spondyloarthropathies. Both rat and mouse models proved that the HLA-B27 gene itself is involved in the disease process, although an environmental trigger is required for the onset of the disease (Hammer et al., 1990; Khare et al., 1995). The disease primarily affected the male rodents, similar to the human disease. However, the spontaneous disease in transgenic rats required a high copy number of HLA-B27 and the human β2-microglobulin (β2m) transgenes (Taurog et al., 1993). B27 transgenic mice also developed spontaneous inflammatory arthritis, but only in the absence of mouse β2m or when human β2m was substituted. Replacement of mouse β2m with human β2m led to several conformational changes in HLA-B27, as observed by using several B27-specific monoclonal antibodies. Mice

expressing B27 and the human β_2m transgene showed normal expression of HLA-B27 molecules and β_2m-free heavy chains of HLA-B27 on the cell surface. It was speculated that β_2m-free heavy chain expressed on the cell surface may present extracellular/exogenous peptides, possibly from a bacterial source (Khare et al., 1996). In the transgenic animal model, it was observed that two of the three peptides derived from the third hypervariable region of HLA-B27 can be presented by endogenous mouse class II molecules to $CD4^+$ T cells (Marker-Hermann et al., 1997). However, MHC class II-deficient HLA-B27 transgenic mice were susceptible to nail disease and arthritis, demonstrating that MHC class II molecules are not involved in the disease pathogenesis in the transgenic mouse model (Khare et al., 1998). However, $CD4^{-/-}$ HLA-B27 transgenic mice do not develop arthritis, suggesting that the disease requires $CD4^+$ T cells for the onset of the disease mediated by the antigen-binding pocket of the B27 molecule.

Being an MHC class I molecule, HLA-B27 will be expected to present endogenous antigens such as viral or cytosolic antigens. However, most human spondyloarthropathies have an onset after an infection with enterobacteria (exogenous antigens). Endogenous peptides are transported to the endoplasmic reticulum with the help of molecules involved in the transport of antigenic peptides (Tap1–Tap2 heterodimer). Tap1 knock-out B27 transgenic mice developed spontaneous arthritis and nail disease, indicating that endogenous processing of antigens may not be required in B27-linked diseases (Khare et al., 2001).

Antigen Presentation

B27 presents antigen like class II molecules. Why is HLA-B27, a class I molecule, linked to an autoimmune disease? The B27 molecule turns out to be a unique class I molecule which can form homodimers in the absence of β2m (Allen et al., 1999b). This may be the reason why HLA-B27 is the only class I molecule linked to a human autoimmune disease. Experimental data from transgenic animals are quite suggestive of the notion that the HLA-B27 molecule is an arthritogenic-peptide-presenting molecule. A β2m-free B27 heavy chain homodimer can form an unusual conformation that probably resembles a class II molecule with open ends and can be transported empty to the cell surface. The dimer can be loaded and can present an exogenous peptide longer than nine amino acids, which can go through a class II antigen-processing pathway. The peptide being presented by the heavy chain dimer could be a self-peptide derived from cartilage antigens that could potentially activate some of the autoreactive $CD4^+$ T cells. There are several bacteria with sequence similarities to joint-derived antigens that can bind B27 (Scofield et al., 1995). Data from studies on human cells have confirmed the mouse studies. In humans it has also been shown that B27 dimers can be formed on the cell surface in patients with spondyloarthropathies (Kollnberger et al., 2002).

Molecular Basis for B27 Association

Even though the B27 molecule has a similar conformation to other class I molecules, the mutation in residue 9 (histidine instead of tyrosine) of B27 could have selective advantage during evolution. The crystal structure of class I molecule shows that β2m binds residue 9 of class I (Madden et al., 1992). This mutation could affect the stability of the complex so that β2m can dissociate from heavy chains, resulting in formation of free heavy chain dimers. Alternatively, this mutation probably results in the faster assembly of the B27 molecule and faster transport to the cell surface than other class I molecules, to counter viral infection better. B27-expressing individuals are slow progressors during HIV infection compared with individuals expressing other class I molecules (Goulder et al., 2001). The molecular basis of B27 association can be explained by differences in amino acids of various subtypes of B27 (Allen et al., 1999a; Hulsmeyer et al., 2002). The critical residues involved in the P2 pocket of peptide binding are Glu45 and Cys67. The unpaired cysteine residue could be one potential source of anomalies in the cellular function of B27. During evolution, the HLA molecules would mutate to combat infection. B2705 is the predominant subtype in most populations and B2704 is the predominant subtype in the Asian population (Khan and Ball, 2002). Most of the other alleles have arisen by mutations and differ by two to seven residues in the α1 and α2 domains of B2705. Two B27 alleles that have been reported to lack association with spondyloarthropathies are B*2706 and B*2709. B2706 differs from B2705 at residues 77, 114, and 116, and B2709 differs at position 116 (His for Asp). Residue 116 is involved in the formation of the F pocket of the peptide-binding cleft that accommodates the C-terminal residue of the peptide. The B and F pockets are critical for peptide binding. The differences in residues of the F pocket may account for differences in the peptides that can bind and their association with disease.

Predisposition to Autoimmunity by Class II Molecules

Similar to class I, there is a high polymorphism in class II alleles; there are more than 370 alleles known for the DRB1 locus and 55 for the DQB1 locus. However, autoimmunity is associated with only a few haplotypes. DR2-DQ6, DR3-DQ2, and DR4-DQ8 are the major haplotypes associated with various autoimmune diseases. Since the specificity and affinity of peptide binding and T-cell recognition is determined by polymorphism in the MHC, it is crucial to study the presentation of putative causative agents for various autoimmune diseases by HLA-DR and -DQ alleles.

Genetic analyses of various autoimmune diseases show that such diseases are more often sporadic than familial, suggesting a multifactorial and multigenic basis. For most organ-specific autoimmune disorders, many susceptibility and resistance genes act in concert to modulate the clinical phenotype; however, MHC is the known major factor, which accounts for 50% of the genetic contribution. Most autoimmune diseases are thought to occur as a sequel to an infection or an unknown trigger, as discussed in other chapters in this book.

TRANSGENIC MICE AS MODELS FOR ROLE OF HLA CLASS II IN AUTOIMMUNITY

The advent of transgenic mice lacking endogenous class II molecules ($A\beta^o$) expressing human HLA-DR and HLA-DQ genes has significantly advanced the understanding of the role of individual HLA class II molecules (Taneja and David, 1999). Introduction of an HLA class II transgene in $A\beta^o$ mice led to the expression of a functional HLA class II molecule and a reconstituted CD4 T-cell compartment, thus resulting in CD4-restricted immune response to various peptides. Thus, the HLA transgene in these mice is self. Experimental data from various laboratories have shown that the function of HLA class II molecules in these mice is similar to that in humans.

The first line of evidence came from *in vivo* and *in vitro* studies with superantigens (SAgs) in HLA transgenic mice. Bacterial SAgs have a lower affinity for mouse MHC class II than for human MHC class II molecules. Because of this biologic characteristic, SAg-induced toxicity is much lower in mouse models than it is in humans. When immunized with staphylococcal enterotoxin B (SEB), HLA-DR3-transgenic mice responded to several log lower concentrations of SEB and secreted higher levels of proinflammatory cytokines than did wild-type mice (DaSilva et al., 2002). The response to SAgs in transgenic mice can result in toxic shock and is dependent on the polymorphism of class II alleles.

The second line of evidence comes from peptide presentation by HLA transgenes in mice. HLA-transgenic mice respond to similar epitopes as observed in humans. In a comparison study DR3.Aβo mice recognized only one epitope comprising of a 1–20 amino acid peptide of heat-shock protein (hsp) 65 of *Mycobacterium tuberculosis*, as observed with human DR3-restricted T cells (Geluk et al., 1998). The response was specific in DR3 mice, as DQ8 mice did not respond to this peptide. These studies suggest that processing and presentation of the antigens in the context of class II molecules is similar in transgenic mice and humans.

The third line of evidence that the function of HLA transgenes in mice is similar to that in humans comes from studies with experimental autoimmune myasthenia gravis (EAMG) in DR3-transgenic mice (Infante et al., 2003). The wild-type mice show a highly conserved TCRBV gene usage and CDR3 sequences in response to acetylcholine receptor (AChR), an autoantigen for myasthenia gravis. However, DR3-restricted murine hybridomas generated from DR3 mice immunized with AChR expressed a diverse set of TCR B chains, which is similar to what is observed in humans. The TCRBV sequences from human MG patients were homologous to those of the DR3-restricted murine clones, suggesting that humans and mice can recognize similar epitopes and use similar CDR3 sequences for the recognition of the same peptide–MHC complex. Thus, HLA-transgenic mice can provide an important insight into the peptide presentation by different class II alleles and the ensuing disease pathogenesis.

HLA-DQ Transgenic Mice as a Model for Rheumatoid Arthritis

The first model of autoimmunity to determine the role of human class II molecules was established by using DQA1*0301, DQB1*0302 (DQ8) transgenic mice. DQ8 occurs in linkage disequilibrium with DR4 and has been shown to be associated with RA in certain ethnic groups (Taneja et al., 1992; Yelamos et al., 1993). Collagen-induced arthritis (CIA), an animal model for RA was studied *in vivo* in $A\beta^o$.DQ8 mice. Immunization of $A\beta^o$.DQ8 mice with heterologous type II collagen (CII) led to a pathogenic autoimmune CD4-mediated response and then severe arthritis and antibodies to self-type II collagen (Nabozny et al., 1996). This is the first model with production of rheumatoid factor (RF), one of the major serologic features of patients with RA (Taneja et al., 2002). In none of the previous models of RA using wild-type mice was there production of RF, resulting in uncertainty whether CIA could serve as a model for RA. The scenario for the development of arthritis in transgenic mice can be compared to that of RA in humans. Both require presentation of an arthritogenic epitope by HLA class II molecules to CD4 T cells, leading to a proliferation of autoreactive cells and production of RF by B cells and subsequent joint pathology. Further studies using CD4- and CD8-deficient mice suggested that the disease was mediated by $CD4^+$ T cells while $CD8^+$ T cells may act as regulatory cells, since CD8-deficient mice transgenic for DQ8 developed high amounts of autoantibodies, including RF, antibodies to CII, and antinuclear antibodies (ANA). A similar situation can be envisaged in RA where production of autoantibodies like RF and ANA could be related to the functional status of CD8 T cells. On the other hand, mice expressing DQA1*0103,DQB1*0601 (DQ6) were resistant to the development of CIA (Bradley et al., 1997). The double transgenic mice expressing both DQ6 and DQ8 developed

moderate CIA comparable with the severe arthritis observed in DQ8 transgenic mice (Bradley et al., 1998), much like patients with RA who bear both susceptible and nonsusceptible HLA haplotypes. These observations have contributed to the concept that polymorphism in DQ may be a major contributing factor in human RA.

Role of HLA-DR in rheumatoid arthritis

Predisposition to RA has been associated with the expression of some subtypes of HLA-DR4 in most human studies ever since the first association of Dw4 and RA shown by Stastny (1983). However, most of the studies in different ethnic groups observed that only two alleles, DRB1*0401 and *0404 occur with increased frequency in RA. Further, while DRB1*0401 and *0404 are associated with predisposition, DRB1*0402 is associated with resistance or protection against RA. However, $A\beta^o$.DRB1*0401 mice were not susceptible to CIA. This was puzzling, but could be explained in at least two ways. First, that DR4 may not be able to present many epitopes of CII. Studies in humans have shown that RA-associated DR alleles bind only few epitopes of CII, while DQ8 can present multiple epitopes of CII (Matsushita et al, 1996). Secondly, $A\beta^o$.DR4 mice express both DR4 and mouse Eβ molecules, and polymorphism in E has been shown to be protective in wild-type susceptible mice (Gonzalez-Gay et al., 1994). To determine the role of DR4 molecules in RA, DRB1*0401 mice were generated in complete MHC knock-out mice (AE^o). DRB1*0401.$AE^{-/-}$ mice develop arthritis that mimics human RA. AE^o mice have a deletion of an 80-kb region of class II such that none of the classical murine class II molecules is expressed. Human T cells differ from those of mice in the expression of MHC class II molecules on their cell surface. Interestingly, similar to humans, T cells from AE^o.DR-transgenic mice express HLA-DR molecules on their cell surface, and can present peptide antigen. Thus, presentation of an antigen in the context of HLA by an activated T cell might also contribute to severity.

To simulate human haplotypes, double transgenic mice expressing autoimmune-associated DQ and DR alleles were generated on an AE^o background. DR4\DQ8.AE^o mice developed arthritis similar to DQ8 mice but also showed gender differences. Gender differences in CIA were not observed in DQ8 mice, suggesting modulation of DQ8-restricted disease by the DR4 molecule. To understand modulation by DR polymorphism, DRB1*0402 mice were studied for their influence on the development of CIA. DRB1*0402 mice were not susceptible to CIA, similar to humans. Mice that expressed both susceptible and resistant DR4 subtypes, 0401/0402, developed arthritis with lower incidence than did DRB1*0401 mice (Taneja et al., 2003a).

The experimental and human data led us to hypothesize that DQ polymorphism may be responsible for susceptibility, while DR may be involved in the modulation of disease. Thus, both DQ and DR alleles in a haplotype can influence the development of disease. On the basis of observations in transgenic mice, the following mechanisms can be suggested. First, polymorphism in DRB1 alleles leads to modulation of DQ-mediated disease because of presentation of hypervariable region 3 (HVR3)-derived peptides by RA-associated DQ molecules (Zanelli et al., 1995). Thus, a high-affinity binding DRB1 HVR3 peptide would negatively select autoreactive T cells, while a low-affinity peptide would positively select potential autoreactive T cells. Secondly, presentation of a peptide by a DR molecule might enhance the autoantigenic immune response. From the studies on DQ and DR transgenic mice, it can be extrapolated that gene complementation or interaction between DQ and DR molecules mediates susceptibility to RA in humans. Depending on the haplotypes carried by an individual, they could be susceptible to severe or mild disease. A homozygous haplotype for predisposing DQ and permissive DR will lead to severe disease. Also, heterozygous RA-susceptible haplotypes (DQ and DR) will result in very severe disease since there will be two predisposing DQ molecules. However, one predisposing and one protective haplotype should show less severity and low incidence.

Interaction of Class II in Experimental Autoimmune Disease

Similar to RA, susceptibility to relapsing polychondritis (RP) is significantly associated with the presence of a class II molecule, HLA-DR4 (Zeuner et al, 1997). The presence of anti-CII-specific antibodies and cell-mediated immunity to cartilage antigens observed in patients with RP indicate that the autoimmune response to CII is important in the pathogenesis (Foidart et al., 1978). In addition to type II collagen, there is also evidence suggesting involvement of other collagens, type IX and type XI, in the pathogenesis of RP (Alsalameh et al, 1993).

When DQ8 and DQ6 mice are immunized with CII, DQ8 mice develop CIA without polychondritis, and DQ6 mice are resistant to both. Mice expressing both DQ6 and DQ8 molecules develop experimental polychondritis, exhibiting both polyarthritis and auricular chondritis (Bradley et al., 1998). In this model, the role of hybrid molecules was suggested in the development of chondritis. This would mean that an α chain of one and a β chain of the other HLA molecule would form a heterodimer that can present unique peptides, leading to pathogenesis. The postulated interaction of DQ6 and DQ8 in generating a hybrid molecule and disease association was strengthened by peptide mapping of type II collagen in double transgenic mice. DQ6α/DQ8β mice

responded to two unique peptides of human CII which did not produce T-cell response in either DQ6 or DQ8 mice (Krco et al., 1999). *In vivo* studies using a hybrid class II molecule in transgenic mice provided support for this hypothesis. However, auricular chondritis was seen in only 25% of the immunized animals and no other features associated with RP were observed. This suggested a role of other genes in disease initiation.

Nonobese diabetic (NOD) mice are prone to autoimmunity that involves multiple genes. DQ8.Aβ^o mice were generated on the NOD and B10 background to determine the role of other autoimmune genes. Transgenic mice expressing DQ8 on the NOD background, but lacking endogenous class II molecules, develop auricular chondritis and polyarthritis with high levels of anti-CIX and anti-CII antibodies following immunization with type II collagen (Taneja et al, 2003b). Conversely, CII-immunized B10.DQ8 mice do not develop auricular chondritis and make lower amounts of antibodies. Although CII is a putative autoantigen for both RP and RA, the cross-reactive response to other collagen types expressed in the outer ear, such as type IX, appears to differ in the two strains. NOD.DQ8 mice mounted a strong response to type IX collagen, while in B10.DQ8 mice no response was observed. This suggests that even though DQ8 can select autoreactive cells in the thymus, non-MHC genes or class I may also be important in susceptibility. However, since disease does not occur in MHC-negative littermates, HLA molecules constitute the major predisposing factor for autoimmunity. These studies suggest that interaction between two class II or class II and other genes can lead to presentation of peptides not seen by either allele individually.

PROTECTIVE ALLELE FOR ONE DISEASE CAN PREDISPOSE TO ANOTHER AUTOIMMUNE DISEASE

HLA-DR2 is associated with protection in RA while the strongest association in MS is with HLA-DR2 haplotypes, DRB1*1501, DRB5*0101, DQA1*0102, and DQB1*0602 (Hillert, 1994). Besides DR2, MS has also been associated with HLA-DR3 and DR4 (Marrosu et al., 1998) and the HLA DR3/DQ2 haplotypes have been shown to confer an increased risk for relapsing remitting MS. The lack of a complete association with a particular HLA allele suggests that MS is a heterogeneous disease at the molecular genetic level. MS is hypothesized (Steinman, 1996) to be mediated by autoreactive T cells against a variety of myelin antigens, including myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocytic glycoprotein (MOG). Immunization of a susceptible strain of mice with autoantigens MBP, PLP or MOG, can lead to development of experimental autoimmune encephalomyelitis (EAE) (Ito et al., 1996; Mangalam et al., 2004; Rich et al., 2004). We and others have shown that HLA transgenic mice can develop EAE using different myelin antigens or by infecting susceptible mice with Theiler virus. DR3 transgenic mice lacking endogenous class II molecules when infected with Theiler virus showed a significantly decreased severity of demyelination, suggesting a DR3 association with benign relapsing MS (Drescher et al., 1998). EAE induction with recombinant MOG (rMOG) in DR2 mice (DRB1*1501, DRB1*1502, and DRB1*1503) showed that all three strains developed EAE; however, the disease incidence and severity was higher in DRB1*1501 transgenic mice. Almost all the T-cell epitopes, identified in HLA transgenic mice using overlapping peptides of MBP, PLP, and MOG, are similar to those identified among MS patients (Kawamura et al., 2000; Khare et al., 2003). Administration of PLP 91–110 peptide induced severe EAE in DR3-transgenic mice (Mangalam et al., 2004). However, disease induced by all the autoantigens in transgenic mice was characterized by paralysis of limbs with mild demyelination, whereas in humans demyelination is the major feature of MS. $CD4^+$ T cells have been shown to infiltrate the central nervous system (CNS) and appear responsible for inflammation and demyelination in MS. It is speculated that class II molecules on T cells may present myelin antigen in the CNS and exacerbate the disease (Authors, unpublished observations). To replicate the human disease, DR3.AE^o transgenic mice were immunized for EAE with PLP peptide. The brain and spinal cord pathology in DR3.AE^o transgenic mice was much more severe than in Aβ^o transgenic mice. The DR3.AE^o mice had severe inflammation and demyelination in the meningial, striatal, and brainstem regions, a hallmark for human MS. Using immunoflourescence staining, MHC class II expression was detected in the CNS, especially on microglial cells. Thus, these experiments suggest that new humanized HLA transgenic mice (AE^o) simulate the human expression of class II molecules and are a better model than Aβ^o mice to study the role of class II molecules in the induction of autoimmune disease.

Data from HLA transgenic mice suggest a role of haplotypes in disease expression. Most DR2/DQ8 double transgenic mice (90%) developed chronic progressive disease with severe inflammatory and demyelinating lesions after immunization with rMOG. DQB1*0601 is stated to be protective in MS (Amirzargar et al., 1998; Marrosu et al., 2001). Disease observed in DR2/DQB1*0601 mice was less frequent (30%) and milder. Neither DQB1*0601 nor DQ8 transgenic mice developed EAE after immunization with rMOG and findings were similar using whole myelin and human PLP peptide 91–110. Also, using whole myelin and PLP peptide 91–110, we have observed that the presence of 1) the HLA-DQ6 or DQ8 molecule alone is not sufficient

for disease induction; 2) disease-susceptible HLA-DR3 transgenic mice bearing HLA-DQ8 had an increased severity and incidence of disease; 3) DQ6 molecules had no effect on disease onset or severity; and 4) transgenic DQ6 or DQ8 molecules on a disease-resistant background had no effect on disease induction, since none of the HLA-DR2/DQ6, DR2/DQ8 or DR4/DQ8 double transgenic mice developed EAE. These data suggest that the presence of more than one susceptibility allele, namely HLA DR2/DQ8 or DR3/DQ8, likely resulted in additional selection and expansion of potential autoreactive T cells, leading to enhanced severity of disease in double transgenic mice. This study also pointed to MS being a heterogeneous disease and to HLA association being specific for various autoantigens that are presumably involved.

REQUIREMENT OF OTHER MOLECULES ALONG WITH MHC FOR AUTOIMMUNE DIABETES

NOD mice develop spontaneous diabetes, which has been linked to IAg7, the class II of the NOD strain. Even though DQ8 is similar in structure to IAg7 of the NOD mice (Wucherpfennig, 2003), DQ8 transgenic mice do not spontaneously develop diabetes as do NOD mice. However, DQ8 transgenic mice lose tolerance to self-GAD65 and potential autoreactive T cells are found in the periphery and pancreas, and cause insulitis but not progression to diabetes (Abraham et al., 1999). As with DQ8 transgenic mice, the presence of a predisposing type I diabetes MHC gene(s) in certain individuals could result in escape of autoreactive T cells against pancreatic antigens. The onset of the disease may require a second injury in the pancreas. This injury could come in the form of a virus/bacterial infection, and/or overproduction of a cytokine or overexpression of an accessory molecule or other genetically-determined variations in immune homeostasis. To simulate such an insult in the pancreas, the HLA-DQ8 (type I-predisposing) and the HLA-DQ6 (diabetes-protective) transgenic mice expressing the costimulatory molecule CD80 (B7.1) in islet β cells under the rat insulin promoter (RIP) were generated. HLA-DQ8/RIP/B7.1 mice developed spontaneous diabetes, while HLA-DQ6/RIP/B7.1 transgenic mice did not (Wen et al, 2000). These studies further confirmed that while HLA is the major predisposing gene in most if not all autoimmune diseases, the presence of the disease-associated HLA allele alone is insufficient for development of autoimmunity in diseased individuals. Further studies using double transgenic mice expressing both DR4 or DR3 and DQ8 suggested that DR4 and DR3 could regulate the diabetogenic effect of DQ8, which in turn suggests interactions between various MHC class II molecules are important in the disease process (Wen et al., 2001; Rajagopalan et al., 2003).

MOLECULAR BASIS FOR DQ ASSOCIATION

Studies using molecular biology and the crystal structure of HLA genes defining the peptide motifs have shown that DQ genes can be important in conferring susceptibility to or protection from diseases, including MG, MS, and T1D. DQ8 is associated with many autoimmune diseases like diabetes, RA, and celiac disease. The crystal structure of the HLA-DQ8 molecule shows that DQ8 differs from DR molecules in the peptide side chain binding pockets for P1, P4 and P9 (Lee et al., 2001). In DQ8, a very polar P1 peptide side chain pocket is observed; the P4 pocket is the largest pocket and differs from that of DR molecules in shape and size. While for DR molecules, P4 is a shallow pocket, the P4 pocket of DQ8 is very deep. The P4 pocket of DQ6, which is associated with protection from diabetes, is similar to DQ8, suggesting no correlation between P4 pocket and diabetes. The major difference in the protecting allele and susceptibility allele lies at residue 57 in the P9 pocket of the DQB1 molecules. The protective DQ7 and DQ6 alleles encode asparagine at position 57 while an alanine, valine or serine at this position characterizes predisposing alleles. Thus, in HLA-DQ8 molecules the presence of alanine at position 57 could interfere with the salt bridge formed between Asp57β and Arg76α, which could potentially make DQ8 a more open molecule and lead to an unstable peptide–MHC complex. This would put fewer constraints on the peptide binding to the DQ8 molecule (Wucherpfennig, 2003). However, a contribution from the P9 pocket of DQ and the P4 pocket of DR have been suggested in disease susceptibility and resistance to T1D (Cucca et al., 2001). Studies in humans and mice have shown that DQ8 is a promiscuous molecule and can bind many more peptides than the DQ6 and DR molecules (Matsushita et al., 1996; Krco et al., 1999; Stratmann et al,, 2000), making it a candidate molecule that can predispose to autoreactivity.

ROLE OF DQ AND DR IN AUTOIMMUNITY

The question arises as to why DQ8 can predispose to so many autoimmune diseases and DR is the regulatory molecule. The association of a particular HLA class II haplotype with disease seems to depend on geographic locations as well as the ethnic composition of the populations. Studies using transgenic mice suggest that DR2-restricted autoimmunity may be directed against MBP, MOG, and PLP, while DR3-restricted autoimmunity may be directed against PLP only. Further, DR2 and DR3 may present different epitopes of MOG and PLP. Evolutionarily, DQ is a stable molecule with less polymorphism than observed in DRB1 locus. The

DQ8 molecule has not undergone many changes evolutionarily and occurs with only three known subtypes. This could be because DQ8 has the advantage of being able to bind and present multiple peptides. On the other hand, DR has many subtypes. For example, DRB1*04 has at least 59 subtypes which differ by one to three amino acids at the peptide-binding groove. However, not all the subtypes are associated with susceptibility to autoimmune diseases. We speculate that DR4 polymorphism has increased to counteract the predisposition to autoimmunity imposed by the DQ8 molecule. Thus, multiple subtypes of DR4 lead to many possible DR4\DQ8 haplotypes. Only those with an advantage of presenting microbial antigens, and clearing infections, and with the capacity to counter the autoimmune responses, may eventually be selected. Thus, the variation in DR subtypes in the peptide-binding region can be important for disease pathogenesis and can be explained by the following proposals.

First, various subtypes of an MHC allele may generate a differential immune response, type 1 helper T cell (Th1) or Th2, after binding similar peptides. For example, some peptides can be bound by both DRB1*0401 and DRB1*0402 even though they differ in the HVR3 region by three peptides and overall charge. The presentation of similar peptides might generate a differential immune response, which would explain the association of DRB1*0401 with predisposition to develop RA and/or the protection provided by DRB1*0402.

Second, the DRB1 HVR3-derived peptides are presented by the DQ molecule that shapes the T-cell repertoire in the thymus; the DR subtypes that are associated with autoimmunity when presented lead to positive selection of an autoreactive TCR that can recognize a self-antigen in the periphery. An infection or any other insult to the immune system could result in exposure of that antigen to the immune system, leading to expansion of T cells.

CONCLUDING REMARKS

From various *in vivo* and *in vitro* studies, it has become clear that MHC and non-MHC genetic components are common elements for various autoimmune diseases and their corresponding animal models. Thus, polymorphism in MHC is an advantage for human survival, but autoimmunity is the price that must be paid for combating infection effectively and for survival. Evolutionarily, only those HLA genes that can generate strong immune responses to infections are selected. The constant mutations observed might be leading to the generation of new subtypes of an allele to circumvent autoimmunity. Haplotypes but not single class II alleles function in the selection of the T-cell repertoire and susceptibility to disease.

References

Abraham, R.S., Wilson, S.B., de Souza, N.F., Jr, Strominger, J.L., Munn, S.R., and David, C.S. (1999). NOD background genes influence T cell responses to GAD 65 in HLA-DQ8 transgenic mice. *Hum. Immunol. 60*, 583–590.

Allen, R.L., Bowness, P., and McMichael, A.J. (1999a). The role of B27 in spondyloarthropathies. *Immunogenetics 50*, 220–227.

Allen, R.L., O'Callaghan C.A., McMichael, A.J., and Bowness, P. (1999b). HLA-B27 can form a novel beta 2-microglobulin-free heavy chain homodimer structure. *J. Immunol. 162*, 5045–5048.

Alsalameh, S., Mollenhauer, J., Scheuplein, F., Stoss, H., Kalden, J.R., Burkhardt, H., and Burmester, G.R. (1993). Preferential cellular and humoral immune reactivities to native and denatured collagen types IX and XI in a patient with fatal relapsing polychondritis. *J. Rheumatol. 20*, 1419–1424.

Amirzargar, A., Mytilineos, J., Yousefipour, A., Farjadian, Sh., Scherer, S., Opelz, G., and Ghaderi, A. (1998). HLA class II (DRB1, DQA1, and DQB1) associated genetic susceptibility in Iranian multiple sclerosis (MS) patients. *Eur. J. Immunogenet. 25*, 297–301.

Ball, E.J. and Khan, M.A. (2001). HLA-B27 polymorphism. *Joint Bone Spine 68*, 378–382.

Bradley, D.S., Nabozny, G.H., Cheng, S., Zhao, P., Griffiths, M.M., Luthra, H.S., and David, C.S. (1997). HLA-DQB1 polymorphism determine incidence, onset, and severity of collagen-induced arthritis (CIA) in transgenic mice: Implications in human rheumatoid arthritis. *J. Clin. Invest. 100*, 2227–2234.

Bradley, D.S., Das, P., Griffiths, M.M., Luthra, H.S.and David, C.S. (1998). HLA DQ6/8 double transgenic mice develop auricular chondritis following type II collagen immunization: a model for human relapsing polychondritis. *J. Immunol. 161*, 5046–5053.

Chicz, R.M, Lane, W.S., Robinson, R.A., Trucco, M., Strominger, J.L., and Gorga, J.C. (1994). Self-peptides bound to the type 1 diabetes associated class II MHC molecules HLA-DQ1 and HLA-DQ8. *Int. Immunol. 6*, 1639–1649.

Cucca, F., Lampis, R., Congia, M., Angius, E., Nutland, S., Bain, S.C., Barnett, A.H., and Todd, J.A. (2001). A correalation between the relative predisposition of the MHC class II alleles to type I diabetes and the structure of their proteins. *Hum. Mol. Genet. 10*, 2025–2037.

DaSilva, L., Welcher, B.C., Ulrich, R.G., Aman, M.J., David, C.S., and Bavari, S. (2002). Human like immune response of human leukocyte-DR3 transgenic mice to staphylococcal enterotoxins: novel model for superantigen vaccines. *J. Infect. Dis. 185*, 1754–1760.

Drescher, K.M., Nguyen, L.T., Taneja, V., Coenen, M.J., Leibowitz, J.L., Strauss G., Hammerling G.J., David C.S., and Rodriguez, M. (1998). Expression of the human histocompatibility leukocyte antigen DR3 transgene reduces the severity of demyelination in a murine model of multiple sclerosis. *J. Clin. Invest. 101*, 1765–1774.

Foidart, J.M., Abe, S., Martin, G.R., Zizic, T.M., Barnett, E.V., Lawley, T.J., and Katz, S.I. (1978). Antibodies to type II collagen in relapsing polychondritis. *N. Engl. J. Med. 299*, 1203–1207.

Geluk, A., Taneja, V., van Meijgaarden, K.E., Zanelli, E., Abou-Zeid, C., Thole, J.E., deVries, R.R., David, C.S., and Ottenhoff, T.H. (1998). Identification of HLA class II-restricted determinants of *Mycobacterium tuberculosis*-derived proteins by using HLA-transgenic, class II-deficient mice. *Proc. Natl. Acad. Sci. USA 95*, 10797–10802.

Gonzalez-Gay, M.A., Nabozny, G.H., Bull, M.J., Zanelli, E., Douhan, J., Griffiths, M.M., Glimcher, L.H., Luthra, H.S., and David, C.S. (1994). Protective role of major histocompatibility complex class II Ebd transgene on collagen-induced arthritis. *J. Exp. Med. 180*, 1559–1564.

Goulder, P.J., Phillips, R.E., Colbert, R.A., McAdam, S., Ogg, G., Nowak, M.A., Giangrande, P., Luzzi, G., Morgan, B., Edwards, A., McMichael, A.J., and Rowland-Jones, S. (1997). Late escape from an

immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat. Med. 3*, 212–217.

Hammer, R.E., Maika, S.D., Richardson, J.A., Tang, J.P., and Taurong, J.D. (1990). Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β2m: an animal model of HLA-B27 associated human disorders. *Cell 63*, 1099–1112.

Hillert, J. (1994). Human leukocyte antigen studies in multiple sclerosis. *Ann. Neurol. 36*, 15–17.

Hulesmeyer, M., Hillig, R.C., Voltz, A., Ruhl, M., Schroder, W., Saenger, W., Ziegler, A., and Uchanska-Ziegler, B. (2002). HLA-B27 subtypes differently associated with disease exhibit subtle structural alterations. *J. Biol. Chem. 277*, 47844–47853.

Infante, A.J., Baillargeon, J., Kraig, E., Lott, L., Jackson, C., Hammerling, G.J., Raju, R., and David, C.S. (2003). Evidence of a diverse T cell receptor repertoire for acetylcholine receptor, the autoantigen of mysathenia gravis. *J. Autoimmun. 21*, 167–174.

Ito, K., Bian, H.J., Molina, M., Han, J., Magram, J., Saar, E., Belunis, C., Bolin, D.R., Arceo, R., Campbell, R., Falcioni, F., Vidovic, D., Hammer, J., and Nagy, Z.A. (1996). HLA-DR4-IE chimeric class II transgenic, murine class II-deficient mice are susceptible to experimental allergic encephalomyelitis. *J. Exp. Med. 183*, 2635–2644.

Kawamura, K., Yamamura, T., Yokoyama, K., Chui, D.H., Fukui, Y., Sasazuki, T., Inoko, H., David, C.S., and Tabira, T. (2000). HLA-DR2-restricted responses to proteolipid protein 95–116 peptide cause autoimmune encephalitis in transgenic mice. *J. Clin. Invest. 105*, 977–984.

Khan, M.A. and Ball, E.J. (2002). Genetic aspects of ankylosing spondylitis. *Best Pract. Res. Clin. Rheumatol. 16*, 675–690.

Khare, M., Rodriguez, M., and David, C.S. (2003). HLA class II transgenic mice authenticate restriction of myelin oligodendrocyte glycoprotein-specific immune response implicated in multiple sclerosis pathogenesis. *Int. Immunol. 15*, 535–546.

Khare, S.D., Luthra, H.S., and David, C.S. (1995). Spontaneous inflammatory arthritis in B27 transgenic mice lacking beta2-microglobulin: a model of human spondyloarthropathies. *J. Exp. Med. 182*, 1153–1158.

Khare, S.D., Hanson, J., Luthra, H.S., and David, C.S. (1996). HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human β2microglobulin (β2m) double transgenic mice with disrupted mouse β2m. *J. Clin. Invest. 98*, 2746–2755.

Khare, S.D., Bull, M.J., Hanson, J., Luthra, H.S., and David C.S. (1998). Spontaneous inflammatory disease in HLA-B27 transgenic mice is independent of MHC class II molecules: A direct role of B27 heavy chains and not B27-derived peptides. *J. Immunol. 160*, 101–106.

Khare, S.D., Lee Bull, M.J., Hanson, J., Luthra, H.S., and David C.S. (2001). Spontaneous inflammatory disease in HLA-B27 transgenic mice does not require transporter of antigenic peptides. *Clin. Immunol. 98*, 364–369.

Kollnberger, S., Bird, L., Sun, M.Y., Retiere, C., Braud, V.M., McMichael, A., and Bowness, P. (2002). Cell surface expression and immune receptor recognition of HLA-B27 homodimers. *Arthritis Rheum. 46*, 2972–2982.

Krco, C.J., Harders, J., Watanabe, S., Griffiths, M., Luthra, H.S., and David, C.S (1999). Identification of T cell epitopes on human type II collagen using HLA-DQ8 and HLA-DQ6 transgenic mice. *J. Immunol. 163*, 1661–1665.

Lee, K.H., Wucherpfennig, K.W., and Wiley, D.C. (2001). Structure of a human insulin peptide-HLA DQ8-complex and susceptibility to type I diabetes. *Nat. Immunol. 2*, 501–507.

Madden, D.R., Gorga, J.C., Strominger, J.L., and Wiley, D.C. (1992). The three dimensional structure of B27 at 2.1A resolution suggests a general mechanism for tight peptide binding to MHC. *Cell 70*, 1035–1048.

Mangalam, A.K., Khare, M., Krco, C., Rodriguez, M., and David, C. (2004). Identification of T cell epitopes on human proteolipid protein and induction of experimental autoimmune encephalomyelitis in HLA class II-transgenic mice. *Eur. J. Immunol. 34*, 280–290.

Marker-Hermann, E., Meyer zum Buschenfelde, K.H., and Wildner, G. (1997). HLA-B27 derived peptides as autoantigens for T lymphocytes in ankylosing spondylitis. *Arthritis Rheum. 40*, 2047–2054.

Marrosu, M.G., Murru, M.R., Costa, G., Murru, R., Muntoni, F., and Cucca, F. (1998). DRB1-DQA1-DQB1 loci and multiple sclerosis predisposition in the Sardinian population. *Hum. Mol. Genet. 7*, 1235–1240.

Marrosu, M.G., Murru, R., Murru, M.R., Costa, G., Zavattar, P., Whalen, M., Cocco, E., Mancosu, C., Schirru, L., Solla, E., Fadda, E., Melis, C., Porru, I., Rolesu, M., and Cucca F. (2001) Dissection of the HLA association with multiple sclerosis in the founder isolated population of Sardinia. *Hum. Mol. Genet. 10*, 2907–2916.

Matsushita, S., Nishi, T., Osio, M., Yamaoka, K., Yoni, K., Kanai, T., and Nishimura, Y. (1996). HLA-DQ binding peptide motifs. Comparative analysis of type II collagen-derived peptides to DR and DQ molecules of rheumatoid arthritis-susceptible and non-susceptible haplotypes. *Int. Immunol. 8*, 757–764.

McMichael, A. and Klenerman, P. (2002). HIV/AIDS: HLA leaves its footprints on HIV. *Science 296*, 1410–1401.

Nabozny, G.H., Baisch, J.M., Cheng S., Cosgrove D., Griffiths M.M., Luthra H.S., and David C.S. (1996). HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. *J. Exp. Med. 183*, 27–37.

Rajagapalan, G., Kudva, Y., Chen, L., Wen, L., and David, C.S. (2003) Autoimmune diabetes in HLA-DR3/DQ8 transgenic mice expressing the costimulatory molecule B-7-1 in the beta cells of islets of Langerhans. *Int. Immunol. 15*, 1035–1044.

Rich, C, Link, J.M., Zamora, A., Jacobsen, H., Meza-Romero, R., Offner, H., Jones, R., Burrows, G.G, Fugger, L., and Vandenbark, A.A. (2004). Myelin oligodendrocyte glycoprotein-35-55 peptide induces severe chronic experimental autoimmune encephalomyelitis in HLA-DR2-transgenic mice. *Eur. J. Immunol. 34*, 1251–1261.

Scofield, R.H., Kurien, B., Gross, T., Warren, W.L., and Harley, J.B. (1995). HLA-B27 binding of peptide from its own sequence and similar peptides from bacteria: implications for spondyloarthropathies. *Lancet 345*, 1542–1544.

Stastny, P. (1983). Rheumatoid arthritis: relationship with HLA-D. *Am. J. Med. 75*, 9–15.

Steinman, L. (1996). Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell 85*, 299–302.

Stratmann, T., Apostolopoulos, V., Mallet-Designe, V., Corper, A.L., Scott, C.A., Wilson, I.A., Kang, A.S., and Teyton, L (2000). The I-Ag7 MHC class II molecule linked to murine diabetes is a promiscuous peptide binder. *J. Immunol. 165*, 3214–3225.

Taneja, V., Mehra, N.K., Chandershekaran, A.N., Ahuja, R.K., Singh, Y.N., and Malaviya A.N. (1992). HLA-DR4-DQw8, but not DR4-DQw7 haplotypes occur in Indian patients with rheumatoid arthritis. *Rheumatol. Int. 11*, 251–255.

Taneja, V. and David, C.S. (1999). HLA class II transgenic mice as models of human diseases. *Immunol. Rev. 169*, 67–80.

Taneja, V., Taneja, N., Paisansinsup, T., Behrens, M., Griffiths, M., Luthra, H., and David, C.S. (2002). CD4 and CD8 T cells in susceptibility/protection to collagen-induced arthritis in HLA-DQ8 transgenic mice: implications for rheumatoid arthritis. *J. Immunol. 168*, 5867–5875.

Taneja, V., Taneja, N., Behrens, M., Suchong, P., Griffiths, M., Luthra, H.S., and David, C.S. (2003a). DRB1*0402 (DW10) transgene protects collagen-induced arthritis susceptible H2Aq and DRB1*0401 (DW4) mice from arthritis. *J. Immunol. 171*, 4431–4438.

Taneja, V., Griffiths, M.M., Behrens, M., Luthra, H.S., and David, C.S. (2003b). Auricular chondritis in NODDQ8 Abetao ($Ag7^{-/-}$) transgenic mice resembles human relapsing polychondritis. *J. Clin. Invest. 112*, 1843–1850.

Taurog, J.D., Maika, S.D., Simmons, W.A., Breban, M., and Hammer, R.E. (1993). Susceptibility to inflammatory disease in HLA-B27 transgenic rat lines correlates with the level of B27 expression. *J. Immunol. 150*, 4168–4178.

Vogt, A.B., Krospshofer, H., Kalbacher, H., Kalbus, M., Rammensee, H.G., Coligan, J.F., and Martin, R. (1994). Ligand motifs of HLA-DRB5*0101 and DRB1*1501 molecules delineated from self-peptides. *J. Immunol. 153*, 1665–1673.

Wen, L., Wong, F.S., Tang, J., Chen, N.Y., Altieri, M., David, C.S., Flavell, R., and Sherwin, R. (2000). *In vivo* evidence for the contribution of human histocompatibilty leukocyte antigen (HLA)-DQ molecules to the development of diabetes. *J. Exp. Med. 191*, 97–104.

Wen, L. Chen, N.,Y., Tang, J., Sherwin, R., and Wong, F.S. (2001). The regulatory role of DR4 in a spontaneous diabetes DQ8 transgenic model. *J. Clin. Invest. 107*, 871–880.

Wucherpfennig, K.W. (2003). MHC-linked susceptibility to type I diabetes: a structural perspective. *N. Y. Acad. Sci. 1005*, 119–127.

Yelamos, J., Garcia-Lozano, J.R., Moreno, I., Aguilera, I., Gonzalez, M.F., Garcia, A., Nunez-Roldan, A., and Sanchez, B. (1993). Association of HLA-DR4-Dw15 (DRB1*0405) and DR10 with rheumatoid arthritis in a Spanish population. *Arthritis Rheum. 36*, 811–814.

Zanelli, E., Gonzale-Gay, M., and David C.S. (1995). Could HLA-DRB1 be the protective locus in rheumatoid arthritis? *Immunol. Today 16*, 274–278.

Zeuner, M., Straub, R.H., Rauh, G., Albert, E.D., Scholmerich, J., and Lang, B. (1997). Relapsing polychondritis: Clinical and immunogenetics analysis of 62 patients. *J. Rheumatol. 24*, 96–101.

CHAPTER

21

Genetics and Autoimmunity: Non-MHC Genes

YARON TOMER

Division of Endocrinology, University of Cincinnati College of Medicine, Cincinnati, USA

Autoimmune diseases are complex diseases in which susceptibility genes and environmental triggers act in concert to initiate the autoimmune response. The paradigm that genetic susceptibility plays a central role in the development of autoimmune diseases is supported by solid epidemiologic data, including family and twin studies. As discussed in Chapters 3, 5 and 20, one of the major genes contributing to the genetic etiology of many autoimmune diseases is the major histocompatiblity complex (MHC) (HLA) gene complex, and in some diseases the HLA genes are believed to contribute most of the genetic susceptibility [e.g., in type 1 diabetes (T1D) (Pociot and McDermott, 2002)]. However, since in most autoimmune diseases the concordance rate for HLA identical siblings is significantly lower than the concordance rate for monozygotic (identical) twins (Stenszky et al., 1985), it is clear that other non-HLA genes must play a major role in the genetic etiology of autoimmune diseases. In this chapter we will focus on the contribution of non-MHC genes to the genetic susceptibility to autoimmune diseases.

FAMILIAL NATURE OF AUTOIMMUNE DISEASES

Family Studies

The first clue that a complex disease has a genetic etiology comes from the observation that the disease clusters in families. Many autoimmune diseases have been reported to show familial clustering. For example, in T1D, siblings and offspring of T1D patients have a much higher frequency of disease than the prevalence in the general population (Melton et al., 1983; Warram et al., 1984). Interestingly, offspring of T1D fathers are at much higher risk for T1D than are offspring of T1D mothers, suggesting that exposure to a diabetic environment in utero may have a protective effect on the offspring, perhaps by inducing immunologic tolerance (Warram et al., 1984; 1991). Similarly, familial clustering has been reported in systemic lupus erythematosus (SLE) (Tsao, 2003), rheumatoid arthritis (RA) (Gregersen,

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

2003), Graves' disease, Hashimoto's thyroiditis (Tomer and Davies, 2003), and multiple sclerosis (MS) (Hochberg, 1987), as well as other autoimmune conditions (Vyse and Todd, 1996). The frequency of autoantibodies is also commonly increased in relatives of patients with autoimmune diseases (Hall and Stanbury, 1967; Chopra et al., 1977; Carey et al., 1980; Tamai et al., 1980; 1986; Burek et al., 1982; Volpe, 1985).

Familial clustering of a disease does not necessarily mean that the disease is genetic. It can be the result of random chance, shared extrinsic (environmental) factors (Tomer and Davies, 1993), shared intrinsic (genetic) factors, or a combination of these. When nongenetic factors are the cause of familial clustering of a disease, the distribution of affected members in families would be random. However, when genetic predisposition is the cause of the familial occurrence of a disease, the distribution of affected members in families would follow a pattern consistent with genetic inheritance (Figure 21.1).

One way to test whether the familial clustering of a disease is consistent with genetic inheritance is by performing a segregation analysis. In this analysis many families in which the disease under study clusters are analyzed to see if the clustering occurs at random or demonstrates Mendelian or a complex pattern of inheritance. Segregation analyses have shown strong genetic influences in several autoimmune diseases, including SLE (Tsao et al., 2002), MS (Rigby et al., 1998; Seldin et al., 1999), RA (Sadovnick et al., 1993), Graves' disease (Villanueva et al., 2000), and thyroid antibody diathesis (Pauls et al., 1993; Phillips et al., 1990; 1991; Jaume et al., 1999).

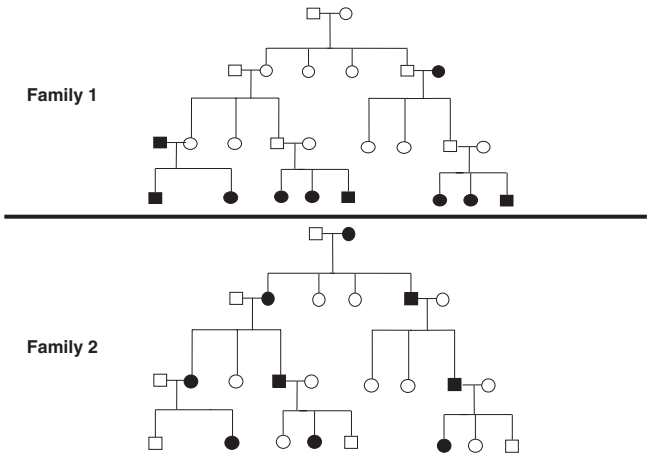


FIGURE 21.1 Clustering of a disease in families does not necessarily mean that the disease has a genetic etiology. Shown are two families in which diseases cluster. In family 1 the clustering is due to an environmental factor (e.g., viral gastroenteritis) and the clustering does not follow Mendelian inheritance. In family 2 the clustering is due to genetic inheritance of the disease and the vertical transmission suggests dominant inheritance (e.g., Huntington disease). In complex diseases, such as type 1 diabetes or systemic lupus erythematosus, the clustering of the disease in families is due to the combined effects of genetic and environmental influences, and therefore, the pedigrees usually do not conform to any of the "interpretable" patterns described above.

The clustering of autoimmune diseases in families is not restricted to a single disease in the same family. Clustering of several autoimmune diseases in the same family has been reported for many autoimmune diseases (Burek et al., 1990; Mumford et al., 1994; Vyse and Todd, 1996; McCanlies et al., 1998; Levin and Tomer, 2003; Tait et al., 2004). These findings are of major importance because they imply that certain genetic variants predispose to autoimmunity in general and are not disease specific. Indeed, an analysis of genetic studies in several autoimmune diseases has revealed overlapping non-MHC susceptibility loci among several autoimmune diseases (Becker et al., 1998).

Sibling Risk Ratio

A very useful method to test the genetic contribution to familial aggregation of a disease is to calculate the sibling risk ratio (λs). The λs is the ratio of the prevalence of the disease in siblings of affected individuals to the prevalence of the disease in the general population (Risch, 1990). The λs expresses the increased risk of developing the disease in an individual who has a sibling with the disease and is a quantitative measure of the genetic contribution to the disease. An λs of $\geqq 5$ usually indicates a significant genetic contribution to the pathogenesis of a disease (Vyse and Todd, 1996). The λs was estimated to be 5–20 in SLE (Hochberg, 1987; Tsao et al., 2002), 20 in MS (Risch, 1987), 17 in autoimmune thyroid disease (AITD) (Villanueva et al., 2003), 15 in T1D (Wagener et al., 1982), and 5 in RA (Rigby et al., 1998). These data support a strong genetic influence on the development of autoimmune diseases.

Twin Studies

Twin studies are the most powerful method for evaluating genetic predisposition to complex diseases. They are based on comparison of the concordance (simultaneous occurrence) of a given disease among monozygotic (MZ; i.e., "identical") twins with the concordance among dizygotic (DZ; i.e., fraternal) twins. MZ twins have nearly identical genetic makeup, whereas DZ twins share 50% of their genes (like siblings). Therefore, if the concordance is higher in the MZ twins when compared to the DZ twins, it suggests that the disease has an inherited component.

Several large twin studies have been reported in autoimmune diseases showing consistently higher concordance in MZ twins compared to DZ twins (Jarvinen and Aho, 1994) (Table 21.1). In RA the concordance rates for MZ and DZ twins have been estimated to be 12–15% and 2–4%, respectively (Seldin et al., 1999). In Graves' disease the concordance rates for MZ twins were higher (17–22%), with much

TABLE 21.1 Twin studies performed in autoimmune diseases

Disease	MZ twin concordance (%)	DZ twin concordance (%)	References
Rheumatoid arthritis	12–15	2–4	Seldin et al. (1999)
Systemic lupus erythematosus	25–57	0–5	Block et al. (1975), Jarvinen et al. (1992), Grennan et al. (1997)
Multiple sclerosis	25–30	0–5	Ebers et al. (1986), Kinnunen et al. (1988), Sadovnick et al. (1993), Mumford et al. (1994)
Crohn's disease	20–50	3–5	Thompson et al. (1996), Orholm et al. (2000)
Type 1 diabetes	35–70	10	Kyvik et al. (1995), Hawa et al. (1997), Redondo et al. (2001)
Graves' disease	17–22	2	Brix et al. (2001), Ringold et al. (2002)
Hashimoto's thyroiditis	38	0	Brix et al. (2000)
Thyroid antibodies	60–80	23–40	Brix et al. (2000), Phillips et al. (2002)

DZ, dizygotic; MZ, monozygotic.

lower concordance rates in DZ twins (2%) (Brix et al., 2001; Ringold et al., 2002). Similar concordance rates have been reported in MS: 25–30% in MZ twins compared to 0–5% in DZ twins (Ebers et al., 1986; Heltberg, 1987; Kinnunen et al., 1988; Sadovnick et al., 1993; Mumford et al., 1994). Even higher concordance rates were reported for SLE among MZ twins (25–57%) and among DZ twins were much lower (0–5%) (Block et al., 1975; Jarvinen et al., 1992; Grennan et al., 1997).

One of the difficulties in twins studies is that reports of concordance rates can vary significantly depending on the age of the proband and the follow-up time since proband diagnosis. For example, cross-sectional twin studies in T1D not taking into account proband age and length of follow-up have shown concordance rates of 35%–70% for MZ twins and approximately 10% for DZ twins (Kyvik et al., 1995). Examination of the length of follow-up since proband diagnosis has shown that the concordance rates were 43% within 12 years of proband diagnosis and 50% within 40 years, demonstrating that the concordance increases as the length of follow-up increases (Hawa et al., 1997). Age of proband diagnosis is also crucial. Diabetes risk approaches 65% for the co-twin of an MZ proband diagnosed before 5 years of age (Kyvik et al., 1995; Redondo et al., 2001). These data suggest that early-onset T1D may have a stronger genetic component that late-onset T1D.

Gender Effects

Most autoimmune diseases are more common in females than in males (see Chapters 3 and 25). The increased female preponderance of autoimmune diseases may be secondary to sex steroid effects or X chromosome effects. Some data suggest that estrogens promote induction of autoimmunity. Autoimmunity is most common in fertile women (Tunbridge et al., 1977), and animal studies show induction of susceptibility to autoimmunity in males by estrogens (Grossman et al., 1991; Paavonen, 1994; Van Griensven et al., 1997). Another possibility is that the increased female susceptibility to autoimmunity is related to the X chromosome. One possible mechanism whereby the X chromosome could influence the development of AITD is probabilistic: females are approximately twice as likely as males to inherit an autoimmunity susceptibility gene since they have two X chromosomes while males have one. Another mechanism is related to the phenomenon of X chromosome inactivation. Even though females have two X chromosomes, only one X chromosome gene is expressed in female somatic cells due to X chromosome inactivation. X chromosome inactivation occurs early in embryonic life and, thereafter, in each cell, either the maternal or paternal chromosome is inactivated. This results in a tissue mosaic of paternally and maternally expressed X chromosomal alleles. Therefore, a female who is heterozygous for an X-linked gene encoding for a self-antigen will have two classes of cells that differ in the transcription of this X chromosome-encoded gene. If these two cell classes extend to the thymic cells responsible for tolerizing T cells in embryonic life, some lymphocytes may not be tolerized to one of the two self-antigens encoded by the X-chromosome. Such lymphocytes would be autoreactive to that antigen and could induce an autoimmune response (Stewart, 1998). Several X chromosome loci have been shown to be linked with autoimmune disease (Barbesino et al., 1998; Imrie et al., 2001; Jawaheer et al., 2001; Zhou et al., 2003), but, so far, no X chromosome susceptibility gene for autoimmunity has been identified.

TOOLS USED TO MAP AND IDENTIFY COMPLEX DISEASE GENES

The two basic tools used for mapping complex disease genes are linkage and association studies. Linkage studies look for cosegregation of markers and disease in families. If a tested marker is close to a disease susceptibility gene, the likelihood that a recombination will occur between them during meiosis is low, and therefore, the marker's alleles will cosegregate with the disease in families (Figure 21.2). Association studies compare the frequency of marker disease alleles in patients and controls. In general, linkage studies are best for screening the whole genome for regions (loci) harboring disease susceptibility genes, while association studies are more powerful for fine mapping the genes in these loci. These tools have been successful in mapping new autoimmune disease genes, e.g., in T1D (Davies et al., 1994) and Crohn's disease (Hugot et al., 2001). While different strategies have been employed to identify complex disease genes, one popular approach is outlined below (Glazier et al., 2002). This strategy is based on five steps:

1. *Identifying linked loci*. This is achieved by whole genome screening using microsatellite markers at an average distance of <10 cM.
2. *Confirming linked loci*. A linked locus should be confirmed by finding evidence for linkage in two independent datasets (Lander and Kruglyak, 1995). Confirmed loci most likely harbor susceptibility genes, e.g., HLA in T1D (Davies et al., 1994).
3. *Fine mapping confirmed loci*. Linked loci can be fine mapped by linkage disequilibrium (LD) mapping. LD mapping is based on association studies with markers that saturate the region of interest. The marker that shows the strongest association with the disease is probably closest to the disease gene. This method can narrow down the region of interest to a few hundred kilobases (Ueda et al., 2003).
4. *Testing genes in the linked region*. After the linked region has been fine mapped the genes in this region can be analyzed. Sequencing of the genes in the fine-mapped loci will identify single nucleotide polymorphisms (SNPs) which are then tested for association with the disease. If a certain SNP shows a consistently significant association with the disease, it may be the susceptibility allele in the region, even though LD with another disease-causing polymorphism cannot be ruled out.
5. *Functional studies*. To demonstrate that an associated allele is a true susceptibility allele it is necessary to show that it affects the function of the gene in a way that increases the risk of developing disease. This provides indirect evidence that it may be the actual susceptibility allele for the disease.

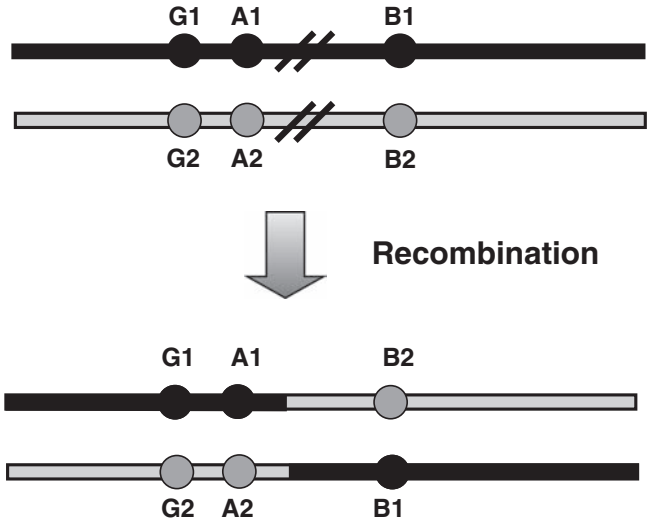


FIGURE 21.2 Principle of linkage. During meiosis, when homologous chromosomes pair, they often break at identical points along their length and switch the distal segments to the breaking point, exchanging identical segments of DNA between two homologous chromosomes (recombination). The principle of linkage analysis is based on the fact that if a marker (A) is close to a mutated gene (G) on a chromosome they will segregate together during recombination, while a marker (B) that is far from the mutated gene will not cosegregate with it. In the example shown, marker allele A1 of marker A is close to the mutated gene (G1) and allele B1 of marker B is far from the mutated gene (G1). Likewise, allele A2 of marker A is close to the normal allele of the gene (G2) and allele B2 of marker B is far from the normal allele of the gene (G2). After recombination, allele A1 cosegregates with the mutated gene (G1), while allele B1 does not. As a result, within a given family a certain allele of marker A (A2) will be seen more frequently than expected by random chance in the affected individuals (i.e., those inheriting the mutated allele of the gene). The closer the marker to the disease gene the stronger the linkage with the disease.

Adapted with permission from Tomer and Davies (2002).

NON-MHC IMMUNE REGULATORY GENES PREDISPOSING TO AUTOIMMUNITY

Several non-MHC immune regulatory genes have been shown to be susceptibility genes for autoimmunity. As expected these genes are not specific for one autoimmune disease but predispose to several autoimmune conditions. However, some variability does exist with certain immune regulatory genes showing a much stronger association with some autoimmune conditions than with others. For example, the CTLA-4 (cytotoxic T-lymphocyte antigen-4) gene shows the strongest association with Graves' disease and a much weaker association with Hashimoto's disease and T1D (Ueda et al., 2003).

CTLA-4 Gene

CTLA-4 is an important costimulatory molecule that plays a key role in the interaction between T cells and antigen-presenting cells (APCs). APCs activate T cells by presenting to the T-cell receptor (TCR) an antigenic peptide bound to an HLA class II protein on the cell surface. However, a second signal is also required for T-cell activation, and these costimulatory signals may be provided by the APCs themselves or other local cells (Reiser and Stadecker, 1996). The costimulatory signals are provided by a variety of proteins that are expressed on the cell surface of APCs (e.g., CD80 (B7-1), CD86 (B7-2), B7h, and CD40) and interact with receptors (CD28, CTLA-4, and CD154 (CD40L)) on the surface of $CD4^+$ T lymphocytes during antigen presentation (Reiser and Stadecker, 1996). The complex interaction between APC proteins and T-cell proteins during antigen presentation is called the "immunologic synapse" (Figure 21.3). Whereas the binding of CD80 (B7) to CD28 on T cells costimulates T cell activation, CTLA-4, which has a higher affinity for CD80 (B7), downregulates T-cell activation by competing for the binding of CD80 (B7) to CD28, and by directly suppressing T-cell activation (Brunner et al., 1999). Indeed, CTLA-4 knock-out mice develop lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction, demonstrating the crucial inhibitory role for CTLA-4 in T-cell activation. The suppressive effects of CTLA-4 on T-cell activation have raised the possibility that mutations/polymorphisms causing reduced CTLA-4 expression and/or function could result in an exaggerated T-cell activation and lead to the development of autoimmunity.

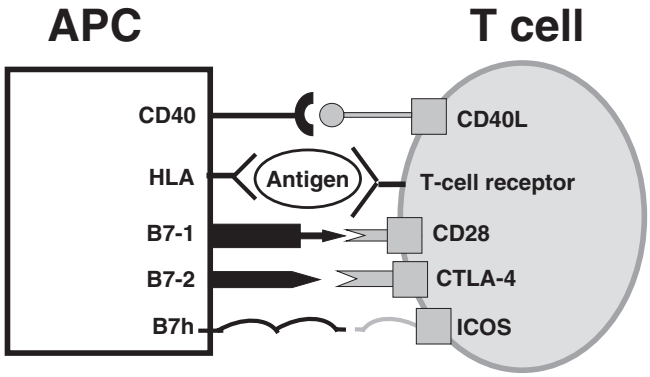


FIGURE 21.3 Immunologic synapse. The antigen-presenting cell (APC) presents a peptide antigen bound to HLA class II molecules, and the peptide is recognized by the T-cell receptor. Engagement of CD80 (B7) molecules with CD28 provides costimulation, while CTLA-4 binding to the CD80 (B7) molecules blocks CD28 activation and also directly suppresses T-cell activation. Engagement of the inducible costimulator (ICOS) with B7h provides additional costimulatory signals. In addition, engagement of CD40 with CD40 ligand activates the APC, and if the APC is a B cell, will result in B-cell proliferation, differentiation, and antibody secretion.

CTLA-4 Association Studies in Autoimmunity

Since the first description of an association between CTLA-4 and Graves' disease in 1995 (Yanagawa et al., 1995) CTLA-4 has been shown to be associated with many autoimmune conditions. This is not unexpected in view of the critical role of CTLA-4 in the immunologic synapse. CTLA-4 has been shown to be associated with both B-cell-mediated autoimmune diseases such as Graves' disease (Donner et al., 1997b) (see below) and T-cell mediated autoimmune diseases such as T1D (Marron et al., 1997) (see below). Additionally, CTLA-4 was shown to be associated with organ-specific autoimmune diseases, e.g., Graves' disease and MS (Donner et al., 1997b), and systemic autoimmune diseases (Ahmed et al., 2001). Thus, it seems that CTLA-4 is a general autoimmunity gene. However, the relative risk conferred by CTLA-4 is low (1.1–1.5) (Ueda et al., 2003), demonstrating that other genes must play a role in the development of autoimmunity, possibly by interactions with CTLA-4 (see below).

Initially, CTLA-4 was reported to be associated with Graves' disease (Yanagawa et al., 1995; Nistico et al., 1996; Donner et al., 1997b; Kotsa et al., 1997; Villanueva et al., 2000; Kouki et al., 2002; Nithiyananthan et al., 2002). The association between Graves' disease and the CTLA-4 3′ untranslated (3′UTR) microsatellite and A/G_{49} SNP has been consistent across populations of different ethnic backgrounds, such as white populations (Yanagawa et al., 1995), Japanese (Yanagawa et al., 1997; Akamizu et al., 2000), and Koreans (Park et al., 2000). Later studies demonstrated associations with Hashimoto thyroiditis, also in populations of diverse ethnic origins, such as white populations (Donner et al., 1997a; Kotsa et al., 1997; Nithiyananthan et al., 2002), and Japanese (Sale et al., 1997; Akamizu et al., 2000). Many other autoimmune diseases have also been shown to be associated with CTLA-4, most notably T1D (Nistico et al., 1996; Marron et al., 1997; Donner et al., 1997b), Addison disease (Vaidya et al., 2000a; Blomhoff et al., 2004), myasthenia gravis (Huang et al., 1998), SLE (Ahmed et al., 2001), MS (Ligers et al., 2001), and primary biliary cirrhosis (Agarwal et al., 2000). Taken together these data strongly support CTLA-4 as a general autoimmunity susceptibility gene (Kristiansen et al., 2000).

CTLA-4 and Antibody Production

Since CTLA-4 does not show disease specificity, it is likely that it is a general autoimmunity gene. To test this hypothesis we tested for linkage of the CTLA-4 locus with the presence of thyroid antibodies (TAbs) without clinical disease. Indeed, we have shown strong evidence for linkage between the CTLA-4 gene region and the production of thyroid antibodies with a maximum LOD score (MLS) of 4.2 (Tomer et al., 2001). Further studies have

confirmed these results (Ban et al., 2003a; Zaletel et al., 2002).

Which CTLA-4 Polymorphism is the Causative One?

The fact that polymorphisms within or near the CTLA-4 gene showed linkage (Vaidya et al., 1999; Tomer et al., 2001) and association (Nistico et al., 1996) with autoimmunity does not necessarily mean that the CTLA-4 is the susceptibility gene in this locus. It is possible that another gene close by in linkage disequilibrium with CTLA-4 is the susceptibility gene in this region. Indeed, the region on chromosome 2q33 containing the CTLA-4 gene is replete with candidate immune regulatory genes for thyroid autoimmunity [e.g., CD28 and inducible costimulator (ICOS)], and it was first unclear whether the CTLA-4 gene itself or another immune regulatory gene in the region was involved in the genetic susceptibility to autoimmunity. Therefore, we (Ban et al., 2003a) and others (Marron et al., 2000; Wood et al., 2002; Ueda et al., 2003) fine mapped the region and have shown that the strongest association was with the CTLA-4 gene, suggesting that it was the susceptibility gene in this locus. That still left the question of which CTLA-4 polymorphism is the causative one. A recent large fine mapping study using over 100 markers in the CTLA-4 region demonstrated that the causative polymorphism is located in the 3′ UTR of the CTLA-4 gene (Ueda et al., 2003). However, several candidate polymorphisms in this 5-kb region still exist, including a microsatellite marker and several SNPs (Ueda et al., 2003), and additional functional studies are needed to identify which of these polymorphism is the causative one (see below).

CD40 Gene

Using a whole genome linkage study we have identified a locus on chromosome 20q11 showing strong evidence for linkage with Graves' disease with a maximum LOD score of 3.5 (Tomer et al., 1998; 1999; 2003). This Graves' disease locus was not linked and associated with Hashimoto's thyroiditis and MS, suggesting that this may be a locus that is specific for Graves' disease. Moreover, in families with individuals with Graves' disease or Hashimoto's thyroiditis, the locus was linked only with Graves' disease, demonstrating its high specificity for Graves' disease (Tomer et al., 1999). This region was also tested by another group from the UK and evidence was reported for linkage with Graves' disease in a subset of their families (Pearce et al., 1999). This further supported the 20q11 locus as an important susceptibility locus for Graves' disease.

The CD40 gene, an important immune modulator, is located within the linked region on chromosome 20q11 and, therefore, it was a likely positional candidate gene for Graves' disease. CD40 is a transmembrane glycoprotein that is expressed predominantly on B cells, monocytes, dendritic cells (DCs), epithelial cells, and other cells (Durie et al., 1994). Binding of CD154 (CD40L) to CD40 induces B cells to proliferate and to undergo immunoglobulin isotype switching (Banchereau et al., 1994). Moreover, *in vivo* blockade of CD40 has been shown to suppress the induction of experimental autoimmune diseases (Carayanniotis et al., 1997; Schaub et al., 1999; Burkly, 2001; Homann et al., 2002). Therefore, we tested whether CD40 could be the Graves' disease susceptibility gene on chromosome 20q11. Sequencing of the CD40 gene revealed a C/T SNP in the 5′UTR region of the gene. The SNP is located within a segment of the 5′UTR that controls the initiation of translation and is designated the Kozak sequence. Analysis of the CD40 Kozak SNP showed an association between the CC genotype and Graves' disease, but with a low relative risk of 1.6 (Tomer et al., 2002a). These results were confirmed by other studies (Kim et al., 2003; Tomer et al., 2005), but not by all studies (Houston et al., 2004). Since the Kozak sequence influences the initiation of translation, we hypothesized that the Kozak SNP might alter CD40 translation, thereby influencing its expression. Expression studies confirmed this hypothesis (see below).

Protein Tyrosine Phosphatase Gene

The protein tyrosine phosphatase (PTPN22) gene is the latest autoimmunity gene to be identified. Several recent studies have shown that PTPN22 is associated with RA (Begovich et al., 2004), SLE (Kyogoku et al., 2004), T1D (Bottini et al., 2004; Smyth et al., 2004), and Graves' disease (Velaga et al., 2004). Thus, like CTLA-4, PTPN22 seems to be a general autoimmunity gene that predisposes to both B- and T-cell-mediated autoimmune diseases. PTPN22 is an important regulator of TCR signaling in memory and effector T cells (Hasegawa et al., 2004). The mouse ortholog (PEP) was shown to be a potent inhibitor of TCR-dependent responses, albeit PEP knock-out mice have a very subtle change in T-cell function. Therefore, additional studies are needed to elucidate the role of PTPN22 in autoimmunity.

Similar to CTLA-4 the relative risk conferred by PTPN22 is relatively low (approximately 2). Therefore, it seems that other autoimmunity genes must play a role in the development of autoimmunity, and it is likely that many genes with small effects (like CTLA-4 and PTPN22) cause predisposition to autoimmunity, and not one or a few major genes.

TISSUE-SPECIFIC GENES IN AUTOIMMUNE DISEASES

While immune regulatory genes may play an important role in the general susceptibility to autoimmunity, it is likely that disease-specific non-immune regulatory genes

contribute to the etiology of specific autoimmune conditions. These disease-specific genes are likely to be expressed in the tissues affected by the autoimmune response in organ-specific autoimmune diseases. Indeed, at least three tissue-specific genes have been identified to date: the insulin variable number of tandem repeats (VNTR) polymorphism in T1D, the NOD2 gene in Crohn's disease, and the thyroglobulin gene in AITD.

Insulin VNTR Polymorphism in Type 1 Diabetes

The first locus, outside the HLA region, which was found to be consistently linked and associated with T1D was the insulin gene region on chromosome 11q, designated insulin-dependent diabetes mellitus (IDDM)-2 (Davies et al., 1994; Hashimoto et al., 1994). This locus conferred a risk that was independent of HLA genotype (Bell et al., 1984; Bain et al., 1992; Lucassen et al., 1993; Van der Auwera et al., 1993; Hashimoto et al., 1994). This locus was estimated to contribute about 10% to disease susceptibility (Davies et al., 1994). It is now known that the polymorphism conferring the susceptibility to IDDM in this locus is the VNTR polymorphism (Undlien et al., 1995). The VNTR is located 365 base pairs upstream from the initiation of transcription of the insulin gene. The polymorphism arises from a variable number of 14 base pair oligonucleotide repeats. Based on the number of repeats, the length of the VNTR can be divided into three classes: class I (approximately 570 bp), class II (approximately 1640 bp), and class III (approximately 2400 bp) (Lucassen et al., 1993; Ahmed et al., 1999). Homozygosity for short class I alleles confers a two- to five-fold increased risk for T1D , while class III alleles are dominantly protective (Nistico et al., 1996; Bennett et al., 1995).

NOD2/CARD15 Gene and Crohn's Disease

The first whole genome scan demonstrating that a locus on chromosome 16q showed strong evidence for linkage with Crohn's disease was reported in 1996 (Hugot et al., 1996). This locus was designated IBD1 and the linkage evidence at IBD1 was replicated by several studies, most notably by an International Genetics Consortium showing a LOD score of 5.8 at IBD1 (Cavanaugh, 2001). Following the mapping of the IBD1 locus, the region was fine mapped and a novel gene, nucleotide-binding oligomerization domain 2 (NOD2), also called caspase activation recruitment domain 15 (CARD15), was identified to within this locus and was shown to be the susceptibility gene at the IBD1 locus (Hugot et al., 2001; Ogura et al., 2001).

NOD2 Polymorphisms Associated with Crohn's Disease

Several polymorphisms were identified within the coding region of the NOD2 gene that showed strong association with Crohn's disease but not with ulcerative colitis (Hugot et al., 2001). The three most important NOD2 polymorphisms that were found to be associated with Crohn's disease are two missense SNPs (i.e., SNPs that change the amino acid sequence of the gene), Arg702Trp and Gly907Arg, and one frameshift mutation (3040insC) causing a premature stop codon (Hugot et al., 2001). The relative risk of developing Crohn's disease in an individual with these polymorphisms ranges from 3 for simple heterozygotes to 38 in homozygotes for the susceptibility allele (Hugot et al., 2001). Therefore, there seems to be a gene dosage effect (McGovern et al., 2001).

Genotype–Phenotype Correlations

The NOD2 SNPs identified to date were found to be associated with Crohn's disease only in white patients but not in Asian (Inoue et al., 2002) or African American patients (Cho, 2004). In addition, the Cly908Arg SNP was found to be more common in Ashkenazi Jewish patients (Sugimura et al., 2003), and a new intronic variant (IVS+158, designated JW1) was reported to be specific for Ashkenazi Jewish Crohn's disease patients (Sugimura et al., 2003). Additional correlations were found with earlier age of onset and ileal disease (Cho, 2004).

NOD2 Function and the Effects of the NOD2 Single Nucleotide Polymorphisms

The NOD2 gene is expressed in peripheral blood monocytes, macrophages, and intestinal epithelial cells (Cho, 2004). NOD2 has three domains: a C-terminal leucine-rich repeat (LRR) domain, a nucleotide oligomerization domain, and an N-terminal region that contains the CARD. The CARD mediates activation of the NF-κB transcription factors, which is a master regulator of inflammatory responses (Watanabe et al., 2004). The C-terminal region of NOD2 contains an LRR that serves as a pattern-recognition receptor for many innate signals such as lipopolysaccharide (Watanabe et al., 2004). Therefore, the LRR domain might be involved in the recognition of microbial products (O'Neill, 2004). Interestingly, the three major SNPs in NOD2 that are associated with Crohn's disease are located in the LRR region of the gene (Cho, 2004). Originally, it was thought that the NOD2 polymorphisms in the LRR domain caused a decreased functional response of NOD2, leading to decreased NF-κB activation (Ogura et al., 2001). However, this model was inconsistent with data pointing to a crucial role for NF-κB activation in the activation of type 1 helper T (Th1) cells and the inflammatory response in Crohn's disease (O'Neill, 2004). Recent data may help

resolve this inconsistency by demonstrating that NOD2 inhibits the activation of the Toll-like receptor 2 (TLR2) by peptidoglycan, and that NOD2 deficiency resulted in increased TLR2-mediated activation of NF-κB (Watanabe et al., 2004). Thus, NOD2 SNPs may predispose to Crohn's disease by decreasing NOD2 activity, which leads to increased TLR2-mediated NF-κB activation and enhanced Th1 inflammatory responses (Watanabe et al., 2004).

Thyroglobulin Gene and Susceptibility to Autoimmune Thyroid Disease

Thyroglobulin (Tg) is the major protein product synthesized in the thyroid gland (Tomer, 1997). There is abundant evidence that Tg plays an important role in the etiology of AITD including: 1) anti-Tg antibodies are detected in most patients with AITD, both Graves' disease and Hashimoto's thyroiditis (Roitt et al., 1958; Ericsson et al., 1985), and there is evidence that Tg antibodies of AITD patients are restricted in their epitope specificity, in contrast to the polyclonal nature of Tg antibodies found in healthy individuals (Kuppers et al., 1992); 2) immunization with Tg induces autoimmune thyroiditis in experimental animals (Vladutiu and Rose, 1971); and 3) spontaneous models of autoimmune thyroiditis in the nonobese diabetic (NOD) mouse and BB/W rat are also characterized by the development of anti-Tg antibodies (Allen et al., 1987; Braley-Mullen et al., 1999). Therefore, Tg is a good candidate gene for thyroid autoimmunity.

Two whole genome screens have shown strong evidence for linkage of AITD with the chromosome 8q24 region which contains the Tg gene (Sakai et al., 2001; Tomer et al., 2002b) (see below). Since the Tg gene was located within the linked region, we proceeded to analyze the Tg gene directly. Analysis of microsatellite markers within intronic regions of the Tg gene gave a significant LOD score of 2.9, confirming that the Tg gene was linked with AITD (Tomer et al., 2002b). Association studies showed an association of Tg microsatellite markers with AITD (Tomer et al., 2002b; Collins et al., 2003; Ban et al., 2004).

The fact that markers within the Tg gene were linked and associated with AITD strongly suggested that the Tg gene was the AITD susceptibility gene on 8q24. Therefore, we sequenced the Tg gene and identified 14 new Tg SNPs. Case–control association studies demonstrated that one SNP cluster in exons 10–12, in strong linkage disequilibrium, and a SNP in exon 33 were significantly associated with AITD (Ban et al., 2003b). Taken together these data suggest that Tg is a major AITD susceptibility gene (Ban et al., 2003b). It can be hypothesized that Tg sequence variants are involved in the susceptibility to AITD; such variants may act by modifying the interaction of Tg peptide profiles, produced by digestion of Tg by cathepsins in endosomes, with HLA class II molecules.

WHOLE GENOME SCREENING IN AUTOIMMUNE DISEASES

As discussed above, whole genome screening is a powerful genetic tool with which to identify loci or genetic regions that might be linked with disease. When a locus which is linked with disease is identified, the region can be fine mapped and the gene identified. This strategy has been used successfully in a number of complex diseases, most notably in the mapping and identification of NOD2 as a susceptibility gene for Crohn's disease (Hugot et al., 2001; Ogura et al., 2001). Numerous genome screens have been performed in autoimmune diseases, resulting in a growing list of loci which might harbor susceptibility genes for autoimmunity.

Genome scans in SLE families identified several loci showing evidence for linkage. Most notable are loci on chromosomes 1q23, 1q41, 2q35, 4p16, and 6p11 (Tsao, 2003). Similarly, loci on chromosomes 1q, 2q, 6p, 9q, 16q, and 18p were identified for MS (Kenealy et al., 2004), loci on chromosomes 1p and 18q were identified for RA (Gregersen, 2003), and loci on chromosomes 2q, 6q, 10q, 12q 14q, and 20q were identified for AITD (Tomer and Davies, 2003; Tomer et al., 2003). Interestingly, significant overlap exists between loci identified for different autoimmune diseases (Becker et al., 1998), suggesting the existence of a significant number of susceptibility genes that predispose to autoimmunity in general and not to any specific autoimmune disease. One locus that was found to be linked with several autoimmune diseases is the 18q locus. The 18q locus was originally identified as a T1D locus but it was later found to be linked with Graves' disease (Vaidya et al., 2000b), as well as MS and RA (Merriman et al., 2001). However, additional studies are necessary to examine whether the 18q locus contains an autoimmunity susceptibility gene. In summary, linkage studies serve as the first step in gene identification. As more linkage studies are being performed it will be possible to examine which loci show the highest level of replication between different centers. These loci represent the best candidates for fine mapping and gene identification.

MECHANISMS BY WHICH GENES CAN INDUCE THYROID AUTOIMMUNITY

General Principles

In classical monogenic diseases the genetic defect changes the action of a gene by decreasing its effectiveness, e.g., the Pendrin gene in Pendred syndrome (Everett et al., 1997), or causing its overactivity, e.g. the RET proto-oncogene in multiple endocrine neoplasia type 2 (Eng, 1996;

Puxeddu and Fagin, 2001). However, in complex diseases, such as the autoimmune diseases, the genetic defect may cause subtle changes in the function of one or more genes that, when combined, increase the risk for disease. Therefore, even when a gene causing a common disease is mapped, proving that the polymorphism is also biologically meaningful can be difficult. Direct and indirect functional studies have been performed for several autoimmunity susceptibility genes, and some of these are summarized below.

CTLA-4 Functional Studies

CTLA-4 gene polymorphisms have been studied extensively for their effects on CTLA-4 function and/or expression. CTLA-4 is an important costimulatory molecule that downregulates T-cell activation and induces tolerance (see Figure 21.3). The suppressive effects of CTLA-4 on T-cell activation have raised the possibility that the CTLA-4 polymorphisms associated with AITD decrease its expression and/or function, thereby promoting the development of autoimmunity.

Several CTLA-4 polymorphisms have been shown to be associated with AITD, including a 3′UTR AT microsatellite (Yanagawa et al., 1995), a 3′UTR SNP (designated CT60) (Ueda et al., 2003), and an A/G polymorphism in the leader sequence of the gene (Donner et al., 1997b). These three polymorphisms have been tested in both direct and indirect functional studies for their effects on the function/expression of CTLA-4. The first functional study examined the effects of the A and G alleles of the CTLA-4 A/G_{49} SNP on the inhibitory function of CTLA-4 (Kouki et al., 2000). The authors showed that blocking of CTLA-4 on T cells isolated from individuals with the G allele had less effect on reducing the inhibitory function of CTLA-4 than blocking CTLA-4 on T cells isolated from individuals with the A allele. Similar results were obtained by our group using a slightly different study design (Ban et al., 2003a). This could imply that the A and G alleles of the CTLA-4 leader sequence influence its function and/or expression. However, since this was an indirect study it cannot rule out that another CTLA-4 polymorphism in linkage disequilibrium with the A/G_{49} SNP is causing this observed effect on CTLA-4 function. We have examined the effects of the CTLA-4 A/G_{49} SNP using an *in vitro* assay by transfecting T-cell lines lacking CTLA-4 with CTLA-4 cDNA having the A or the G allele: no difference was seen in the expression and inhibitory function of CTLA-4 (Xu et al., 2002). Thus, this study demonstrated that the A and G alleles of the CTLA-4 A/G_{49} SNP did not directly influence its function. Other polymorphisms must be responsible for the association of CTLA-4 with AITD. The CT60 3′UTR SNP was also tested by indirect functional studies for its effects on the levels of a splice variant of CTLA-4. The results demonstrated that the G allele of the CT60 SNP allele that predisposes to autoimmunity was associated with reduced production of a splice variant of CTLA-4, resulting in decreased soluble CTLA-4 levels (Ueda et al., 2003). Studies of the 3′UTR AT microsatellite have demonstrated that the long repeats (which predispose to autoimmunity) reduced inhibitory function of CTLA-4 (Takara et al., 2003). Moreover, preliminary data in myasthenia gravis showed that the long repeats of the AT microsatellite at the 3′UTR of the CTLA-4 gene decreased the half life of the CTLA-4 mRNA (Huang et al., 2000; Holopainen and Partanen, 2001). This could provide an attractive explanation for the association between the long repeats of the 3′UTR AT microsatellite and autoimmunity, whereby the long repeats decrease the half life of the CTLA-4 mRNA, causing reduced CTLA-4 expression and hyperactivation of T cells, and leading to autoimmunity (Tomer, 2001).

Insulin VNTR Gene

The insulin VNTR gene is located adjacent to regulatory DNA sequences affecting insulin gene expression. Therefore, it was postulated that the polymorphism influenced disease disposition through influence on insulin gene expression (Docherty, 1992). Indeed, the insulin VNTR was shown to influence gene expression *in vitro* (Kennedy et al., 1995) and *in vivo* (Bennett et al., 1995), with class I alleles being associated with 1.5–3.0-fold increase in insulin gene transcription in the pancreas. However, it was difficult to reconcile lower insulin mRNA levels in the pancreas, associated with class III alleles, with a protective effect on T1D development. Several groups have since reported that class III VNTR alleles are associated with significantly higher insulin mRNA levels in the human fetal thymus (Vafiadis et al., 1997; Puglise et al., 1997). These authors postulated that higher levels of thymic insulin expression in individuals with class III alleles promoted the negative selection of insulin-specific T lymphocytes, thus facilitating immune tolerance induction and protection from T1D. These findings, if confirmed, may represent a general principle in autoimmunity and may apply to other autoimmune conditions.

CONCLUSIONS AND FUTURE DIRECTIONS

The autoimmune diseases are complex diseases believed to be caused by the combined effects of multiple susceptibility genes and environmental triggers. There are sufficient epidemiologic data to support an important genetic contribution to the development of autoimmunity, and in the past few years several loci and genes have shown evidence for linkage and/or association with autoimmune diseases. Thus, the genetic susceptibility to autoimmunity seems to involve

several genes with varying effects. With the completion of the human genome project and the establishment of large SNP databases, the identification of additional non-MHC autoimmunity susceptibility genes will become more feasible.

The autoimmunity loci identified so far show that some putative autoimmune susceptibility genes may be immune-modifying genes that increase the susceptibility to autoimmunity in general (e.g., CTLA-4 and PTPN22), while others may be specific to a certain disease (e.g., insulin VNTR). The next step in investigating the role of these genes in the development of autoimmunity is by functional studies and genotype–phenotype correlations. It is most likely that the susceptibility loci for autoimmune diseases interact and that their interactions may influence disease phenotype and severity (Tomer et al., 1999). The molecular basis for the interactions between susceptibility genes in complex diseases is unknown. These interactions could represent the cumulative effect of increased statistical risk, or alternatively, there may be molecular interactions between the susceptibility genes or their products, which ultimately determine disease phenotype. Another unresolved question is how environmental factors interact with susceptibility genes to modify the risk for disease, as well as the disease phenotype. We are slowly progressing towards identification of the autoimmunity susceptibility genes and once they are identified we will begin to understand the underlying molecular mechanisms by which they induce systemic and organ-specific autoimmunity.

Acknowledgments

I thank Dr David Greenberg for helpful discussions. This work was supported in part by grants DK61659 & DK67555 from NIDDKD.

References

Agarwal K., Jones, D.E., Daly, A.K., James, O.F., Vaidya, B., Pearce, S., and Bassendine, M.F. (2000). CTLA-4 gene polymorphism confers susceptibility to primary biliary cirrhosis. *J. Hepatol. 32*, 538–541.

Ahmed, S., Bennett, S.T., Huxtable, S.J., Todd, J.A., Matthews, D.R., and Gough, S.C. (1999). INS VNTR allelic variation and dynamic insulin secretion in healthy adult non-diabetic Caucasian subjects. *Diabet. Med. 16*, 910–917.

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 (Oxf.) 40*, 662–667.

Akamizu, T., Sale, M.M., Rich, S.S., Hiratani, H., Noh, J.Y., Kanamoto, N., Saijo, M., Miyamoto, Y., Saito, Y., Nakao, K., and Bowden, D.W. (2000). Association of autoimmune thyroid disease with microsatellite markers for the thyrotropin receptor gene and CTLA-4 in Japanese patients. *Thyroid 10*, 851–858.

Allen, E.M., Appel, M.C., and Braverman, L.E. (1987). Iodine-induced thyroiditis and hypothyroidism in the hemithyroidectomized BB/W rat. *Endocrinology 121*, 481–485.

Bain, S.C., Prins, J.B., Hearne, C.M., Rodrigues, N.R., Rowe, B.R., Pritchard, L.E., Ritchie, R.J., Hall, J.R.S., Undlien, D.E., Ronningen, K.S., Dunger, D.B., Barnett, A.H., and Todd, J.A. (1992). Insulin gene region-encoded susceptibility to type 1 diabetes is not restricted to HLA-DR4-positive individuals. *Nat. Genet. 2*, 212–215.

Ban, Y., Davies, T.F., Greenberg, D.A., Kissin, A., Marder, B., Murphy, B., Concepcion, E.S., Villanueva, R.B., Barbesino, G., Ling, V., and Tomer, Y. (2003a). Analysis of the CTLA-4, CD28 and inducible costimulator (ICOS) genes in autoimmune thyroid disease. *Genes Immun. 4*, 586–593.

Ban, Y., Greenberg, D.A., Concepcion, E., Skrabanek, L., Villanueva, R., and Tomer, Y. (2003b). Amino acid substitutions in the thyroglobulin gene are associated with susceptibility to human and murine autoimmune thyroid disease. *Proc. Natl. Acad. Sci. USA 100*, 15119–15124.

Ban, Y., Tozaki, T., Taniyama, M., Tomita, M., and Ban, Y. (2004). Association of a thyroglobulin gene polymorphism with Hashimoto's thyroiditis in the Japanese population. *Clin. Endocrinol. (Oxf.) 61*, 263–268.

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.

Barbesino, G., Tomer, Y., Concepcion, E.S., Davies, T.F., and Greenberg, D. (1998). Linkage analysis of candidate genes in autoimmune thyroid disease: 2. Selected gender-related genes and the X-chromosome. *J. Clin. Endocrinol. Metab. 83*, 3290–3295.

Becker, K.G., Simon, R.M., Bailey-Wilson, J.E., Freidlin, B., Biddison, W.E., McFarland, H.F., and Trent, J.M. (1998). Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. *Proc. Natl. Acad. Sci. USA 95*, 9979–9984.

Begovich, A.B., Carlton, V.E., Honigberg, L.A., Schrodi, S.J., Chokkalingam, A.P., Alexander, H.C., Ardlie, K.G., Huang, Q., Smith, A.M., Spoerke, J.M., Conn, M.T., Chang, M., Chang, S.Y., Saiki, R.K., Catanese, J.J., Leong, D.U., Garcia, V.E., McAllister, L.B., Jeffery, D.A., Lee, A.T., Batliwalla, F., Remmers, E., Criswell, L.A., Seldin, M.F., Kastner, D.L., Amos, C.I., Sninsky, J.J., and Gregersen, P.K. (2004). A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am. J. Hum. Genet. 75*, 330–337.

Bell, G.I., Horita, S., and Karam, J.H. (1984). A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes 33*, 176–183.

Bennett, S.T., Lucassen, A.M., Gough, S.C.L., Powell, E.E., Undlien, D.E., Pritchard, D.E., Merriman M.E., Kawaguchi, Y., Dronsfield, M., Pociot, F., Nerup, J., Bouzekri, N., Cambon-Thomsen, A., Ronningen, K.S., Barnett, A.H., Bain, S.C., and Todd J.A. (1995). Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet. 9*, 284–292.

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.

Blomhoff, A., Lie, B.A., Myhre, A.G., Kemp, E.H., Weetman, A.P., Akselsen, H.E., Huseby, E.S., and Undlien, D.E. (2004). Polymorphisms in the cytotoxic T lymphocyte antigen-4 gene region confer susceptibility to Addison's disease. *J. Clin. Endocrinol. Metab. 89*, 3474–3476.

Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., MacMurray, J., Meloni, G.F., Lucarelli, P., Pellecchia, M., Eisenbarth, G.S., Comings, D., and Mustelin, T. (2004). A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat. Genet. 36*, 337–338.

Braley-Mullen, H., Sharp, G.C., Medling, B., and Tang, H. (1999). Spontaneous autoimmune thyroiditis in NOD.H–2h4 mice. *J. Autoimmun. 12*, 157–165.

Brix, T.H., Kyvik, K.O., Christensen, K., and Hegedus, L. (2001). Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *J. Clin. Endocrinol. Metab. 86*, 930–934.

Brunner, M.C., Chambers, C.A., Chan, F.K., Hanke, J., Winoto, A., and Allison, J.P. (1999). CTLA-4-mediated inhibition of early events of T cell proliferation. *J. Immunol. 162*, 5813–5820.

Burek, C.L., Hoffman, W.H., and Rose, N.R. (1982). The presence of thyroid autoantibodies in children and adolescents with AITD and in their siblings and parents. *Clin. Immunol. Immunopathol. 25*, 395–404.

Burek, C.L., Rose, N.R., Guire, K.E., and Hoffman, W.H. (1990). Thyroid autoantibodies in black and in white children and adolescents with type 1 diabetes mellitus and their first degree relatives. *Autoimmunity 7*, 157–167.

Burkly. L.C. (2001). CD40 pathway blockade as an approach to immunotherapy. *Adv. Exp. Med. Biol. 489*, 135–152.

Carayanniotis, G., Masters, S.R., and Noelle, R.J. (1997). Suppression of murine thyroiditis via blockade of the CD40–CD40L interaction. *Immunology 90*, 421–426.

Carey, C., Skosey, C., Pinnamaneni, K.M., Barsano, C.P., and DeGroot, L.J. (1980). Thyroid abnormalities in children of parents who have Graves' disease: possible pre-Graves' disease. *Metabolism 29*, 369–376.

Cavanaugh, J. (2001). International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set: Crohn disease and chromosome 16. *Am. J. Hum. Genet. 68*, 1165–1171.

Cho, J.H. (2004). Advances in the genetics of inflammatory bowel disease. *Curr. Gastroenterol. Rep. 6*, 467–473.

Chopra, I.J., Solomon, D.H., Chopra, U., Yodhihara, E., Tersaki, P.L., and Smith, F. (1977). Abnormalities in thyroid function in relatives of patients with Graves' disease and Hashimoto's thyroiditis: lack of correlation with inheritance of HLA-B8. *J. Clin. Endocrinol. Metab. 45*, 45–54.

Collins, J.E., Heward, J.M., Carr-Smith, J., Daykin, J., Franklyn, J.A., and Gough, S.C.L. (2003). Association of a rare thyroglobulin gene microsatellite variant with autoimmune thyroid disease. *J. Clin. Endocrinol. Metab. 88*, 5039–5042.

Davies, J.L., Kawauchi, Y., Bennet, S.T., Copeman, J.B., Cordell, H.J., Pritchard, L.E., Reed, P.W., Gough, S.C.L., Jenkins, S.C., Palmer, S.M., Balfour, K.M., Rowe, B.R., Farrall, M., Barnett, A.H., Baln, S.C., and Todd, J.A. (1994). A genome-wide search for human type1 diabetes susceptibility genes. *Nature 371*, 130–136.

Docherty, K. (1992). 1992 R. D. Lawrence Lecture. The regulation of insulin gene expression. *Diabet. Med. 9*, 792–798.

Donner, H., Braun, J., Seidl, C., Rau, H., Finke, R., Ventz, M., Walfish, P.G., Usadel, K.H., and Badenhoop, K. (1997a). Codon 17 polymorphism of the cytotoxic T lymphocyte antigen 4 gene in Hashimoto's thyroiditis and Addison's disease. *J. Clin. Endocrinol. Metab. 82*, 4130–4132.

Donner, H., Rau, H., Walfish, P.G., Braun, J., Siegmund, T., Finke, R., Herwig, J., Usadel, K.H., and Badenhoop, K. (1997b). CTLA4 alanine-17 confers genetic susceptibility to Graves' disease and to type 1 diabetes mellitus. *J. Clin. Endocrinol. Metab. 82*, 143–146.

Durie, F.H., Foy, T.M., Masters, S.R., Laman, J.D., and Noelle, R.J. (1994). The role of CD40 in the regulation of humoral and cell-mediated immunity. *Immunol. Today 15*, 406–411.

Ebers, G.C., Bulman, D.E., Sadovnick, A.D., Paty, D.W., Warren, S., Hader, W., Murray, T.J., Seland,, T.P., Duquette, P., and Grey, T. (1986). A population-based study of multiple sclerosis in twins. *N. Engl. J. Med. 315*, 1638–1642.

Eng, C. (1996). Seminars in medicine of the Beth Israel Hospital, Boston. The RET proto-oncogene in multiple endocrine neoplasia type 2 and Hirschsprung's disease. *N. Engl. J. Med. 335*, 943–951.

Ericsson, U.B., Christensen, S.B., and Thorell, J. (1985). A high prevalence of thyroglobulin autoantibodies in adults with and without thyroid disease as measured with a sensitive solid-phase immunosorbent radioassay. *Clin. Immunol. Immunopathol. 37*, 154–162.

Everett, L.A., Glaser, B., Beck, J.C., Idol, J.R., Buchs, A., Heyman, M., Adawi, F., Hazani, E., Nassir, E., Baxevanis, A.D., Sheffield, V.C., and Green, E.D. (1997). Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat. Genet. 17*, 411–422.

Glazier, A.M., Nadeau, J.H., and Aitman, T.J. (2002). Finding genes that underlie complex traits. *Science 298*, 2345–2349.

Gregersen, P.K. (2003). Teasing apart the complex genetics of human autoimmunity: lessons from rheumatoid arthritis. *Clin. Immunol. 107*, 1–9.

Grennan, 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. Markers 13*, 93–98.

Grossman, C.J., Roselle, G.A., and Mendenhall, C.L. (1991). Sex steroid regulation of autoimmunity. *J. Steroid Biochem. Mol. Biol. 40*, 649–659.

Hall, R. and Stanbury, J.B. (1967). Familial studies of autoimmune thyroiditis. *Clin. Exp. Immunol. 2*, 719–725.

Hasegawa, K., Martin, F., Huang, G., Tumas, D., Diehl, L., and Chan, A.C. (2004). PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science 303*, 685–689.

Hashimoto, L., Habita, C., Beressl, J.B., Delepine, M., Besse, C., Cambon-Thomsen, A., Deschamps, I., Rotter, J.I., Djouiah, S., James, M.R., Froguel, P., Weissenbach, J., Lathrop, G.M., and Juiler, C. (1994). Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. *Nature 371*, 161–164.

Hawa, M., Rowe, R., Lan, M.S., Notkins, A.L., Pozzilli, P., Christie, M.R., and Leslie, R.D. (1997). Value of antibodies to islet protein tyrosine phosphatase-like molecule in predicting type 1 diabetes. *Diabetes 46*, 1270–1275.

Heltberg, A. (1987). Twin studies in multiple sclerosis. Ital. *J. Neurol. Sci. (Suppl 6)*, 35–39.

Hochberg, M.C. (1987). The application of genetic epidemiology to systemic lupus erythematosus. *J. Rheumatol. 14*, 867–869.

Holopainen, P.M. and Partanen, J. (2001). Technical note: Linkage disequilibrium and disease-associated CTLA-4 gene polymorphisms. *J. Immunol. 167*, 2457–2458.

Homann, D., Jahreis, A., Wolfe, T., Hughes, A., Coon, B., van Stipdonk, M.J., Prilliman, K.R., Schoenberger, S.P., and von Herrath, M.G. (2002). CD40L blockade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells. *Immunity 16*, 403–415.

Houston, F.A., Wilson, V., Jennings, C.E., Owen, C.J., Donaldson, P., Perros, P., and Pearce, S.H. (2004). Role of the CD40 locus in Graves' disease. *Thyroid 14*, 506–509.

Huang, D., Liu, L., Noren, K., Xia, S.Q., Trifunovic, J., Pirskanen, R., and Lefvert, A.K. (1998). Genetic association of CTLA-4 to myasthenia gravis with thymoma. *J. Neuroimmunol. 88*, 192–198.

Huang, D., Giscombe, R., Zhou, Y., Pirskanen, R., and Lefvert, A.K. (2000). Dinucleotide repeat expansion in the CTLA-4 gene leads to T cell hyper-reactivity via the CD28 pathway in myasthenia gravis. *J. Neuroimmunol. 105*, 69–77.

Hugot, J.P., Lauren-Puig, P., Gower-Rousseau, C., Olson, J.M., Lee, J.C., Beaugerie, L., Naom, I., Dupas, J.-L., Van Gossum, A., Orholm, M., Bonaiti-Pellie, C., Weissenbach, J., Mathew, C.G., Lennard-Jones, J.E., Cortot, A., Colombel J.-F., and Tomas G. (1996). Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature 379*, 821–823.

Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., and Gassull, M. (2001).

Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature 411*, 599–603.

Imrie, H., Vaidya, B., Perros, P., Kelly, W.F., Toft, A.D., Young, E.T., Kendall-Taylor, P., and Pearce, S.H.S. (2001). Evidence for a Graves' disease susceptibility locus at chromosome Xp11 in a United Kingdom population. *J. Clin. Endocrinol. Metab.* 86, 626–630.

Inoue, N., Tamura, K., Kinouchi, Y., Fukuda, Y., Takahashi, S., Ogura, Y., Inohara, N., Nunez, G., Kishi, Y., Koike, Y., Shimosegawa, T., Shimoyama, T., and Hibi, T. Lack of common NOD2 variants in Japanese patients with Crohn's disease. *Gastroenterology* 123, 86–91.

Jarvinen, P. and Aho, K. (1994). Twin studies in rheumatic diseases. *Semin. Arthritis Rheum. 24*, 19–28.

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.

Jaume, J.C., Guo, J., Pauls, D.L., Zakarija, M., McKenzie, J.M., Egeland, J.A., Burek, C.L., Rose, N.R., Hoffman, W.H., Rapoport, B., and McLachlan, S.M. (1999). Evidence for genetic transmission of thyroid peroxidase autoantibody epitopic "fingerprints". *J. Clin. Endocrinol. Metab. 84*, 1424–1431.

Jawaheer, D., Seldin, M.F., Amos, C.I., Chen, W.V., Shigeta, R., Monteiro, J., Kern, M., Criswell, L.A., Albani, S., Nelson, J.L., Clegg, D.O., Pope, R., Schroeder, H.W., Jr., Bridges, S.L., Jr., Pisetsky, D.S., Ward, R., Kastner, D.L., Wilder, R.L., Pincus, T., Callahan, L.F., Flemming, D., Wener, M.H., and Gregersen, P.K. (2001). A genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with other autoimmune diseases. *Am. J. Hum. Genet. 68*, 927–936.

Kenealy, S.J., Babron, M.C., Bradford, Y., Schnetz-Boutaud, N., Haines, J.L., Rimmler, J.B., Schmidt, S., Pericak-Vance, M.A., Barcellos, L.F., Lincoln, R.R., Oksenberg, J.R., Hauser, S.L., Clanet, M., Brassat, D., Edan, G., Yaouanq, J., Semana, G., Cournu-Rebeix, I., Lyon-Caen, O., and Fontaine, B. (2004). A second-generation genomic screen for multiple sclerosis. *Am. J. Hum. Genet. 75*, 1070–1078.

Kennedy, G.C., German, M.S., and Rutter, W.J. (1995). The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. *Nat. Genet. 9*, 293–298.

Kim, T.Y., Park, Y.J., Hwang, J.K., Song, J.Y., Park, K.S., Cho, B.Y., and Park, D.J. (2003). A C/T polymorphism in the 5′-untranslated region of the CD40 gene is associated with Graves' disease in Koreans. *Thyroid* 13, 919–926.

Kinnunen, E., Juntunen, J., Ketonen, L., Koskimies, S., Konttinen, Y.T., Salmi, T., Koskenvuo, M., and Kaprio, J. (1988). Genetic susceptibility to multiple sclerosis. A co-twin study of a nationwide series. *Arch. Neurol. 45*, 1108–1111.

Kotsa, K., Watson, P.F., and Weetman, A.P. (1997). A CTLA-4 gene polymorphism is associated with both Graves' disease and autoimmune hypothyroidism. *Clin. Endocrinol. 46*, 551–554.

Kouki, T., Sawai, Y., Gardine, C.A., Fisfalen, M.E., Alegre, M.L., and DeGroot, L.J. (2000). CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. *J. Immunol. 165*, 6606–6611.

Kouki, T., Gardine, C.A., Yanagawa, T., and DeGroot, L.J. (2002). Relation of three polymorphisms of the CTLA-4 gene in patients with Graves' disease. *J. Endocrinol. Invest. 25*, 208–213.

Kristiansen, O.P., Larsen, Z.M., and Pociot, F. (2000). CTLA-4 in autoimmune diseases—a general susceptibility gene to autoimmunity? *Genes Immun. 1*, 170–184.

Kuppers, R.C., Bresler, H.S., Lynne Burek, C., Gleason, S.L., and Rose, N.R. (1992). Immunodominant determinants of thyroglobulin associated with autoimmune thyroiditis. In C. A. Bona and A. K. Kaushik (eds), *Molecular Immunobiology of Self-Reactivity* (pp. 247–284). New York: Marcel Dekker.

Kyogoku, C., Langefeld, C.D., Ortmann, W.A., Lee, A., Selby, S., Carlton, V.E., Chang, M., Ramos, P., Baechler, E.C., Batliwalla, F.M., Novitzke, J., Williams, A.H., Gillett, C., Rodine, P., Graham, R.R., Ardlie, K.G., Gaffney, P.M., Moser, K.L., Petri, M., Begovich, A.B., Gregersen, P.K., and Behrens, T.W. (2004). Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am. J. Hum. Genet. 75*, 504–507.

Kyvik, K.O., Green, A., and Beck-Nielsen, H. (1995). Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins. *BMJ 311*, 913–917.

Lander, E. and Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat. Genet. 11*, 241–247.

Levin, L and Tomer, Y. (2003). The etiology of autoimmune diabetes and thyroiditis: evidence for common genetic susceptibility. *Autoimmun. Rev. 2*, 377–386.

Ligers, A., Teleshova, N., Masterman, T., Huang, W.X., and Hillert, J. (2001). CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. *Genes Immun. 2*, 145–152.

Lucassen, A.M., Julier, C., Beressi, J.P., Boitard, C., Froguel, P., Lathrop, M., and Bell, J.I. (1993). Susceptibility to insulin-dependent diabetes mellitus maps to a 4.1 kb segment of DNA spanning the insulin gene and associated VNTR. *Nat. Genet. 4*, 305–310.

Marron, M.P., Raffel, L.J., Garchon, H.J., Jacob, C.O., SerranoRios, M., Martinez Larrad M.T., Teng, W.P., Park, Y., Zhang, Z.X., Goldstein, D.R., Tao, Y.W., Beaurain, G., Bach, J.F., Huang, H.S., Luo, D.F., Zeidler, A., Rotter, J.I., Yang, M.C.K., Modilevsky, T., Maclaren, N.K., and She, J.X. (1997). Insulin-dependent diabetes mellitus (IDDM) is associated with CTLA4 polymorphisms in multiple ethnic groups. *Hum. Mol. Genet. 6*, 1275–1282.

Marron, M.P., Zeidler, A., Raffel, L.J., Eckenrode, S.E., Yang, J.J., Hopkins, D.I., Garchon, H.J., Jacob, C.O., Serrano-Rios, M., Martinez Larrad, M.T., Park, Y., Bach, J.F., Rotter, J.I., Yang, M.C., and She, J.X. (2000). Genetic and physical mapping of a type 1 diabetes susceptibility gene (IDDM12) to a 100-kb phagemid artificial chromosome clone containing D2S72-CTLA4-D2S105 on chromosome 2q33. *Diabetes 49*, 492–499.

McCanlies, E., O'Leary, L.A., Foley, T.P., Kramer, M.K., Burke, J.P., Libman, A., Swan, J.S., Steenkiste, A.R., McCarthy, B.J., Trucco, M., and Dorman, J.S. (1998). Hashimoto's thyroiditis and insulin-dependent diabetes mellitus: differences among individuals with and without abnormal thyroid function. *J. Clin. Endocrinol. Metab. 83*, 1548–1551.

McGovern, D.P., van Heel, D.A., Ahmad, T., and Jewell, D.P. (2001). NOD2 (CARD15), the first susceptibility gene for Crohn's disease. *Gut 49*, 752–754.

Melton, L.J., 3, Palumbo, P.J., and Chu, C.P. (1983). Incidence of diabetes mellitus by clinical type. *Diabet. Care* 6, 75–86.

Merriman, T.R., Cordell, H.J., Eaves, I.A., Danoy, P.A., Coraddu, F., Barber, R., Cucca, F., Broadley, S., Sawcer, S., Compston, A., Wordsworth, P., Shatford, J., Laval, S., Jirholt, J., Holmdahl, R., Theofilopoulos, A.N., Kono, D.H., Tuomilehto, J., Tuomilehto-Wolf, E., Buzzetti, R., Marrosu, M.G., Undlien, D.E., Ronningen, K.S., Ionesco-Tirgoviste, C., Shield, J.P., Pociot, F., Nerup, J., Jacob, C.O., Polychronakos, C., Bain, S.C., and Todd, J.A. (2001). Suggestive evidence for association of human chromosome 18q12–q21 and its orthologue on rat and mouse chromosome 18 with several autoimmune diseases. *Diabetes 50*, 184–194.

Mumford, C.J., Wood, N.W., Kellar-Wood, H., Thorpe, J.W., Miller, D.H., and Compston, D.A. (1994). The British Isles survey of multiple sclerosis in twins. *Neurology 44*, 11–15.

Nistico, L., Buzzetti, R., Pritchard, L.E., Van der Auwera, B., Giovannini, C., Bosi, E., Larrad, M.T., Rios, M.S., Chow, C.C., Cockram, C.S., Jacobs, K., Mijovic, C., Bain, S.C., Barnett, A.H., Vandewalle, C.L.,

Schuit, F., Gorus, F.K., Tosi, R., Pozzilli, P., and Todd, J.A. (1996). The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum. Mol. Genet. 5*, 1075–1080.

Nithiyananthan, R., Heward, J.M., Allahabadia, A., Franklyn, J.A., and Gough, S.C. (2002). Polymorphism of the CTLA-4 gene is associated with autoimmune hypothyroidism in the United Kingdom. *Thyroid 12*, 3–6.

O'Neill, L.A. (2004). How NOD-ing off leads to Crohn disease. *Nat. Immunol. 5*, 776–778.

Ogura, Y., Bonen, D.K., Inohara, N., Nicolase, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T,, Karaliuskas, R., and Duerr, R.H. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411, 603–606.

Paavonen, T. (1994). Hormonal regulation of immune responses. *Ann. Med. 26*, 255–258.

Park, Y.J., Chung, H.K., Park, D.J., Kim, W.B., Kim, S.W., Koh, J.J., and Cho, B.Y. (2000). Polymorphism in the promoter and exon 1 of the cytotoxic T lymphocyte antigen-4 gene associated with autoimmune thyroid disease in Koreans. *Thyroid 10*, 453–459.

Pauls, D.L., Zakarija, M., McKenzie, J.M., and Egeland, J.A. (1993). Complex segregation analysis of antibodies to thyroid peroxidase in Old Order Amish families. *Am. J. Med. Genet. 47*, 375–379.

Pearce, S.H., Vaidya, B., Imrie, H., Perros, P., Kelly, W.F., Toft, A.D., McCarthy, M.I., Young, E.T., and Kendall-Taylor, P. (1999). Further evidence for a susceptibility locus on chromosome 20q13.11 in families with dominant transmission of Graves disease [letter]. *Am. J. Hum. Genet. 65*, 1462–1465.

Phillips, D., McLachlan, S., Stephenson, A., Roberts, D., Moffitt, S., McDonald, D., Ad'Hiah, A., Stratton, A., Young, E., and Clark, F. (1990). Autosomal dominant transmission of autoantibodies to thyroglobulin and thyroid peroxidase. *J. Clin. Endocrinol. Metab. 70*, 742–746.

Phillips, D.I.W., Prentice, L., McLachlan, S.M., Upadhyaya, M., Lunt, P.W., and Rees Smith, B. (1991). Autosomal dominant inheritance of the tendency to develop thyroid autoantibodies. *Exp. Clin. Endocrinol. 97*, 170–172.

Pociot, F and McDermott, M.F. (2002). Genetics of type 1 diabetes mellitus. *Genes Immun. 3*, 235–249.

Puglise, A., Zeller, M., Fernandez, A., Jr., Zalcberg, L.J., Bartlett, R.J., Ricordi, C., Pietropaolo, M., Eisenbarth, G.S., Bennett, S.T., and Patel, D.D. (1997). The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat. Genet. 15*, 293–297.

Puxeddu, E. and Fagin, J.A. (2001). Genetic markers in thyroid neoplasia. *Endocrinol. Metab. Clin. North. Am. 30*, 493–513, x.

Redondo, M.J., Yu, L., Hawa, M., Mackenzie, T., Pyke, D.A., Eisenbarth, G.S., and Leslie, R.D. (2001). Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia 44*, 354–362.

Reiser, H. and Stadecker, M.J. (1996). Costimulatory B7 molecules in the pathogenesis of infectious and autoimmune diseases. *N. Engl. J. Med. 335*, 1369–1377.

Rigby, A.S., MacGregor, A.J., and Thomson, G. (1998). HLA haplotype sharing in rheumatoid arthritis sibships: risk estimates subdivided by proband genotype. *Genet. Epidemiol. 15*, 403–418.

Ringold, D.A., Nicoloff, J.T., Kesler, M., Davis, H., Hamilton, A., and Mack, T. (2002). Further evidence for a strong genetic influence on the development of autoimmune thyroid disease: The California twin study. *Thyroid 12*, 647–653.

Risch, N. (1987). Assessing the role of HLA-linked and unlinked determinants of disease. *Am. J. Hum. Genet. 40*, 1–14.

Risch, N. (1990). Linkage strategies for genetically complex traits. II. The power of affected relative pairs. *Am. J. Hum. Genet. 46*, 229–241.

Roitt, I.M., Campbell, P.N., and Doniach, D. (1958). The nature of the thyroid autoantibodies present in patients with Hashimoto's thyroiditis (lymphadenoid goitre). *Biochem. J. 69*, 248–254.

Sadovnick, A.D., Armstrong, H., Rice, G.P., Bulman, D., Hashimoto, L., Paty, D.W., Hashimoto, S.A., Warren, S., Hader, W., and Murray, T.J. (1993). A population-based study of multiple sclerosis in twins: update. *Ann. Neurol. 33*, 281–285.

Sakai, K, Shirasawa, S, Ishikawa, N, Ito, K, Tamai, H, Kuma, K, Akamizu, T, Tanimura, M, Furugaki, K, Yamamoto, K, and Sasazuki, T. (2001). Identification of susceptibility loci for autoimmune thyroid disease to 5q31–q33 and Hashimoto's thyroiditis to 8q23–q24 by multipoint affected sib-pair linkage analysis in Japanese. *Hum. Mol. Genet. 10*, 1379–1386.

Sale, M.M., Akamizu, T., Howard, T.D., Yokota, T., Nakao, K., Mori, T., Iwasaki, H., Rich, S.S., Jennings-Gee, J.E., Yamada, M., and Bowden, D.W. (1997). Association of autoimmune thyroid disease with a microsatellite marker for the thyrotropin receptor gene and CTLA-4 in a Japanese population. *Proc. Assoc. Am. Physicians 109*, 453–461.

Schaub, M., Issazadeh, S., Stadlbauer, T.H., Peach, R., Sayegh, M.H., and Khoury, S.J. (1999). Costimulatory signal blockade in murine relapsing experimental autoimmune encephalomyelitis. *J. Neuroimmunol. 96*, 158–166.

Seldin, M.F., Amos, C.I., Ward, R., and Gregersen, P.K. (1999). The genetics revolution and the assault on rheumatoid arthritis. *Arthritis Rheum. 42*, 1071–1079.

Smyth, D., Cooper, J.D., Collins, J.E., Heward, J.M., Franklyn, J.A., Howson, J.M., Vella, A., Nutland, S., Rance, H.E., Maier, L., Barratt, B.J., Guja, C., Ionescu-Tirgoviste, C., Savage, D.A., Dunger, D.B., Widmer, B., Strachan, D.P., Ring, S.M., Walker, N., Clayton, D.G., Twells, R.C., Gough, S.C., and Todd, J.A. (2004). Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. *Diabetes 53*, 3020–3023.

Stenszky, V., Kozma, L., Balazs, C., Rochlitz, S., Bear, J.C., and Farid, N.R. (1985). The genetics of Graves' disease: HLA and disease susceptibility. *J. Clin. Endocrinol. Metab. 61*, 735–740.

Stewart, J.J. (1998). The female X-inactivation mosaic in systemic lupus erythematosus. *Immunol. Today 19*, 352–357.

Sugimura, K, Taylor, K.D., Lin, Y.C., Hang, T., Wang, D., Tang, Y.M., Fischel-Ghodsian, N., Targan, S.R., Rotter, J.I., and Yang, H. (2003). A novel NOD2/CARD15 haplotype conferring risk for Crohn disease in Ashkenazi Jews. *Am. J. Hum. Genet. 72*, 509–518.

Tait, K.F., Marshall, T., Berman, J., Carr-Smith, J., Rowe, B., Todd, J.A., Bain, S.C., Barnett, A.H., and Gough, S.C. (2004). Clustering of autoimmune disease in parents of siblings from the Type 1 diabetes Warren repository. *Diabet. Med. 21*, 358–362.

Takara, M., Kouki, T., and DeGroot, L.J. (2003). CTLA-4 AT-repeat polymorphism reduces the inhibitory function of CTLA-4 in Graves' disease. *Thyroid 13*, 1083–1089.

Tamai, H., Ohsako, N., Takeno, K., Fukino, O., Takahashi, H., Kuma, K., Kumagai, L.F., and Nagataki, S. (1980). Changes in thyroid function in euthyroid subjects with family history of Graves' disease; a followup study of 69 patients. *J. Clin. Endocrinol. Metab. 51*, 1123–1128.

Tamai, H., Kumagai, L.F., and Nagataki, S. (1986). Immunogenetics of Graves' disease. In A.M. McGregor (ed.), *Immunology of Endocrine Diseases* (pp. 123–141). Lancaster: MTP Press.

Tomer, Y. (1997). Anti-thyroglobulin autoantibodies in autoimmune thyroid diseases: cross-reactive or pathogenic? *Clin. Immunol. Immunopathol. 82*, 3–11.

Tomer, Y (2001). Unraveling the genetic susceptibility to autoimmune thyroid diseases: CTLA-4 takes the stage. *Thyroid 11*, 167–169.

Tomer, Y. and Davies, T.F. (1993). Infection, thyroid disease and autoimmunity. *Endocr. Rev.* 14, 107–120.

Tomer, Y. and Davies, T.F. (2002). Genetic factors relating to the thyroid with emphasis on complex diseases. In: J.A.H. Waas and S.M. Shalet (eds), *Oxford Textbook of Endocrinology and Diabetes* (p358). Oxford: Oxford University Press.

Tomer, Y. and Davies, T.F. (2003). Searching for the autoimmune thyroid disease susceptibility genes: From gene mapping to gene function. Endocr. Rev. 24, 694–717.

Tomer, Y., Barbesino, G., Greenberg, D.A., Concepcion, E.S., and Davies, T.F. (1998). A new Graves disease-susceptibility locus maps to chromosome 20q11.2. *Am. J. Hum. Genet. 63*, 1749–1756.

Tomer, Y., Barbesino, G., Greenberg, D.A., Concepcion, E.S., and Davies, T.F. (1999). Mapping the major susceptibility loci for familial Graves' and Hashimoto's diseases: Evidence for genetic heterogeneity and gene interactions. *J. Clin. Endocrinol. Metab. 84*, 4656–4664.

Tomer, Y., Greenberg, D.A., Barbesino, G., Concepcion, E.S., and Davies, T.F. (2001). CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. *J. Clin. Endocrinol. Metab. 86*, 1687–1693.

Tomer, Y., Concepcion, E., and Greenberg, D.A. (2002a). A C/T single nucleotide polymorphism in the region of the CD40 gene is associated with Graves' disease. *Thyroid 12*, 1129–1135.

Tomer, Y., Greenberg, D.A., Concepcion, E., Ban, Y., and Davies, T.F. (2002b). Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. *J. Clin. Endocrinol. Metab. 87*, 404–407.

Tomer, Y., Ban, Y., Concepcion, E., Barbesino, G., Villanueva, R., Greenberg, D.A., and Davies, T.F. (2003). Common and unique susceptibility loci in Graves and Hashimoto diseases: Results of whole-genome screening in a data set of 102 multiplex families. *Am. J. Hum. Genet. 73*, 736–747.

Tomer, Y., Davies, T.F., and Greenberg, D.A. (2005). What is the contribution of a Kozak SNP in the CD40 gene to Graves' disease? *Clin. Endocrinol. 62*, 258.

Tsao, B.P. (2003). The genetics of human systemic lupus erythematosus. *Trends Immunol. 24*, 595–602.

Tsao, B.P., Grossman, J.M., Riemekasten, G., Strong, N., Kalsi, J., Wallace, D.J., Chen, C.J., Lau, C.S., Ginzler, E.M., Goldstein, R., Kalunian, K.C., Harley, J.B., Arnett, F.C., Hahn, B.H., and Cantor, R.M. (2002). Familiality and co-occurrence of clinical features of systemic lupus erythematosus. *Arthritis Rheum. 46*, 2678–2685.

Tunbridge, W.M.G., Evered, D.C., Hall, R., Appleton, D., Brewis, M., Clark, F., Evans, J.G., Young, E., Bird, T., and Smith, P.A. (1977). The spectrum of thyroid disease in a community: the Whickham survey. *Clin. Endocrinol. (Oxf.) 7*, 481–493.

Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., Rainbow, D.B., Hunter, K.M., Smith, A.N., Di Genova, G., Herr, M.H., Dahlman, I., Payne, F., Smyth, D., Lowe, C., Twells, R.C., Howlett, S., Healy, B., Nutland, S., Rance, H.E., Everett, V., Smink, L.J., Lam, A.C., Cordell, H.J., Walker, N.M., Bordin, C., Hulme, J., Motzo, C., Cucca, F., Hess, J.F., Metzker, M.L., Rogers, J., Gregory, S., Allahabadia, A., Nithiyananthan, R., Tuomilehto-Wolf, E., Tuomilehto, J., Bingley, P., Gillespie, K.M., Undlien, D.E., Ronningen, K.S., Guja, C., Ionescu-Tirgoviste, C., Savage, D.A., Maxwell, A.P., Carson, D.J., Patterson, C.C., Franklyn, J.A., Clayton, D.G., Peterson, L.B., Wicker, L.S., Todd, J.A., and Gough S.C. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423, 506–511.

Undlien, D.E, Bennet, S.T, Todd, J.A, Akselsen, H.E, Ikaheimo, I., Reijonen, H., Knip, M., Thorsby, E., and Ronningen, K.S. (1995). Insulin gene region-encoded susceptibility to IDDM maps upstream of the insulin gene. *Diabetes 44*, 620–625.

Vafiadis, P., Bennett, S.T., Todd, J.A., Nadeau, J., Grabs, R., Goodyer, C.G., Wickramasinghe, S., Colle, E., and Polychronakos, C. (1997). Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat. Genet. 15*, 289–292.

Vaidya, B., Imrie, H., Perros, P., Young, E.T., Kelly, W.F., Carr, D., Large, D.M., Toft, A.D., McCarthy, M.I., Kendall-Taylor, P., Pearce, S.H. (1999). The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. *Hum. Mol. Genet. 8*, 1195–1199.

Vaidya, B., Imrie, H., Geatch, D.R., Perros, P., Ball, S.G., Baylis, P.H., Carr, D., Hurel, S.J., James, R.A., Kelly, W.F., Kemp, E.H., Young, E.T., Weetman, A.P., Kendall-Taylor, P., and Pearce, S.H. (2000a). Association analysis of the cytotoxic T lymphocyte antigen-4 (CTLA-4) and autoimmune regulator-1 (AIRE-1) genes in sporadic autoimmune Addison's disease. *J. Clin. Endocrinol. Metab. 85*, 688–691.

Vaidya, B., Imrie, H., Perros, P., Young, E.T., Kelly, W.F., Carr, D., Large, D.M., Toft, A.D., Kendall-Taylor, P., and Pearce, S.H. (2000b). Evidence for a new Graves disease susceptibility locus at chromosome 18q21. *Am. J. Hum. Genet. 66*, 1710–1714.

Van der Auwera, B., Heimberg, H., Schrevens, A.F., Van Wayenberge, C., Flament, J., and Schuit, F.C. (1993). 5′ insulin gene polymorphism confers risks to IDDM independently of HLA class II susceptibility. *Diabetes 42*, 851–854.

Van Griensven, M., Bergijk, E.C., Baelde, J.J., De Heer, E., and Bruijn, J.A. (1997). Differential effects of sex hormones on autoantibody production and proteinuria in chronic graft-versus-host disease-induced experimental lupus nephritis. *Clin. Exp. Immunol. 107*, 254–260.

Velaga, M.R., Wilson, V., Jennings, C.E., Owen, C.J., Herington, S., Donaldson, P.T., Ball, S.G., James, R.A., Quinton, R., Perros, P., and Pearce, S.H. (2004). The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J. Clin. Endocrinol. Metab. 89*, 5862–5865.

Villanueva, R.B., Inzerillo, A.M., Tomer, Y., Barbesino, G., Meltzer, M., Concepcion, E.S., Greenberg, D.A., Maclaren, N., Sun, Z.S., Zhang, D.M., Tucci, S., and Davies, T.F. (2000). Limited genetic susceptibility to severe Graves' ophthalmopathy: No role for CTLA-4 and evidence for an environmental etiology. *Thyroid 10*, 791–798.

Villanueva, R., Greenberg, D.A., Davies, T.F., and Tomer, Y. (2003). Sibling recurrence risk in autoimmune thyroid disease. *Thyroid 13*, 761–764.

Vladutiu, A.O. and Rose, N.R. (1971). Autoimmune murine thyroiditis: relation to histocompatibiltiy (H-2) type. *Science 174*, 1137–1139.

Volpe, R. (1985). Autoimmune thyroid disease. In R. Volpe (ed.), *Autoimmunity and Endocrine Disease* (pp. 109–285). New York: Marcel Dekker.

Vyse T.J. and Todd J.A. (1996). Genetic analysis of autoimmune disease. *Cell 85*, 311–318.

Wagener, D.K., Sacks, J.M., LaPorte, R.E., and MacGregor, J.M. (1982). The Pittsburgh study of insulin-dependent diabetes mellitus. Risk for diabetes among relatives of IDDM. *Diabetes 31*, 136–144.

Warram, J.H., Krolewski, A.S., Gottlieb, M.S., and Kahn, C.R. (1984). Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N. Engl. J. Med. 311*, 149–152.

Warram, J.H., Martin, B.C., and Krolewski, A.S. (1991). Risk of IDDM in children of diabetic mothers decreases with increasing maternal age at pregnancy. *Diabetes 40*, 1679–1684.

Watanabe, T., Kitani, A., Murray, P.J., and Strober, W. (2004). NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat. Immunol. 5*, 800–808.

Wood, J.P., Pani, M.A., Bieda, K., Meyer, G., Usadel, K.H., and Badenhoop, K. (2002). A recently described polymorphism in the CD28 gene on chromosome 2q33 is not associated with susceptibility to type 1 diabetes. *Eur. J. Immunogenet. 29*, 347–349.

Xu, Y., Graves, P., Tomer, Y., and Davies, T. (2002). CTLA-4 and autoimmune thyroid disease: lack of influence of the A49G signal peptide polymorphism on functional recombinant human CTLA-4. *Cell. Immunol. 215*, 133–140.

Yanagawa, T., Hidaka, Y., Guimaraes, V., Soliman, M., and DeGroot, L.J. (1995). CTLA-4 gene polymorphism associated with Graves' disease in a caucasian population. *J. Clin. Endocrinol. Metab. 80*, 41–45.

Yanagawa, T., Taniyama, M., Enomoto, S., Gomi, K., Maruyama, H., Ban, Y., and Saruta, T. (1997). CTLA4 gene polymorphism confers susceptibility to Graves' disease in Japanese. *Thyroid 7*, 843–846.

Zaletel, K., Krhin, B., Gaberscek, S., Pirnat, E., and Hojker S. (2002). The influence of the exon 1 polymorphism of the cytotoxic T lymphocyte antigen 4 gene on thyroid antibody production in patients with newly diagnosed Graves' disease. *Thyroid 12*, 373–376.

Zhou, X., Tan, F.K., Wang, N., Xiong, M., Maghidman, S., Reveille, J.D., Milewicz, D.M., Chakraborty, R., and Arnett, F.C. (2003). Genome-wide association study for regions of systemic sclerosis susceptibility in a Choctaw Indian population with high disease prevalence. *Arthritis Rheum. 48*, 2585–2592.

CHAPTER

22

Infections, Immunity, and Autoimmunity

ROLF M. ZINKERNAGEL AND HANS HENGARTNER
Institute of Experimental Immunology, University Hospital, Zurich, Switzerland

In this chapter we review the evidence that some autoimmune diseases are triggered by infections. While infection may not explain the complete pathophysiology of autoimmunity, it could be important in a fair proportion of autoimmune diseases, and therefore, have a great impact on our thinking about prevention and treatment.

Long-term epidemiologic studies are essential to establish the role of infection in autoimmunity. While such studies will take many years to produce results, collections of sera or epidemiologic data, assembled for other reasons, may be usable for such analyses of causes of autoimmunity. We postulate that the encounter between infectious agents and maternal antibodies acquired systematically via the placenta or via milk in the gastrointestinal tract is of major importance in setting the overall host–infection balance for later in life. Therefore, changing hygiene practices, vaccinations, and other epidemiologic parameters may be useful in reversing the increasing predisposition to autoimmune diseases in the developed and developing world.

There is no doubt that the immune system functions to protect the species from infection (Mims, 1987). The specific, adaptive immune system is essentially complemented by a wide range of innate resistance mechanisms, which are probably responsible for greater than 95% of the general resistance against infections. While we usually identify the adaptive immune system with protection against cytopathic acute infections, there is a cost to the system biology of the immune system—as is always the case in biology—in the form of rare immunopathology and even rarer autoimmune disease. The beneficial aspects and strength of the system provide 50–95% protection, but the cost for effective immunity against infectious diseases, such as polio or measles, is that immunity against noncytopathic agents may sometimes produce more immunopathology than immunoprotection (Zinkernagel, 1997). Nevertheless, in the present coevolutionary equilibrium, this cost represents a small price compared to the overall benefit. Similarly, autoimmunity by autoantibodies or autoreactive T cells is part of the cost of the specific reactivity patterns of the immune system to react quickly and efficiently against acute cytopathic agents or toxins. Responses are optimal but not maximal; otherwise coevolutionary selection would not continue, since a genetically fixed system will always be selected against. Thus, immune protection by antibodies or T cells is essential for the survival of the species and immunopathology represents the cost of this. Immunopathology is most easily seen during immune responses of immunocompetent hosts against noncytopathic and persistent infectious agents. If we do not recognize these responses, or do not know about an infection, they eventually (in 1–20 years) cause an immunopathologic disease, often called an "autoimmune" disease (Zinkernagel,

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

1997). Of course, it is impossible to generalize to all autoimmune diseases but, if only a certain percentage of autoimmune diseases could be related to or even clearly associated with infections, then prevention by avoidance or vaccination, or early treatment of infection could at least be envisaged.

While such etiopathophysiology of autoimmunity has been envisaged for many years, concrete examples and results have been slow in coming. But then who would have thought 30 years ago that gastric ulcers are not caused by stress but usually by infection? Or who would have predicted that some neurologic and psychiatric diseases are associated with viral or bacterial infection, such as Borna disease virus or *Borrelia burgdorferi*. While occult infection explains only a few cases of autoimmunity today, many more may be so explained tomorrow.

IMPORTANT PARAMETERS OF THE IMMUNE SYSTEM

Role of Antigen Dose, Time Kinetics, and Distribution

T cells react against antigens that enter and remain in initially few of the secondary lymphoid organs for 3–10 days. T cells ignore antigens that stay strictly outside of secondary lymphatic organs. For example, papilloma virus infections in skin or some peripheral solid tumors are ignored by T cells as long as relevant antigens do not reach draining lymph nodes or spleen. If antigens, infectious or self-antigens, were always expressed in lymphoid organs and if all T cells were then induced either very early in the thymus or later in lymphatic organs, then all T cells would eventually be deleted or exhausted [reviewed by Zinkernagel (1997; 2002b)]. If antigen remains systemically available, new precursor T cells will be deleted immediately and nonreactivity against such antigens will be maintained. T cells engage in specific reactivity that, together with nonspecific resistance mechanisms, including complement, cytokines, and chemokines, will help to rid the host of infections. Antibody responses are called T-independent if the antigen can trigger B cells directly, e.g., by cross-linking surface receptors that are usually repetitive and identical on infectious agents, or alternatively, when antigen is associated with lipopolysacharides (LPS) or other Toll-like receptor (TLR) ligands (reviewed by [Pitha, 2004]).

Immune responses are regulated by various positive and negative regulation mechanisms. These mechanisms are, however, largely unclear and the word ‘regulation’ alone does not solve this problem. On the other hand, there is little argument that antigen itself, including its time of presence and its localization, is a key regulator of the immune response (Zinkernagel, 1997). If antigen is everywhere, including in all lymphatic organs, then no immune response usually ensues. If antigen is strictly extralymphatic, it is ignored. If antigen is temporarily within the lymphatic system, it usually triggers an immune response and will eventually be eliminated. This perhaps oversimplified view can be illustrated with mice that lack the spleen, or all secondary lymphatic organs except the spleen, or have neither spleen nor any secondary lymph nodes or Peyer patches (Zinkernagel, 1997; 2002b). In the absence of draining lymph nodes a subcutaneous infection with a noncytopathic virus renders the host a carrier within a few days. Even though the splenic response may be triggered initially, it will quickly collapse due to the generalized abundance of antigen. The absence of a spleen renders the host more susceptible to generalized infections with encapsulated bacteria. However, because the usual entry point of infections is via skin or mucosa, the initial trigger of the immune response is in the draining lymph nodes or Peyer patches, so that an asplenic host can do well immunologically.

Specificity of Humoral and Cell-Mediated Immunity

Classically T-cell-independent IgM is pentameric and short-lived (1 day), and therefore provides early and immediate protection. Because of its short half-life, IgM is usually nonpathogenic as an autoantibody. In contrast, a switch of T-cell responses to long-lived IgG (half life 7–21 days) usually requires T-cell help, at least initially. As IgG has a considerably longer half life, it provides longer protection against infections, and can be transferred from mother to offspring by Fc receptors in the placenta, or in some species via the gastrointestinal tract. A disadvantage, however, is that the long-lived IgG autoantibodies may cause disease. Therefore, a switch to IgG autoantibodies is efficiently prevented by the usual absence of autoreactive helper T cells.

Specificity of antibodies or T cells is best defined by the discriminatory capacity of the immune response against infections. Neutralizing antibodies that protect against polio virus serotype I will not protect against infections with polio virus II, and vice versa. Similarly cytotoxic T cells (CTLs) specific for hepatitis C virus (HCV) will not protect against hepatitis B virus (HBV) infections and vice versa. It is noteworthy that, in general, antibody or T-cell specificities are imnmunologically defined by either enzyme-linked immunosorbent assays (ELISAs) or T-cell proliferation assays, but not by protection. While these parameters usually correlate well with protection, often they do not.

Antibody specificities defined by ELISAs or coprecipitation assays detect lower avidity antibodies (10^{-5} to perhaps 10^{-7} M). In contrast, protective neutralizing antiviral antibodies require binding affinities of the order of 10^{-8}–10^{-10} M

to be effective. Unfortunately, for most other biologic activities of antibodies, e.g., those involved in autoimmune disease or in immune complex disease or other immunopathologies, these parameters are often unknown.

We have little knowledge about the binding qualities of the T-cell receptor. The peptide specificity of T cells is usually tested at 10^{-6}M, but probably is closer to 10^{-9}–10^{-10}M under physiologic conditions. These parameters have been much less well determined, if at all, for T-cell reactivity with bacterial toxins or parasitic antigens, such as those of the malaria parasite. The various immunoglobulin classes are distinctly regulated. As stated, IgM and IgG are essentially controlling systemically distributed antigens, whereas local mucosal IgA controls commensal flora and local IgE controls ecto-parasitic infections. While the rules of T-cell help for the IgM to IgG switch have been well analyzed, its conditions and parameters are much less clear for IgA or IgE responses.

Immunologic Memory

Immunologic memory has been reviewed repeatedly in the past 10 years (Mackay, 1993; Sprent, 1993; Ahmed and Gray, 1996; Zinkernagel, 2002a) and discussed over the past 100 years. It is defined in textbooks as earlier and higher responses in hosts that have had contact with the same antigen earlier in life. The important question is whether this quality actually corresponds to protection against disease after reinfection? While most immunologists would answer positively, the details of this question are largely unexplored. In fact, all contemporary vaccines that provide excellent protection are vaccines that induce neutralizing antibody responses, whereas all the vaccines that we do not possess yet will need to induce strong and lasting T-cell-mediated immunity. A major and important reason for this discrepancy is that, for coevolutionary reason, only antibodies can be transferred from mother to immunoincompetent newborns, whereas T cells cannot. Because of paternal major histocompatiblity complex (MHC) incompatibilities, a fetus is always a foreign graft within the mother; it is not rejected because of MHC-negative barriers and the immunoincompetence of the offspring. The adoptively acquired maternal antibodies against all relevant infections are therefore essential to help the offspring survive long enough for its own immune system to mature. Experiments with calves deprived of colostral milk, and with mutant mice that lack all antibodies, have clearly shown that without adoptively transferred maternal antibodies the newborn is highly susceptible to many infections (Brambell, 1970). In contrast, under the umbrella of maternal antibodies, any infection encountered by the infant within the first few weeks of life and even up to 1–2 years, will be attenuated by maternal protective antibodies; this process means that these infections simulate a vaccine (Zinkernagel, 2001) by naturally attenuating the infection which can then activate the immune system to produce a protective state.

T-CELL IMMUNOPATHOLOGY: AUTOIMMUNITY

It is remarkable how inert the immune system can be in general, particularly the T-cell component. The following experiments illustrate this convincingly. Transgenic mice that express the lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) under the rat insulin promoter (RIP) express the LCMV-GP only in insulin producing β cells (see Chapter 26). If these mice are left unchallenged, they do not develop diabetes. However, when infected with a low dose of LCMV, these same mice develop diabetes within 10 days and do not recover (Ohashi et al., 1991; Oldstone et al., 1991). Such experiments clearly show that a strictly extralymphatic self-antigen is ignored by the immune system. LCMV-GP-specific T cells are present but are not usually positively induced to become effector cells to cause pathology. However, this experiment also shows that such potentially autoreactive T cells are neither deleted nor negatively selected to establish tolerance. If a hitherto strictly extralymphatic antigen were later to enter secondary lymphatic organs for a few days, as would occur after infection of the transgenic mice with the original "parental" LCMV, CTLs are induced efficiently. These effector T cells will emigrate to the islet β cells and destroy them under the experimental conditions.

If these RIP-GP mice are infected with a recombinant vaccinia virus expressing the same LCMV-GP (VACC-GP_{LCMV}) they do not develop diabetes despite the fact that CTLs have been primed (Ohashi et al., 1993). In this model the difference in the severity of autoimmunity caused by LCMV infection versus VACC-GP_{LCMV} infection indicates that priming of CTL responses of itself does not automatically cause autoimmune disease. Infection with intact LCMV induces about a hundred to a thousand times more effector CTLs over 14 days compared with the VACC-GP_{LCMV} infection. However, if RIP-GP mice also express CD80 (B7.1) in their islet cells (Harlan et al., 1994) or, alternatively, a D^bGP_{33-41}-specific transgenic T-cell receptor (TCR), VAC-GP_{LCMV} does readily induce diabetes (Ehl et al., 1998). Thus, inertia depends on a physiologically low level of precursor T cells and the absence of so-called "second signals" in the extralymphatic periphery. The threshold for autoimmune disease via these induced T-cell responses is therefore very high.

Recently, we have extended these studies with RIP-GP mice to evaluate the conditions under which $CD8^+$ T cells specific for a transgenic β islet antigen can induce disease (Lang et al., 2005). In contrast to infection with a replicating LCMV, immunization with LCMV-GP peptide did not

induce disease despite induction of large numbers of autoreactive CTLs. When this immunization protocol was subsequently supplemented with the administration of TLR ligands, overt diabetes developed. This difference correlated with the interferonally triggered MHC class I upregulation in the target organ. Thus, not only induction of immunopathologic immune responses, but also "target quality" plays a major role in autoimmune disease development (Lang et al., 2005). Within this framework "epitope spreading" needs to be discussed (see Chapter 14). This concept postulates that destruction or decay of cells that are usually strictly extralymphatic may induce autoimmune T- or B-cell responses, leading to autoimmunity involving several of the autoantigens expressed in these cells. For example, virus-infected islet cells may release self-antigen in sufficient amounts and for a sufficient time period to induce T cells not only against viral but also against self-antigen. While such responses have been demonstrated under model situations to be theoretically possible, they have not been shown in human autoimmune disease directly, either for T or B cells. Autoimmune responses either during acute or (more likely) chronic infections may, however, trigger such slowly developing disease. This discussion of "epitope spreading," as later for "mimicry," is rendered so difficult and nonsatisfying because of the following general problems of analytic experimental investigations. Thus, in immunology, it is virtually impossible to definitely exclude postulated mechanisms, but we have to search for pathogenetic principles that are important and frequent. From this point of view, "epitope spreading," which triggers pathology, on the one hand may involve antigens that have been immunologically ignored so far, and on the other hand must be a rare process that usually only occurs after 25–30 years of age. Overall, therefore, epitope spread may represent one of the coevolutionary costs of immunity against acute or chronic infections (Gammon et al., 1991; Vanderlugt and Miller, 2002).

Recombinant vaccinia immunizations are considered usually to be quite strong, and are used successfully to immunize hosts against tumors. However, if the transgenic islet β cells were to be considered as very small tumors, the above example illustrates that, to reject such strictly extralymphatic small tumors, an enormous T-cell immune response would be required. This was formally illustrated by combining the RIP-GP model with a sarcoma expressing the same model tumor antigen LCMV-GP (Ochsenbein et al., 2001). A mouse transgenic for LCMV-GP in islet β cells carrying a tumor expressing the same GP tumor antigen was treated with various vaccines, including recombinant vaccinia virus or dendritic cells expressing the relevant GP-epitope, to assess whether the tumor could be rejected without causing autoimmune diabetes. By means of careful titrations, we found that within a factor of two or three there was no therapeutic window permitting tumor rejection (a wanted result) without induction of autoimmunity causing diabetes (an unwanted result) (Ludewig et al., 1998). From these experiments we can conclude that a high threshold for T-cell induction and activation is an important basis for prevention of autoimmunity or immunopathology against strictly extralymphatic self-antigens, organs or cells. The same threshold also applies to strictly extralymphatic tumors, unfortunately. Overall these examples illustrate why T-cell-mediated autoimmune disease is rare before the age of 25 (a necessity for survival of the human species), and why induction and maintenance of an antiperipheral solid tumor T-cell immune response is apparently very difficult.

B-CELL TOLERANCE AND AUTOANTIBODY (PLUS T-CELL)-DEPENDENT AUTOIMMUNE DISEASES

While T-cell tolerance is relatively clearly defined, at least for deletional forms where specific T cells are simply absent, this is not true to the same extent for B-cell tolerance; there is still a debate as to whether true deletional B-cell tolerance exists and to what level (Goodnow et al., 1989; Nemazee and Buerki, 1989; Bachmann et al., 1993) (see Chapters 11 and 13). This is particularly important because the turnover and regeneration of B cells is more rapid than that of T cells; probably about 10% of B cells are renewed each day (Goldsby et al., 2000). Also, the question of whether B cells are present or absent against intracellular antigens differs according to whether these self-antigens are extracellular and/or membrane expressed, and their densities. While autoantigen-specific B cells probably are present against intracellular antigens, and probably also against rare serum self-antigens, they may be deleted or at least be largely absent against highly expressed membrane antigens. This conclusion is based largely on experiments with antibody in mice carrying a transgene for lysozyme or an alloantigen (Goodnow et al., 1989; Nemazee and Buerki, 1989).

However, studies on mice expressing a virus glycoprotein probably at low density under various promoters have shown that they can promptly respond with a neutralizing antibody response against this particular new self-antigen (Zinkernagel et al., 1990; Bachmann et al., 1993). In the latter case, the IgM response is particularly interesting because it is T-cell independent. In this model, therefore, the question arose whether the B cells themselves were present or absent or whether the absence of T cells was responsible for the absent autoantibody response. Experiments showed that transgenic mice expressing the vesicular stomatis virus

glycoprotein (VSV-G) on host cells promptly responded first with a T-cell-independent IgM response, and then a subsequent T-cell-dependent switched IgG response when immunized with live or ultraviolet (UV)-inactivated VSV virus. In contrast, these same mice did not respond with a neutralizing antibody response if immunized with purified VSV-G in various adjuvants. From these findings we concluded that B cells did not respond to monomeric self-antigens, but did promptly react against repetitive polymeric corpuscular self-antigens, as would be expressed on a virus (Bachmann et al., 1993). The switch to IgG was classically T-cell help dependent. In this VSV-G example, T-cell help was negatively selected against, or deleted, for specific T cells recognizing VSV-G, but of course was still inducible against internal antigens present in replication-competent or UV-inactivated virus. Therefore, it was concluded, as many times previously, that autoantibody responses against self-antigens that usually are available systemically within the host as monomers or oligomers are very rare; this is because T-cell help is deleted, and because T-cell help is obligatory for induction of an IgM and IgG autoantibody response, particularly against monomeric antigen. If, however, self-antigens were somehow to be linked to infectious agents, e.g., on the surface of infected cells or cell fragments, then such autoantibodies could well be induced by appropriate cognate foreign antigen-specific T-cell help.

On the other hand, autoreactive B cells may be readily induced to produce IgM by a highly repetitive antigen. Interestingly, such polymeric highly repetitive self-structures are usually not accessible to B cells in the intact host. For example, acetylcholine receptors expressed in a highly polymeric form in the motor neuronal endplate (Vincent et al., 2003), or DNA or type 2 collagen (Holmdahl et al., 1990), which express repetitive polymeric structures, are not accessible to B cells under normal circumstances. If, however, cells decay or aggregated acetylcholine receptors are expressed without being covered by the endplate, as in thymomas, for example, such structures may become accessible to B cells. They may then induce an IgM response and even get switched to IgG, particularly under circumstances in which hitherto ignored self-antigens have induced helper T cells.

A number of additional experiments support the notion that B cells are probably not as solidly absent or tolerant as T cells. Classical experiments in the 1970s showed that polyclonal B-cell activators, such as lipopolysaccharides, can regularly induce autoantibodies in mice (Moller, 1975). Similarly, highly persistent viral infections in mice and humans have been shown to induce hypergammaglobulinemia, which often includes autoantibody. IgM autoantibodies may be induced, perhaps quite often, but without serious consequences because of the short half life of IgM (1–2 days). In contrast, once the T-cell-help-dependent switch to IgG has occurred, the prolonged half life of IgG may have serious pathologic consequences.

Another important aspect, the binding characteristics of autoantibodies necessary to cause pathology, is largely unknown. As discussed above, for neutralizing antibodies that protect against viral infections, it has been shown that rather high avidities/affinities are necessary, of the order of 10^8-$10^{10}\,M^{-1}$. Since autoantibodies are usually measured by ELISAs or immunohistology, then binding properties are probably, or so we speculate, rather low, of the order of 10^5-$10^{-7}\,M^{-1}$. Direct and well-characterized evidence for autoimmune disease caused by antibodies exists for myasthenia gravis (Vincent et al., 2003), and for a novel antibody-dependent rheumatoid arthritis model (Monach et al., 2004).

ROLE OF ANTIGEN AND INFECTION

As mentioned above, the structure of antigen plays a major role in inducing B-cell responses; thus, highly repetitive structures induce T-cell-help-independent IgM but not IgG responses, whereas monomeric antigens cannot induce a B-cell response independent of T-cell help.

An additional hypothesis, that of mimicry, has been discussed repeatedly during the past 20 years (Oldstone, 1998), and in many chapters in this volume. This concept proposes that a self-antigen resembles antigens expressed or induced by infectious agents. The streptococcal antigen that cross-reacts with heart antigen provides a classical example (see Chapter 62), but many others have been invoked. Of course, such mimicry could be an important cause of autoimmune disease and immunopathologies if the binding qualities and the direct disease-causing correlations were clearly established. However, mimicry is rarely, if ever, demonstrated in the form of pathology. Often only ELISAs, or other antibody-binding assays, including histology, are used as proof. As is often the case in biology, nothing is impossible and mimicry sounds like a good idea, but whether this process is really involved and can explain the role of infectious disease in triggering and causing autoimmune disease, or maintenance of disease, remains to be demonstrated directly. If it does occur, it must be rare or coevolution of host and parasites would not have been successful. What are the alternatives?

It is likely that infections destroy cells that hitherto were ignored immunologically, because self-antigens of those cells did not reach the lymphohemopoeitic system at sufficient levels to either induce an immune response or for the reactive lymphocytes to be completely deleted. Infections of such cells or organs could cause direct destruction of ignored cells to release antigens, or could induce a T-cell-mediated immune response that via immunopathology causes the destruction of these extralymphatic cells. The released

antigen will probably be picked up locally by macrophages and dendritic cells that eventually become trapped in local lymph nodes or the spleen. Antigen that so far had been ignored thus reaches secondary lymphatic organs, usually in association with MHC class II. Above a certain threshold it will induce CD4 T-cell-dependent help and antibody responses. Substantial periods of time and quantities of antigens are required to induce an immune response and to maintain it. The impact of this view on so-called "epitope spreading" is discussed above.

ROLE OF ORGANIZED LYMPHATIC TISSUES

Convincing experiments indicate that antigen must transiently reach secondary lymphatic organs to induce any immune response. If the reverse happens, i.e., secondary lymphatic tissue is exported or newly formed in extralymphatic peripheral organs, an autoimmune response may be maintained (Ludewig et al., 2000). When analyzed by immunohistology, it is striking that many autoantibody-dependent autoimmunities exhibit expression of secondary lymphatic tissues within the diseased target organ. A classical example is Hashimoto's thyroiditis (see Chapter 35), but type 1 diabetes, rheumatoid arthritis, Sjögren syndrome, and various other autoimmune diseases also follow this pattern. To reiterate, there is basically no induction of an immune (or autoimmune) response in the absence of secondary lymphatic organs. However, if secondary lymphatic organs do become organized within a site hitherto immunologically ignored, either islet cells, parotid gland or synovial tissue, the self-antigen expressed there can maintain an autoantibody response for as long as autoantigen remains available and the secondary lymphatic structure is intact. In fact, lymphatic structures may be seen during acute or chronic autoimmune diseases but once all parenchymal cells have been destroyed ("burned out"), these secondary lymphatic structures also disappear.

HORMONAL INFLUENCE

There is roughly a 5 : 1 ratio of females-to-males in most autoantibody-dependent autoimmune diseases. As previously argued, this may correlate with the evolutionary necessity for immune mothers to hand down protective antibodies at optimal levels to their immunoincompetent offspring. Several studies indicate that antibody levels in mice are regulated at least partially by female hormones. Whether this alone is a sufficient explanation of the female bias remains to be seen (see Chapter 25), but in experimental animals, including mice, the preponderance of autoimmune disease in females is striking. The question of hormonal influences on autoimmune diseases remains worthy of further evaluation.

CONCLUSION

Analysis of immunity against infections provides good insights into understanding immunologic tolerance and specificity. While immune protection is the desired result, there is no absolutely safe and entirely beneficial system available in biology: there are always costs. Therefore, there is a balance between protective and immunopathologic aspects of immunity, but this is definitely on the beneficial side for the host. However, the outcome of some ill-balanced situations, which may lead to immunopathology and in some cases to classical autoimmunity, reflects these coevolutionary costs. It is important to note that protection by the immune system guarantees survival of a sufficient number of hosts to assure procreation; for humans this means operationally 20–30 years of age. In fact, both immune reactivities causing autoimmunities and chronic immunopathologies usually appear in patients older than 30, but the inefficiencies of the immune system in dealing with strictly peripheral solid tumors also reflect this similar age barrier. If both the infectious agent and the characteristics of the immune response that is directed against peripheral infections are known, such as cytomegalovirus (CMV), HBV, leprosy, and human immunodeficiency virus (HIV), then an unfavorable balance is called immunopathology and, as a personal viewpoint, if we do not recognize infectious agents associated with a chronic or slowly progressing, often called idiopathic or degenerative, disease then we might tend to call the ensuing immunopathology an autoimmune disease. Even if only a few cases of this category of idiopathic, chronic degenerative autoimmune disease are caused by infections, directly or indirectly, and possibly with very long incubation times, it is worth our greatest efforts to define such relationships, because they are potentially amenable to beneficial actions, be it by avoidance or vaccination, or antibacterial, antiviral or antiparasitic treatments.

References

Ahmed, R. and Gray, D. (1996). Immunological memory and protective immunity: understanding their relation. *Science 272*, 54–60.

Bachmann, M.F., Rohrer, U.H., Kundig, T.M., Burki, K., Hengartner, H. and Zinkernagel, R.M. (1993). The influence of antigen organization on B cell responsiveness. *Science 262*, 1448–1451.

Brambell, R.W.R. (1970). *The Transmission of Immunity from Mother to Young*. Amsterdam: North Holland Publishing Corporation.

Ehl, S., Hombach, J., Aichele, P., Rulicke, T., Odermatt, R., Hengartner, H., Zinkernagel, R., and Pircher, H. (1998). Viral and bacterial infections interfere with peripheral tolerance induction and activate $CD8^+$ T cells to cause immunopathology. *J. Exp. Med. 187*, 763–774.

Gammon, G., Sercarz, E.E., and Benichou, G. (1991). The dominant self and the cryptic self: shaping the autoreactive T-cell repertoire. *Immunol. Today* 12, 193–195.

Goldsby, R.A., Kindt, T.J., and Osborne, B.A. (eds) (2000). *Kuby's Immunology*, 4th ed (ch. 11). New York: W. H. Freeman.

Goodnow, C.C., Crosbie, J., Jorgensen, H., Brink, R.A., and Basten, A. (1989). Induction of self-tolerance in mature peripheral B lymphocytes. *Nature 342*, 385–391.

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.

Holmdahl, R., Andersson, M., Goldschmidt, T.J., Gustafsson, K., Jansson, L., and Mo, J.A. (1990). Type II collagen autoimmunity in animals and provocations leading to arthritis. *Immunol. Rev. 118*, 193–232.

Lang, K.S., Recher, M., Junt, T., Navarini, A.A., Harris, N.L., Freigang, S., Odermatt, B., Conrad, C., Ittner, L.M., Bauer, S., Luther, S.A., Vematsu, S., Akira, S., Hengartner, H., and Zinkernagel, R.M. (2005). Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat. Med.* 11, 138–145.

Ludewig, B., Ochsenbein, A.G., Odermatt, B., Paulin, D., Hengartner, H., Zinkernagel, R.M. (2000). Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. *J. Exp. Med. 191*, 795–804.

Ludewig, B., Odermatt, B., Landermann, S., Hengartner, H., and Zinkernagel, R.M. (1998). Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. *J. Exp. Med. 188*, 1493–1501.

Mackay, C.R. (1993). Immunological memory. *Adv. Immunol. 53*, 217–265.

Mims, C.A. (1987). *Pathogenesis of Infectious Disease*. London, Academic Press.

Moller, G. (1975). One non-specific signal triggers B lymphocytes. *Transplant. Rev. 23*, 126–137.

Monach, P.A., Benoist, C., and Matthis, D. (2004). The role of antibodies in mouse models of rheumatoid arthritis, and relevance to human disease. *Adv. Immunol. 82*, 217–248.

Nemazee, D.A. and Buerki, K. (1989). Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature 337*, 562–566.

Ochsenbein, A.F., Sierro, S., Odermatt, B., Pericin, M., Karrer, U., Hermans, J., Hemmi, S., Hengartner, H., and Zinkernagel, R.M. (2001). Roles of tumour localization, second signals and cross priming in cytotoxic T cell induction. *Nature 411*, 1058–1064.

Ohashi, P.S., Oehen, S., Aichele, P., Pircher, H., Odermatt, B., Herrera, P., Higuchi, Y., Buerki, K., Hengartner, H., and Zinkernagel, R.M. (1993). Induction of diabetes is influenced by the infectious virus and local expression of MHC class I and tumor necrosis factor-alpha. *J. Immunol. 150*, 5185–5194.

Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., Malissen, R., Zinkernagel, R.M., and Hengartner, R.M. (1991). Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell 65*, 305–317.

Oldstone, M.B. (1998). Molecular mimicry and immune-mediated diseases. *FASEB J. 12*, 1255–1265.

Oldstone, M.B., Nerenberg, M., Southern, P., Price, J., and Lewicki, H. (1991). Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell 65*, 319–331.

Pitha, P.M. (2004). Unexpected similarities in cellular responses to bacterial and viral invasion. *Proc. Natl. Acad. Sci. USA 101*, 695–696.

Sprent, J. (1993). Lifespans of naive, memory and effector lymphocytes. *Curr. Opin. Immunol. 5*, 433–438.

Vanderlugt, C.L. and Miller, S.D. (2002). Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat. Rev. Immunol. 2*, 85–95.

Vincent, A., McConville, J., Farrugia, M.E., Bowen, J., Plested, P, Tang, T., Evoli, E., Matthews, I., Sims, G., Dalton, P, Jacobson, L., Polizzi, A., Blaes, F., Lang, B., Beeson, D., Willcox, N., Newson-Davis, J., and Hoch, W. (2003). Antibodies in myasthenia gravis and related disorders. *Ann. N. Y. Acad. Sci. 998*, 324–335.

Zinkernagel, R.M. (1997). The Nobel Lectures in Immunology. The Nobel Prize for Physiology or Medicine, 1996. Cellular immune recognition and the biological role of major transplantation antigens. *Scand. J. Immunol. 46*, 421–436.

Zinkernagel, R.M. (2001). Maternal antibodies, childhood infections and autoimmune diseases. *N. Engl. J. Med. 345*, 1331–1335.

Zinkernagel, R.M. (2002a). On differences between immunity and immunological memory. *Curr. Opin. Immunol. 14*, 523–536.

Zinkernagel, R.M. (2002b). Uncertainties–discrepancies in immunology. *Immunol. Rev. 185*, 103–125.

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 344*, 68–71.

CHAPTER

23

Noninfectious Environmental Agents and Autoimmunity

FREDERICK W. MILLER

Environmental Autoimmunity Group, Office of Clinical Research, National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, Maryland, USA

Although the mechanisms for the development of autoimmune diseases remain obscure, accumulating evidence suggests that these increasingly recognized disorders result from environmental exposures in genetically susceptible individuals (Luppi et al. 1995; Cooper et al., 1999; Miller 1999). Despite the great progress that has been made in understanding a number of major histocompatibility complex (MHC) and non-MHC genetic risk factors for autoimmune diseases (see Chapters 5, 20 and 21), relatively little information is available regarding the role of specific environmental agents in the development of these disorders. This is partly due to the lack of validated exposure biomarkers and environmental assessment tools; difficulties inherent in defining which of the many environmental exposures are related to disease; the little formal training in environmental medicine; the few resources dedicated to this area; and the lack of consensus approaches for the definition of environmentally-associated diseases. As a result, in most autoimmune conditions, the specific environmental triggers remain unknown.

Just as multiple genes are likely to be needed to induce autoimmune disease, multiple environmental exposures may need to occur in a particular sequence, or in tandem, to provoke the chronic immune activation that leads to autoimmunity. It is also possible that epigenetics, referring to modifications in gene expression that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure, may be influenced by environmental exposures. In this regard, many lessons might be learned from studies and registries of cancers which, like autoimmune diseases, are multifactorial disorders in which multiple genetic and environmental risk factors must interact in a correct sequence, with occasional long latencies, before development of disease (Sarasin, 2003). Thus, in some cases, a change induced by one exposure may be necessary before a subsequent exposure can have its effect. Alternatively, mixtures of exposures, including possible infectious and noninfectious agents, perhaps occurring during critical windows when persons may be more susceptible to them (i.e., in utero, in childhood, during puberty, pregnancy or lactation), may be necessary in order to overcome tolerance. Other general principles from cancer that might be relevant to autoimmunity include the pathogenetic heterogeneity of currently defined disorders, the likely low effect sizes from many environmental exposures, and the possible requirement for inducers, promoters, and sustainers of disease at different points in the pathogenetic process (Cooper et al., 2002). This is particularly borne out by investigations suggesting that autoantibodies precede the development of clinical disease by months to years and are antigen driven (Miller et al., 1990; Leslie et al., 2001; Arbuckle et al., 2003). This suggests that certain

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

environmental exposures may be necessary to overcome tolerance in genetically susceptible individuals and induce autoantibody formation, while other agents may be necessary to promote expression of clinical disease.

If environmental agents are defined as everything outside the genome, then the many exposures that have been suspected of being involved in the pathogenesis of autoimmunity may be divided into two general categories: infectious and noninfectious agents. This chapter will focus on the evidence for the role of noninfectious environmental agents in the pathogenesis of human autoimmune diseases, while infectious agents are reviewed in Chapter 22.

EVIDENCE FOR THE ROLE OF ENVIRONMENTAL AGENTS IN AUTOIMMUNE DISEASE

Evidence for the role of environmental agents in autoimmune disease comes from a variety of investigative approaches (Box 23.1). Although many of these methods are indirect, anecdotal, or may be only applicable to single patients, taken together these diverse findings strongly support the contention that most autoimmune diseases do have an important environmental component (Cooper et al., 1999).

One of the strongest lines of evidence for the role of the environment is that, for autoimmune diseases studied to date, there is generally less than 50% disease concordance in monozygotic twins (Leslie and Hawa, 1994; Cooper et al, 1999). While it has been suggested that this could be due to stochastic or other events, this consistent low level of disease concordance in genetically identical persons among all autoimmune disorders studied, argues against this. These findings also hint that, even if all the genetic risk factors for a given autoimmune disease could be fully identified, this would not allow for disease to be predicted with any greater accuracy than the flip of a coin without the incorporation of environmental or other factors.

Box 23.1
Lines of evidence supporting the role of environmental agents in the development of autoimmune disease

- Less than 50% disease concordance in monozygotic twins
- Strong temporal associations with some environmental exposures and disease onset
- Dechallenge (disease resolution or improvement after removal of the suspect agent)
- Rechallenge (disease recurrence or worsening after re-exposure to the suspect agent)
- Seasonality in birth dates and disease onset
- Geographic clustering in disease incidence or prevalence
- Changes in the prevalence or incidence of disease over time and when genetically similar cohorts move to different geographic locations
- Strong biologic plausibility from animal models
- Epidemiologic associations between particular exposures and certain diseases

For certain agents it can be relatively clear when a given exposure is inducing a disease in an individual patient. The definition of an environmental disease in an individual can be accomplished by identifying a new clinical disorder, which develops soon after a novel exposure, resolves when the exposure is removed (dechallenge), and then recurs after reintroduction of the same exposure (rechallenge). This approach is most easily applied in case of exposures to defined chemical entities, such as drugs, foods, and topical or inhaled toxicants. Unfortunately, many xenobiotics (compounds not naturally found in the body) cannot easily be removed from an organism after exposure, and therefore, for these agents this approach is not usually helpful. Exposures in this category include inhaled silica, vaccines, fat-soluble oils, and collagen or silicone implants.

The nonrandom distribution in time and space of some autoimmune illnesses also implies that nongenetic factors are important in disease development. Studies in these areas are preliminary, and sometimes have not been reproduced, leading critics to posit that referral or other biases might explain some of the findings. Nonetheless, intriguing investigations have suggested that certain autoimmune disorders have a seasonal onset (Samuelsson and Carstensen, 2003) or that there is a seasonal association with subsets of patients based on disease-specific autoantibodies (Leff et al., 1991). Furthermore, studies of type 1 diabetes have found significant associations with birth dates (Ursic-Bratina et al., 2001; Willis et al., 2002), implying that certain exposures at certain times of the year may alter the target tissues or immune systems of fetuses or neonates, resulting in later autoimmunity. While infectious agents are often presumed to be the source of such seasonal or geographic associations, the immune system, like other organ systems, appears to have cyclic or rhythmic patterns (Haus and Smolensky, 1999), possibly related to light exposure and mediated by melatonin or other neurohormones (Nelson and Drazen, 2000). Additionally, many occupational or other exposures are seasonal, including exposures to certain pesticides, chemicals in sunscreens, and some air or water pollutants, so it is possible that noninfectious agents could account for some of these data in ways that have not been accounted for. Geographic clustering or gradients in disease prevalence or

incidence have also been found for some autoimmune diseases. These investigations have primarily shown associations with latitude, suggesting a role of ultraviolet (UV) radiation in inducing disease, as may be the case for dermatomyositis (Okada et al., 2003); altering mortality, as may be the case in systemic lupus erythematosis (SLE) (Grant, 2004); or protecting from disease, as may be the case in multiple sclerosis and type 1 diabetes (Ponsonby et al., 2002).

Measurable increases or decreases in the incidence or prevalence of disease over time imply a nongenetic etiology given the slow rate of genetic change in a population. Although data are limited in this area—and several studies are possibly confounded as a result of improvements in the ability to diagnose some conditions over time—it appears that type 1 diabetes, multiple sclerosis, lupus, and myositis are becoming increasingly prevalent, while rheumatoid arthritis in adults and children may be decreasing in frequency in some populations (Oddis et al., 1990; Onkamo et al., 1999; Uramoto et al., 1999; Cooper and Stroehla, 2003). Studies of genetically similar populations living under different conditions are also illuminating. The incidences of both multiple sclerosis and type 1 diabetes have changed as members of a population move to new regions (Dahlquist, 1998; Noseworthy et al., 2000).

Epidemiologic studies linking specific exposures to autoimmunity are limited and usually consist of relatively small, often underpowered investigations, resulting in low effect sizes and large confidence limits. Much larger, well-designed, multicentered, and sometimes international studies, using appropriate controls and collecting adequate information to minimize confounding, will be needed to more fully define the specific environmental risk factors for disease.

IDENTIFYING AND DEFINING ENVIRONMENTALLY-ASSOCIATED AUTOIMMUNE DISEASES

One of the limitations in making progress toward identifying and defining environmental triggers for autoimmune disorders has been the lack of general consensus on the necessary and sufficient evidence needed to define an environmentally-associated condition. Medicolegal issues that surround many environmental exposures have further complicated this area. A group of experts in the field—who are members of the American College of Rheumatology Environmentally Associated Rheumatic Disease Study Group—have developed consensus on a general approach to overcome this problem (Miller et al., 2000). In their scheme, the overall process, from the identification of the first possible patient who develops a disease after an exposure, to the refinement of classification criteria for the disease, is divided into four stages (Table 23.1).

The first stage begins with the identification of a single case, or a series of cases, which are suspected of resulting from a given exposure. The consensus proposal is that these cases need to meet certain criteria to assure a minimum number of attribution elements are present (Miller et al., 2000). A total of at least four of the eight possible attribution elements need to be present, including at least three of five primary elements. The five primary elements are: temporal plausibility (taking into account the pharmacokinetics/pharmacodynamics of the agent, minimum induction time, and maximum latency); exclusion of other likely causes for the syndrome; dechallenge (if possible); rechallenge (if appropriate); and biologic plausibility. The additional three secondary elements are: identification of prior

TABLE 23.1 Proposed stages for identifying and defining environmentally-associated autoimmune diseases*

Stage	Description	Nomenclature (example)
Stage 1—Proposing the association	Case reports, defined by ascertainment criteria, propose a possible association of a specific clinical syndrome with a given exposure	Syndrome following exposure (rheumatoid arthritis following hepatitis B vaccination)
Stage 2—Testing the association	After a number of such cases are reported, surveillance criteria are proposed and epidemiologic and laboratory studies test that hypothesis	Cardinal signs, symptoms, and laboratory findings, but without the putative exposure (eosinophilia myalgia syndrome)
Stage 3—Defining criteria for the condition	If studies above are positive, then specific preliminary classification and other criteria are defined for that specific environmental disease	Exposure-associated disorder (L-tryptophan-associated eosinophilia myalgia syndrome)
Stage 4—Refining criteria for the condition	Criteria are reassessed and refined as additional data are obtained about the disease	Exposure-induced disorder (hydralazine-induced lupus-like disorder)

Modified from Miller et al. (2000).

reports of similar cases (analogy); identification of prior reports of nearly identical cases (specificity); and evidence for a dose–response effect. In addition to meeting these criteria, it is suggested that complete information regarding the history and examination, laboratory reports, core demographic data, family history, prior infections or physiology-altering exposures, all prior diagnoses, and the type/route/dose/duration of the exposure are detailed in the report.

The second stage involves testing the possible association. This should include epidemiologic studies, using surveillance criteria, to evaluate the relationship between a given exposure and a given syndrome. *In vitro*, *in vivo*, and animal studies should also assess the biologic effects of the agent and plausibility of the development of the syndrome. Other approaches, such as clinical, laboratory or genetic risk factor studies, could determine in case–control settings if cases of environmental disease differ from those with similar diseases without the exposure or from those similarly exposed who do not develop disease.

If data from the second stage result in convincing evidence that the assocation is real, then the third stage will be the development of preliminary criteria for that environmentally-associated disease. Classification criteria will define, with reasonable sensitivity and specificity, groups of patients with one disorder from closely related diseases. Expert committee consensus, mathematical algorithms or other approaches could develop these criteria. Symptom, sign, and laboratory criteria should be expressed in clinically sensible and practical formats with precise definitions of constituent elements. Diagnostic, prognostic, and outcome criteria, and disease activity and damage indexes should be considered when adequate data exist. The fourth stage repeats the same processes used in the third stage if new information is collected to warrant a redefinition of the disease.

Although this proposed staging structure has limitations, in that the decisions as to when to progress to the next stage remain somewhat subjective, it nonetheless provides an overall framework to plan for future studies, and it allows the classification of the current environmental agents into groups with different levels of evidence for their association with specific syndromes. Unfortunately, most environmental agents suspected of being associated with autoimmune diseases today remain in stages 1 or 2.

TABLE 23.2 Selected drugs associated in case reports or case series with autoimmune disorders*

Drug	Associated autoimmune disorder
α-Methyldopa	Lupus-like syndrome, hemolytic anemia, thrombocytopenia
Allopurinol	Lupus-like syndrome, vasculitis
Bleomycin	Scleroderma
Captopril	Lupus-like syndrome, vasculitis, membranous glomerulopathy
Chlorpromazine	Lupus-like syndrome, hemolytic anemia
D-penicillamine	Lupus-like syndrome, myositis, hypothyroidism, Goodpasture syndrome
Erythromycins	Lupus-like syndrome, myositis, hepatitis
Estrogens	Lupus-like syndrome, myositis
Gold salts	Lupus-like syndrome, membranous glomerulopathy
Halothane	Hepatitis
Hydralazines	Lupus-like syndrome, vasculitis, hepatitis
Interferon-α	Lupus-like syndrome, antiphospholipid syndrome, arthritis, hemolytic anemia, thrombocytopenia, hepatitis, hypothyroidism
Interferon-γ	Lupus-like syndrome, myositis, arthritis, hypothyroidism
Interleukin-2	Scleroderma, antiphospholipid syndrome, arthritis, hypothyroidism
Iodine	Hypothyroidism
Isoniazid	Lupus-like syndrome, arthritis, hepatitis, vasculitis, hypothyroidism
L-trytophan	Eosinophilia myalgia syndrome, scleroderma, myositis, neuropathies
Lipid-lowering agents	Lupus-like syndrome, myositis, hepatitis
Penicillins	Anemia, lupus-like syndrome, hepatitis
Phenytoin	Scleroderma, lupus-like syndrome, hepatitis, thrombocytopenia
Procainamide	Lupus-like syndrome
Propylthiouracil	Lupus-like syndrome, ANCA vasculitis, myositis
Quinidine	Lupus-like syndrome, arthritis, thrombocytopenia
Rifampicin	Thrombocytopenia, vasculitis
Sulfonamides	Lupus-like syndrome, vasculitis
Tetracyclines	Lupus-like syndrome, arthritis, vasculitis

*Reviewed in Love and Miller (1993), Mackay (1999), Bigazzi (1997), D'Cruz (2000), Hess (2002), and Liu and Kaplowitz (2002).

ANCA, antineutrophil cytoplasmic antibodies.

SPECIFIC NONINFECTIOUS AGENTS ASSOCIATED WITH AUTOIMMUNE DISEASES

Drugs

Of all the noninfectious environmental exposures associated with autoimmunity, drugs are the best recognized and most often reported (Table 23.2). This is partly due to their widespread use and careful monitoring by clinicians, the strict regulatory oversight and adverse event reporting systems in many countries, and the ease of collection of dechallenge and rechallenge evidence to make associations in individual patients. Despite the fact that several hundred drugs have been associated in case reports or case series with a number of immune-mediated or autoimmune illnesses, few of the publications have met the consensus criteria described

above to allow exclusion of confounding factors, and few have been studied in epidemiologic investigations. Chemical agents have been the most often reported drugs preceding the development of autoimmunity, although in recent years, with their increasing use, there has been more focus on biologic agents.

The most commonly recognized drug-related syndromes are lupus-like disorders (Hess, 1991). These are characterized by autoantibodies to histones and single-stranded DNA, rather than autoantibodies to double-stranded DNA as are found more often in idiopathic lupus. Drug-related SLE also differs from idiopathic SLE in having more frequent arthritis and less frequent neurologic and renal involvement, as well as having possibly different genetic risk factors. Virtually all of the 80 or more autoimmune diseases have been anecdotally reported to be associated with one or more drugs. In some of these cases drug-linked disorders differ from the idiopathic forms in clinical, serologic or genetic features, while in other cases they do not.

It is noteworthy that although some drugs appear to be associated with a number of autoimmune conditions (Table 23.2), they do not consistently share common structures, mechanisms of action, metabolites or other features, which would allow toxicity to be predicted or an understanding to be gained of the pathogenesis of these syndromes. The challenge is to begin to decipher the genetic and other risk factors that interact with exposure to these drugs and result in disease, so that disease can be predicted and prevented.

Occupational Exposures

Limited but growing epidemiologic and experimental data have linked a number of occupational exposures to autoimmune diseases (Table 23.3). The most studied of these include silica, solvents, pesticides, and UV radiation (Cooper et al., 2002). One of the first occupationally-associated rheumatic diseases identified was Caplan syndrome, which is seropositive rheumatoid arthritis associated with a specific form of pneumoconiosis that develops in anthracite coal miners and in persons exposed to silica and asbestos (Williams, 1991). The strongest occupational associations with autoimmune disease (i.e., relative risks of 3.0 and higher) have been documented in investigations of silica dust and rheumatoid arthritis, SLE, scleroderma, and anti-neutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis (Parks et al., 1999; Khuder et al., 2002). Weaker associations are seen, however, for solvent exposures (in systemic sclerosis (SSc), undifferentiated connective tissue disease, and multiple sclerosis) and for farming or pesticide exposures (in rheumatoid arthritis). Vinyl chloride has been linked to the development of a scleroderma-like disease characterized by skin thickening, Raynaud phenomenon, acroosteolysis, and pulmonary involvement. This observation, and the publication of several case reports, stimulated research into associations between SSc and other chlorinated solvents (e.g., trichloroethylene and trichloroethane) with varying results (Cooper et al., 2002).

TABLE 23.3 Occupational exposures associated with autoimmune diseases in epidemiologic studies*

Exposure	Disease	Summary of results
Silica	Systemic sclerosis	3-fold increased risk in four occupational cohort studies; mixed results in five population-based case-control studies
	Rheumatoid arthritis	3-fold (or higher) increased risk in five occupational cohort studies
	SLE	>10-fold increased risk in three occupational cohort studies
	ANCA vasculitis	4-fold increased risk in three case-control studies
Solvents	Systemic sclerosis	Mixed results, but some evidence of 2–3-fold increased risk with specific solvents (e.g., paint thinners and removers, trichloroethylene) and with "any" solvent
	Undifferentiated connective tissue disease	2-fold increased risk with paint thinners and removers, mineral spirits; 3-fold increased risk with specific solvent-related occupations
	Rheumatoid arthritis	Weak or no association with specific solvents, but 2-fold increased risk among spray painters and lacquer workers
	Multiple sclerosis	2–3-fold increased risk with solvent exposures in most studies
Pesticides	Rheumatoid arthritis	Weak associations (relative risks <2.0) seen with pesticide exposure and in farmers and horticultural workers
Ultraviolet radiation	Multiple sclerosis	Reduced risk (OR 0.74) of multiple sclerosis and mortality with increased occupational exposure to sunlight
	Dermatomyositis	Positive correlation of the proportion of dermatomyositis with global surface sunlight intensity at 15 referral centers on four continents

*Modified from Cooper et al. (2002).

ANCA, antineutrophil cytoplasmic antibodies; OR, odds ratio.

There are many difficulties in assessing the role of occupational exposures in disease. First, there are few biomarkers for such exposures and essentially none that allows an estimate of lifetime cumulative exposure. Secondly, there are few validated occupational exposure questionnaires and those that do exist are awkward and costly to apply. Thirdly, most studies in this area have been underpowered given the rarity of the diseases and the occupations of interest, resulting in imprecise risk estimates. Finally, there is possible confounding from multiple exposures that makes it difficult to ascertain whether different effects can be attributed to different chemicals. Thus, it may not be surprising that in some cases discrepancies exist in different investigations, making it difficult to assess the true overall risks of most occupational exposures.

Other Exposures

A wide variety of other exposures with greatly different properties have been proposed to be associated with autoimmune diseases (Table 23.4). The evidence supporting these proposed associations range from case reports to epidemiologic studies and many are speculative at this time. These are listed below under the major categories into which they may be classified.

Foods

Foods represent some of the better examples of environmental agents associated with autoimmune diseases. The best example is celiac disease (see Chapter 51), which is characterized by an immune response to ingested wheat gluten and related proteins of rye and barley, which leads to autoantibodies to transglutaminase and inflammation, villous atrophy, and crypt hyperplasia in the intestine (Alaedini and Green, 2005). These is also good evidence for gene–environment interaction in celiac disease with human leukocyte antigens DQ2 and DQ8 being the major known genetic risk factors. Celiac disease is one of the few medical conditions for which dietary intervention is the main treatment modality and a gluten-free diet markedly decreases symptoms in most individuals. Although there is less support for an autoimmune etiology of other food-associated inflammatory disorders, including the L-tryptophan-

TABLE 23.4 Other exposures proposed as possible risk factors for autoimmune diseases

Exposure	Disease	Comments (reference)
Foods (gluten)	Celiac disease	Celiac disease develops after ingestion of foods containing gluten and related proteins in some genetically susceptible persons (Alaedini and Green, 2005)
Cigarette smoking	Rheumatoid arthritis	Studies suggest relative risks of 1.5–3 with a greater effect in men and seropositive disease (Krishnan, 2003; Stolt et al., 2003)
	Autoimmune thyroid disease	Meta-analyses suggest 2–3 fold increased risks of Graves' and Hashimoto's (Vestergaard, 2002)
	Inflammatory bowel disease	Smoking increases risks for Crohn's disease but decreases risks for ulcerative colitis (Timmer, 2003)
Heavy metals	Multiple syndromes	"Pink disease" (acrodynia) and glomerulopathy from mercury toxicity; related syndromes with elements of autoimmunity from cadmium and gold salt toxicity; granulomatous pneumonitis from beryllium exposure; support for genetic risk factors in animal models (Bigazzi, 1994; Dally, 1997; Fontenot and Kotzin, 2003)
Microchimerism	SSc, SLE, primary biliary cirrhosis, autoimmune thyroid disease	Fetal cells detected in maternal blood or target tissue specimens years after pregnancy—not all findings have been reproduced, possibly due to different methodologies with different sensitivities to detect rare microchimeric cells (Sarkar and Miller, 2004)
	Juvenile myositis	Maternal cells detected in children who developed myositis (Artlett et al., 2000)
Vaccines	Multiple syndromes	Arthritis after rubella virus vaccines; thrombocytopenia after measles vaccines; Guillain–Barré syndrome after swine flu vaccine and tetanus; controversy remains over others (Wraith et al., 2003)
Collagen implants	Myositis	In one study, OR = 5.05; 95% CI = 2.31–9.59 for all forms of myositis (Cukier et al., 1993)
Silicone implants	Multiple syndromes	Most studies do not find associations with common autoimmune diseases (Janowsky et al., 2000; Tugwell et al., 2001); rare or atypical connective tissue disease and fibromyalgia remain inadequately studied (Brown et al., 1998; Brown 2002)
Stress	Graves' disease	Stressful life events in the 12 months preceding the diagnosis were significantly higher than controls (OR = 6.3, CI = 2.7–14.7 (Winsa et al., 1991); other diseases poorly studied

ANCA, antineutrophil cytoplasmic antibodies; CI, 95% confidence interval; OR, odds ratio.

associated eosinophilia myalgia syndrome (Sullivan et al., 1996) and the contaminated rapeseed oil-associated toxic oil syndrome (Gelpi et al., 2002), it is possible that some of these represent cases of food-associated autoimmunity.

Tobacco Smoke

Tobacco smoke has been associated epidemiologically with an increased risk of rheumatoid arthritis, autoimmune thyroid disease, and Crohn's disease in several studies, but inconsistent results were found in studies of smoking and systemic lupus erythematosus (SLE). Smoking may be associated with a reduced risk of ulcerative colitis, an inflammatory bowel disease, implying that the complex mix of chemicals in tobacco smoke may have different effects in different genetic backgrounds.

Heavy Metals

Exposures to heavy metals, including mercury, cadmium, gold salts, and beryllium have been associated with a variety of pathologic syndromes, some of which have features of autoimmunity. A recent study of communities in Amazonian Brazil with well-characterized exposures to mercury was the first to document immunologic changes, indicative of autoimmune dysfunction, in persons exposed to mercury (Silva et al., 2004). Additionally, many animal models have documented inflammatory, and sometimes highly specific, autoimmune responses to heavy metals, even at subtoxic doses, and which appear to differ in different genetic backgrounds (Bagenstose et al., 1999). The mechanisms for these many effects remain unclear, but possibilities include changing the response repertoire by direct and indirect means via changes in cytokine profiles, influencing expression of new antigens, new peptides, and/or antigen presentation by modifying the antigen-presenting complex (Rowley and Monestier, 2005).

Microchimerism

Microchimerism is the persistence of a low level of nonhost stem cells or their progeny in an individual. A possible role of microchimerism in the pathogenesis of some (systemic sclerosis, SLE, primary biliary cirrhosis, autoimmune thyroid diseases, and juvenile myositis), but not all, autoimmune diseases has been suggested by recent studies (Sarkar and Miller, 2004). The initial impetus to explore this exposure was that many of the diseases associated with microchimerism have features that are shared with graft-versus-host disease, suggesting a possible mechanism. Although an appealing hypothesis, controversy in the area continues due to the lack of reproducible studies and the lack of proof of the role of microchimeric cells in the pathogenesis of these disorders. That cells of multiple origins and different genetic backgrounds may combine to result in functional organ systems, both in mothers and their offspring, requires a re-evaluation of many current paradigms. It is possible that such chimeric mixtures play a role in autoimmunity, tissue repair, and other areas. Further research—using standardized, sensitive, and validated methods—is needed to address the many questions that the early findings in this field have raised.

Vaccines

Because vaccines are foreign proteins often injected with adjuvants into muscle to induce immune responses, it may not be surprising that immune-mediated adverse events have been reported after a wide variety of immunizations. Although a number of autoimmune diseases have been found to develop following vaccinations, in the US only a few have been deemed associated with disease by the Advisory Committee on Immunization Practices (1996) and are now compensated by the National Vaccine Injury Compensation Program (http://www.hrsa.gov/osp/vicp/INDEX.HTM). These include cases of arthritis after rubella virus vaccines, thrombocytopenic purpura after measles vaccines, and Guillain–Barré syndrome after swine flu or tetanus vaccines. There remains significant controversy over other illnesses possibly caused by immunizations, but most epidemiologic studies in this area have not shown significant associations (Wraith et al., 2003) (See Chapter 24).

Implants

Bovine collagen implants are biomaterials used for the correction of dermal contour deformities. The use of bovine collagen implants in patients with a personal history of autoimmune diseases is contraindicated by the manufacturer due to concerns that they may induce adverse immune responses, since anticollagen autoantibodies are present in some patients with connective tissue diseases. Few epidemiologic studies have been performed in this area, although one study has evaluated the development of myositis in nine patients following collagen implants (Cukier et al., 1993). Eight of the nine patients had a delayed-type hypersensitivity response at the test or treatment sites, and five of six patients tested were found to have increased serum antibodies to collagen. Compared with the general population, the incidence of dermatomyositis or polymyositis among collagen-treated patients was significantly increased.

Silicone implants remain some of the most controversial environmental agents proposed to be associated with connective tissue disorders. Studies in this area have been hampered by the extensive litigation involved in adverse events following silicone breast implants and the lack of adequate regulatory review prior to their initial use. Most studies have

not found associations with common autoimmune diseases (Janowsky et al., 2000; Tugwell et al., 2001); however, some investigators believe that rare or atypical connective tissue diseases and fibromyalgia remain inadequately studied (Brown et al., 1998; Brown, 2002). Of interest, women who develop myositis after silicone implants appear to be an immunogenetically distinct subgroup with allelic associations different from those seen in women who develop myositis without implants (O'Hanlon et al., 2004).

Stress

Anecdotes have been reported that stressful life events have preceded the development of many autoimmune diseases. A large population-based, case–control study of Graves' disease showed that patients had more negative life events in the 12 months preceding the diagnosis of Graves', and negative life-event scores were also significantly higher (Winsa et al., 1991). Other diseases have not been adequately studied. Although the mechanisms by which stress may play a role remain unclear, it has been hypothesized that, under certain conditions, stress hormones may boost immune responses through induction of tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-8, and by inhibiting TGF-β production (Elenkov and Chrousos, 1999). Therefore, conditions that are associated with significant changes in stress system activity may modulate the neuroendocrine–immune axis and perturb systemic cytokine balances, resulting in proinflammatory changes and disease induction.

POSSIBLE MECHANISMS BY WHICH ENVIRONMENTAL AGENTS MAY INDUCE AUTOIMMUNE DISEASES

Although the mechanisms for the development of autoimmune diseases associated with noninfectious agent exposures remain poorly understood, a variety of theories have been postulated to explain how xenobiotics may induce disease (Box 23.2). The wide range of these theories emphasizes the lack of understanding of mechanisms even for the most carefully defined environmentally-associated diseases. This also suggests that different pathogenic mechanisms are likely at work in different syndromes.

Whatever the specific mechanisms for the development of an autoimmune disease, it has been suggested that an overall framework should include the concept of heterogeneity within the currently defined diseases. A working hypothesis that addresses this issue has been termed the "elemental disorder hypothesis," which posits that each autoimmune disease as currently recognized contains many elemental disorders (Shamim and Miller, 2000). In this scenario, an elemental disorder is defined as a unique sign–symptom–laboratory complex (syndrome) that results from a distinct pathogenesis as a result of the interaction of the necessary and sufficient genetic and environmental risk factors (Figure 23.1). If this concept is true, elemental disorders are likely confounding most studies of disease by inducing "comparisons of apples and oranges." If identified, elemental disorders should greatly increase the homogeneity of populations under study and thus decrease the numbers of individuals needed for genetic, environmental, and therapeutic studies. In the future, elemental disorder identification could allow for the prevention of some illnesses by avoidance of environmental risk factors or via gene therapy to correct deleterious genetic risk factors.

Box 23.2
Possible mechanisms by which environmental agents may induce autoimmunity and promote and sustain autoimmune diseases (possible examples in parentheses)*

- Alteration of target tissue autoantigen structure (bystander drugs, heavy metals)
- Upregulation or altered locations of normally sequestered autoantigens (UV radiation)
- Cytotoxic, inhibitory or stimulatory effects on components of the immune system (interferons, interleukins)
- Molecular mimicry—structures shared between environmental agent and self (infectious agents)
- Other effects and combinations of the above

*Initiators of autoimmunity may differ in action from promoters and sustainers of autoimmune disease.

SUMMARY AND FUTURE DIRECTIONS

The multifactorial nature of autoimmune diseases has inhibited understanding of the mechanisms that initiate and sustain them. Autoimmune syndromes are believed to arise, however, from a complex and ill-understood interplay of predisposing genetic and environmental risk factors. While some progress is being made in defining the genetic risk factors, we are in our infancy in the identification of the environmental risk factors for autoimmune illnesses. Understanding the interactions of those elements that are necessary for disease development offers the promise of preventing or treating autoimmune diseases in novel ways. But before that can be accomplished, important questions remain to be answered. Which specific gene–environment interactions lead to which specific clinical syndromes? What are the pathogenic mechanisms involved? Is every autoimmune disease, as currently understood, actually composed

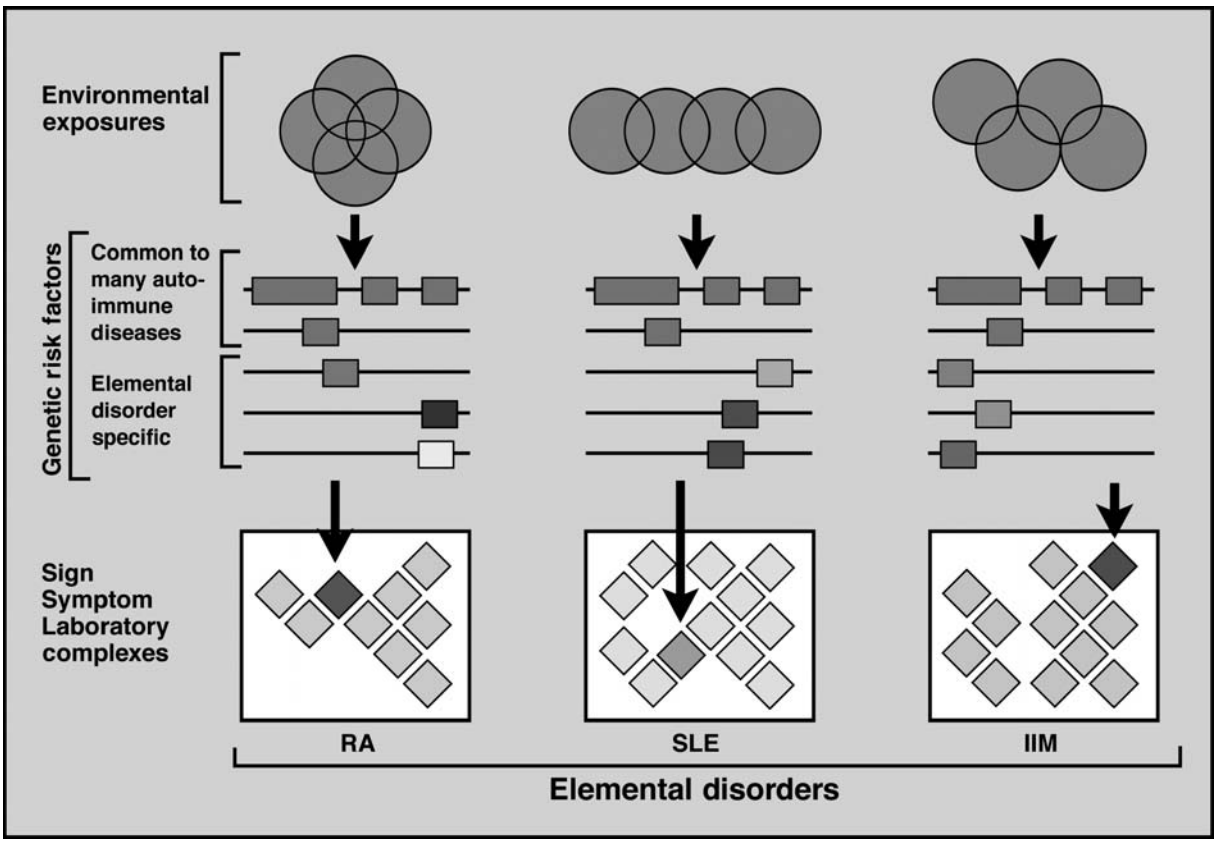


FIGURE 23.1 Possible mechanisms by which autoimmune diseases may arise—the elemental disorder hypothesis. In this view, every autoimmune disease, as currently classified, is a heterogeneous collection of clinical signs, symptoms, and laboratory findings, and is composed of many elemental disorders. Elemental disorders are defined as unique clusters of signs, symptoms, and laboratory features that result from a distinct pathology induced by the interaction of necessary and sufficient environmental and genetic risk factors for that elemental disorder. IIM, idiopathic inflammatory myopathies; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

of many subsets or "elemental disorders," each of which may be defined by a unique pathogenesis resulting from interactions of the necessary and sufficient risk factors? Can selected autoimmune diseases be better treated, cured, or even prevented through answers to some of the above questions?

We live in an increasingly complex sea of xenobiotics, which complicates exposure assessments. More than 80,000 chemicals are registered for use in commerce in the US, and an estimated 2000 new ones are introduced annually to be included in our foods, personal care products, drugs, household cleaners, and a host of industrial processes. The long-term effects of most of these chemicals on human health are unknown, yet we may be exposed to them during the manufacture, distribution, use, and disposal of products or as pollutants in our air, water, or soil. As a result, none of us knows the full range of environmental agents we are exposed to on a daily basis.

Many challenges have prevented further understanding of the environmental risk factors that might trigger autoimmune diseases in genetically susceptible individuals. These include: inadequate validated exposure assessment tools and bioassays; poor training in the evaluation of environmental exposures; the lack of population-based incidence, prevalence, demographic information and databases or repositories for most diseases; inadequate funding; and the lack of accepted and standardized approaches for defining the minimal criteria for an environmental disease. A number of coordinated initiatives may be useful in overcoming these obstacles and making more progress in the future (Box 23.3). Central to all these efforts are greater attention to and funding for understanding the essential environmental exposures that initiate, promote or sustain autoimmune disorders. Such investments are likely very cost-effective since they would have important clinical and financial implications for improving the public health.

Box 23.3
Possible approaches to enhance identification of environmental risk factors for autoimmune diseases

- Foster national and international collaborations and coordination to integrate existing and newly developed clinical databases, registries, specimen repositories, and other resources
- Develop and validate clinically useful standardized environmental exposure assessment tools
- Develop and validate standardized biomarkers for environmental exposures
- Increase support for well-designed, population-based, and case–control hypothesis-testing studies for suspected environmental agents
- Increase support for hypothesis-generating studies to identify new agents and syndromes
- Collect systematic descriptive epidemiologic data for all autoimmune diseases as a baseline for future comparisons
- Increase use of information technology and other novel approaches to enhance communications, coordinate efforts, and facilitate clinical studies
- Improve coordination between animal model and epidemiologic studies
- Develop novel mathematic, statistical, and bioinformatic approaches to enhance epidemiology studies
- Define gene–gene, gene–environment, and environment–environment interactions
- Establish an international coordinating committee to oversee and facilitate the above, encourage multidisciplinary research, and prepare for and respond to epidemics of environmentally-induced immune-mediated diseases

References

Advisory Committee on Immunization Practices (1996). Update: Vaccine side effects, adverse reactions, contraindications, and precautions. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep 45*, 1–35.

Alaedini, A. and Green, P.H. (2005). Narrative review: celiac disease: understanding a complex autoimmune disorder. *Ann. Intern. Med. 142*, 289–298.

Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., and Harley, J.B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med. 349*, 1526–1533.

Artlett, C.M., Ramos, R., Jiminez, S.A., Patterson, K., Miller, F.W., and Rider, L.G. (2000). Chimeric cells of maternal origin in juvenile idiopathic inflammatory myopathies. Childhood Myositis Heterogeneity Collaborative Group. *Lancet 356*, 2155–2156.

Bagenstose, L.M., Salgame, P., and Monestier, M.(1999). Murine mercury-induced autoimmunity: a model of chemically related autoimmunity in humans. *Immunol. Res. 20*, 67–78.

Bigazzi, P.E. (1994). Autoimmunity and heavy metals. *Lupus 3*, 449–453.

Bigazzi, P.E. (1997). Autoimmunity caused by xenobiotics. *Toxicology 119*, 1–21.

Brown, S.L. (2002). Epidemiology of silicone-gel breast implants. *Epidemiology 13* (Suppl.), S34–S39.

Brown, S.L., Langone, J.J., and Brinton, L.A. (1998). Silicone breast implants and autoimmune disease. *J. Am. Med. Womens Assoc. 53*, 21–4, 40.

Cooper, G.S., Miller, F.W., and Germolec, D.R. (2002). Occupational exposures and autoimmune diseases. *Int. Immunopharmacol. 2*, 303–313.

Cooper, G.S., Miller, F.W., and Pandey, J.P. (1999). The role of genetic factors in autoimmune disease: Implications for environmental research. *Environ. Health Perspect. 107* (Suppl), 693–700.

Cooper, G.S. and Stroehla, B.C. (2003). The epidemiology of autoimmune diseases. *Autoimmun. Rev. 2*, 119–125.

Cukier, J., Beauchamp, R.A., Spindler, J.S., Spindler, S., Lorenzo, C., and Trentham, D.E. (1993). Association between bovine collagen dermal implants and a dermatomyositis or a polymyositis-like syndrome. *Ann. Int. Med. 118*, 920–928.

D'Cruz, D. (2000). Autoimmune diseases associated with drugs, chemicals and environmental factors. *Toxicol. Lett.* 112–113, 421–432.

Dahlquist, G. (1998). The aetiology of type 1 diabetes: an epidemiological perspective. *Acta Paediatr 425* (Suppl), 5–10.

Dally, A. (1997). The rise and fall of pink disease. *Soc. Hist . Med. 10*, 291–304.

Elenkov, I.J. and Chrousos, G.P. (1999). Stress, cytokine patterns and susceptibility to disease. *Baillières Best Pract. Res. Clin. Endocrinol. Metab* 13, 583–595.

Fontenot, A.P. and Kotzin, B.L. (2003). Chronic beryllium disease: immune-mediated destruction with implications for organ-specific autoimmunity. *Tissue Antigens 62*, 449–458.

Gelpi, E., de la Paz, M.P., Terracini, B., Abaitua, I., de la Camara, A.G., Kilbourne, E.M., Lahoz, C., Nemery, B., Philen, R.M., Soldevilla, L., and Tarkowski, S. (2002). The Spanish toxic oil syndrome 20 years after its onset: a multidisciplinary review of scientific knowledge. *Environ. Health Perspect. 110*, 457–464.

Grant, W.B. (2004). Solar UV-B radiation is linked to the geographic variation of mortality from systemic lupus erythematosus in the USA. *Lupus* 13, 281–282.

Haus, E. and Smolensky, M.H. (1999). Biologic rhythms in the immune system. *Chronobiol. Int. 16*, 581–622.

Hess, E.V. (1991). Drug-related lupus. *Curr. Opin. Rheumatol. 3*, 809–814.

Hess, E.V. (2002). Environmental chemicals and autoimmune disease: cause and effect. *Toxicology* 181–182, 65–70.

Janowsky, E.C., Kupper, L.L., and Hulka, B.S. (2000). Meta-analyses of the relation between silicone breast implants and the risk of connective-tissue diseases. *N. Engl. J. Med. 342*, 781–790.

Khuder, S.A., Peshimam, A.Z., and Agraharam, S. (2002). Environmental risk factors for rheumatoid arthritis. *Rev. Environ. Health 17*, 307–315.

Krishnan, E. (2003). Smoking, gender and rheumatoid arthritis–epidemiological clues to etiology. Results from the behavioral risk factor surveillance system. *Joint Bone Spine 70*, 496–502.

Leff, R.L., Burgess, S.H., Miller, F.W., Love, L.A., Targoff, I.N., Dalakas, M.C., Joffe, M.M., and Plotz, P.H. (1991). Distinct seasonal patterns in the onset of adult idiopathic inflammatory myopathy in patients with anti-Jo-1 and anti-signal recognition particle autoantibodies. *Arthritis Rheum.* 34, 1391–1396.

Leslie, D., Lipsky, P., and Notkins, A.L. (2001). Autoantibodies as predictors of disease. *J. Clin. Invest.* 108, 1417–1422.

Leslie, R.D. and Hawa, M. (1994). Twin studies in auto-immune disease. *Acta Genet. Med. Gemellol. (Roma)* 43, 71–81.

Liu, Z.X. and Kaplowitz, N. (2002). Immune-mediated drug-induced liver disease. *Clin. Liver Dis.* 6, 755–774.

Love, L.A. and Miller, F.W. (1993). Noninfectious environmental agents associated with myopathies. *Curr. Opin. Rheumatol.* 5, 712–718.

Luppi, P., Rossiello, M.R., Faas, S., and Trucco, M. (1995). Genetic background and environment contribute synergistically to the onset of autoimmune diseases. *J. Mol. Med.* 73, 381–393.

Mackay, I.R. (1999). Immunological perspectives on chronic hepatitis: virus infection, autoimmunity and xenobiotics. *Hepatogastroenterology* 46, 3021–3033.

Miller, F.W. (1999). Genetics of environmentally-associated rheumatic disease. In L.D. Kaufman and J Varga (eds), *Rheumatic Diseases and the Environment* (pp. 33–45). London: Arnold.

Miller, F.W., Hess, E.V., Clauw, D.J., Hertzman, P.A., Pincus, T., Silver, R.M., Mayes, M.D., Varga, J., Medsger, T.A., Jr., and Love, L.A. (2000). Approaches for identifying and defining environmentally associated rheumatic disorders. *Arthritis Rheum. 43*, 243–249.

Miller, F.W., Waite, K.A., Biswas, T., and Plotz, P.H. (1990). The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. *Proc. Natl. Acad. Sci. USA.* 87, 9933–9937.

Nelson, R.J. and Drazen, D.L. (2000). Melatonin mediates seasonal changes in immune function. *Ann. N. Y. Acad. Sci.* 917, 404–415.

Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., and Weinshenker, B.G. (2000). Multiple sclerosis. *N. Engl. J. Med.* 343, 938–952.

O'Hanlon, T., Koneru, B., Bayat, E., Love, L., Targoff, I., Malley, J., Malley, K., and Miller, F. (2004). Immunogenetic differences between Caucasian women with and those without silicone implants in whom myositis develops. *Arthritis Rheum.* 50, 3646–3650.

Oddis, C.V., Conte, C.G., Steen, V.D., and Medsger, T.A., Jr. (1990). Incidence of polymyositis-dermatomyositis: a 20-year study of hospital diagnosed cases in Allegheny County, PA 1963–1982. *J. Rheumatol*. 17, 1329–1334.

Okada, S., Weatherhead, E., Targoff, I.N., Wesley, R., and Miller, F.W. (2003). Global surface ultraviolet radiation intensity may modulate the clinical and immunologic expression of autoimmune muscle disease. *Arthritis Rheum.* 48, 2285–2293.

Onkamo, P., Vaananen, S., Karvonen, M., and Tuomilehto, J. (1999). Worldwide increase in incidence of Type I diabetes—the analysis of the data on published incidence trends. *Diabetologia* 42, 1395–1403.

Parks, C.G., Conrad, K., and Cooper, G.S. (1999). Occupational exposure to crystalline silica and autoimmune disease. *Environ. Health Perspect. 107* (Suppl 5), 793–802.

Ponsonby, A.L., McMichael, A., and van der Mei, I (2002). Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology 181–182*, 71–78.

Rowley, B. and Monestier, M. (2005). Mechanisms of heavy metal-induced autoimmunity. *Mol. Immunol. 42*, 833–838.

Samuelsson, U. and Carstensen, J. (2003). Space-time clustering at birth and at diagnosis of type 1 diabetes mellitus in relation to early clinical manifestation. *J. Pediatr. Endocrinol. Metab.* 16, 859–867.

Sarasin, A. (2003). An overview of the mechanisms of mutagenesis and carcinogenesis. *Mutat. Res.* 544, 99–106.

Sarkar, K. and Miller, F.W. (2004). Possible roles and determinants of microchimerism in autoimmune and other disorders. *Autoimmun. Rev. 37*, 291–294.

Shamim, E.A. and Miller, F.W. (2000). Familial autoimmunity and the idiopathic inflammatory myopathies. *Curr. Rheumatol. Rep. 2*, 201–211.

Silva, I.A., Nyland, J.F., Gorman, A., Perisse, A., Ventura, A.M., Santos, E.C., de Souza, J.M., Burek, C.L., Rose, N.R., and Silbergeld, E.K. (2004). Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in amazon populations in Brazil: a cross-sectional study. *Environ. Health 3*, 11.

Stolt, P., Bengtsson, C., Nordmark, B., Lindblad, S., Lundberg, I., Klareskog, L., and Alfredsson, L. (2003). Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann. Rheum. Dis. 62*, 835–841.

Sullivan, E.A., Kamb, M.L., Jones, J.L., Meyer, P., Philen, R.M., Falk, H., and Sinks, T. (1996). The natural history of eosinophilia-myalgia syndrome in a tryptophan-exposed cohort in South Carolina. *Arch. Intern. Med. 156*, 973–979.

Timmer, A. (2003). Environmental influences on inflammatory bowel disease manifestations. Lessons from epidemiology. *Dig. Dis. 21*, 91–104.

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 rheumatologic disorders? A systematic review for a court-appointed national science panel. *Arthritis Rheum. 44*, 2477–2484.

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, 1950–1992. *Arthritis Rheum. 42*, 46–50.

Ursic-Bratina, N., Battelino, T., Krzisnik, C., Laron-Kenet, T., Ashkenazi, I., and Laron, Z. (2001). Seasonality of birth in children (0–14 years) with type 1 diabetes mellitus in Slovenia. *J. Pediatr. Endocrinol. Metab. 14*, 47–52.

Vestergaard, P. (2002). Smoking and thyroid disorders—a meta-analysis. *Eur. J. Endocrinol. 146*, 153–161.

Williams, W.J. (1991). Caplan's syndrome. *Br. J. Clin. Pract. 45*, 285–288.

Willis, J.A., Scott, R.S., Darlow, B.A., Lewy, H., Ashkenazi, I., and Laron, Z. (2002). Seasonality of birth and onset of clinical disease in children and adolescents (0–19 years) with type 1 diabetes mellitus in Canterbury, New Zealand. *J. Pediatr. Endocrinol. Metab. 15*, 645–647.

Winsa, B., Adami, H.O., Bergstrom, R., Gamstedt, A., Dahlberg, P.A., Adamson, U., Jansson, R., and Karlsson, A. (1991). Stressful life events and Graves' disease. *Lancet 338*, 1475–1479.

Wraith, D.C., Goldman, M., and Lambert, P.H. (2003). Vaccination and autoimmune disease: what is the evidence? *Lancet 362*, 1659–1666.

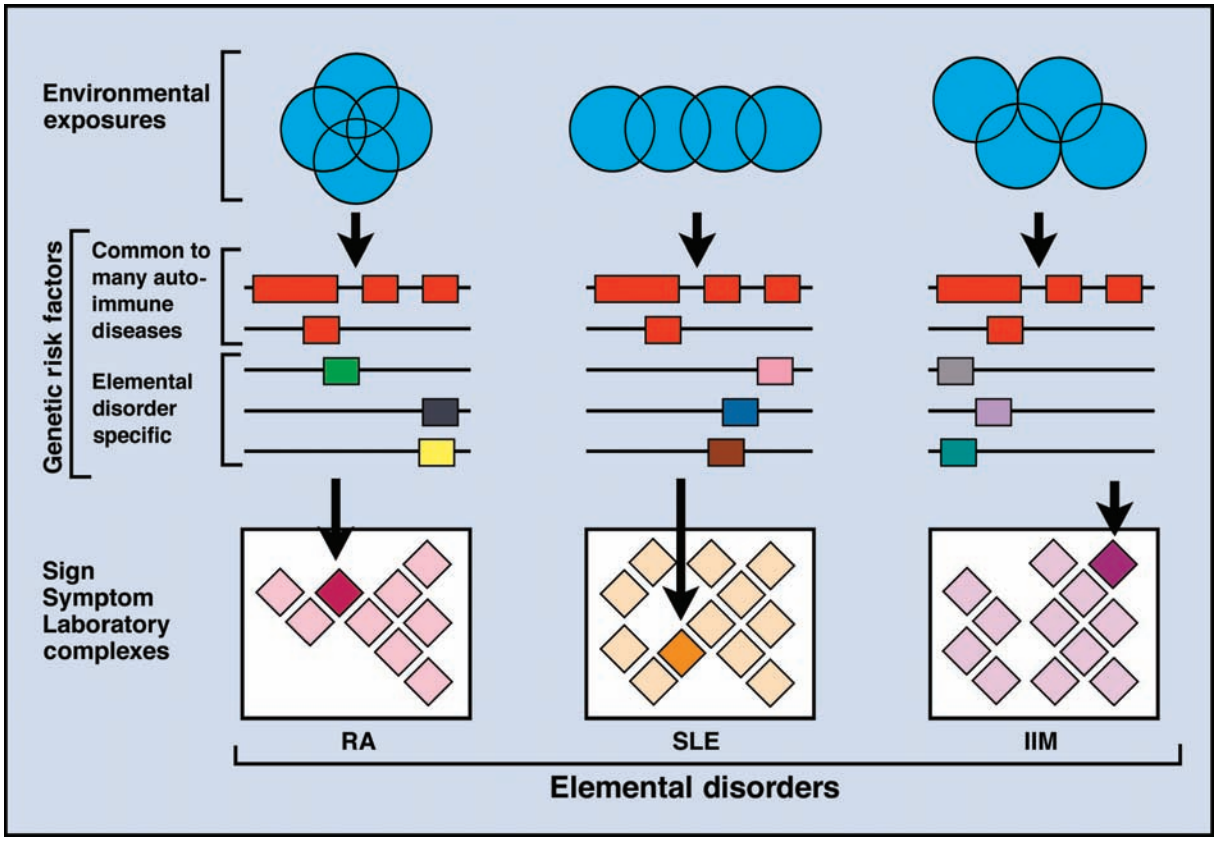


FIGURE 23.1 Possible mechanisms by which autoimmune diseases may arise—the elemental disorder hypothesis. In this view, every autoimmune disease, as currently classified, is a heterogeneous collection of clinical signs, symptoms, and laboratory findings, and is composed of many elemental disorders. Elemental disorders are defined as unique clusters of signs, symptoms, and laboratory features that result from a distinct pathology induced by the interaction of necessary and sufficient environmental and genetic risk factors for that elemental disorder. IIM, idiopathic inflammatory myopathies; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

CHAPTER

24

Vaccines and Autoimmunity

MOSHE TISHLER*

**Department of Medicine 'B', Asaf-Harofe Medical Center, Zrifin, Israel, Sackler Faculty of Medicine, Tel Aviv University*

YEHUDA SHOENFELD†

†Department of Medicine 'B' and Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel, Sackler Faculty of Medicine, Tel Aviv University, Incumbent of the Chair of Laura-Schwartz-Kipp for Research of Autoimmune Diseases, Tel Aviv University

Autoimmune diseases affect approximately 5–10% of the population in Europe and North America. Although the overall prevalence of most autoimmune diseases is quite low, the individual incidence of several such diseases has been steadily increasing in recent years, as documented for type 1 diabetes and multiple sclerosis (Wynn et al., 1990; EURODIAB ACE Study Group, 2000). Some autoimmune disorders occur in age groups for which vaccination programs are required. Therefore, the question of a connection between vaccination and autoimmune occurrences is of importance.

The history of vaccination started with Jenner's inoculation of humans with cow serum as a means of immunization against smallpox. Pasteur further developed the technique and added new vaccines. Vaccination is clearly one of medicine's greatest achievements and a cornerstone of disease prevention, significantly reducing mortality and morbidity. The vaccines contain either a live generally attenuated infectious agent, an inactivated infectious agent, or sometimes products obtained by genetic recombination. Usually vaccines contain not just the specific infectious antigens but also adjuvants such as aluminum salts or carrier proteins. The purpose of vaccination is to induce immunization, a process by which the immune system provides the host with protection against disease.

Nevertheless, since the mid-1990s, reports have accumulated on various side effects of vaccines that had not been observed previously, or perhaps not acknowledged (Cohen and Shoenfeld, 1996; Shoenfeld and Aharon-Maor, 2000; Shoenfeld et al., 2000). These effects include autoimmune expressions as well as actual autoimmune disease. The association between vaccination and autoimmunity has stimulated debate as to whether autoimmune diseases could be triggered by a vaccine. In this chapter the possibility of a link between infection, vaccination and autoimmunity will be discussed.

PATHOGENESIS OF VACCINE-RELATED AUTOIMMUNITY

The autoimmune process in the human being is a result of complex interactions between environmental factors, genetic background, hormones and immunologic defects, thus forming the "mosaic of autoimmunity" (Shoenfeld and

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Isenberg, 1989). Autoimmune responses are not usually followed by any clinical manifestation unless additional factors cause disease expression. A key role in the occurrence of immune responses is played by infectious agents. Today there is solid evidence that infections can either prevent, or more commonly initiate, autoimmune disorders (Bach, 2002; Shoenfeld and Rose, in press).

An infection can initiate autoimmune disease by antigen-specific or non–antigen-specific pathways: these mechanisms can operate in the host either singly or together. The disease itself will be manifested only in an individual who is genetically predisposed. A popular and appealing possibility for the initiation of autoimmunity by an infectious agent is molecular mimicry (Wucherpfennig, 2001). According to this process, antigenic determinants of the microorganisms are recognized by the host's immune system as similar to its own antigenic determinants (Albert and Inman, 1999) and, because of the structural resemblance, antibodies and autoreactive T cells not only destroy the invading pathogen but can react with host tissues as well. Molecular mimicry is exemplified by infections with microorganisms that express sugar structures, thus leading to antibody-mediated neuropathies (Willison and Yuki, 2002). This theory is well represented by the Guillain–Barré syndrome (GBS) in which approximately one third of cases are preceded by *Campylobacter jejuni* infection (Carpo et al., 1999) (see Chapter 47). This bacterium expresses a lipopolysaccharide molecule that mimics various gangliosides presented in high concentration in peripheral nerves. Thus, in sera taken from GBS patients in the acute phase of the disease, antibodies against gangliosides are present. Furthermore, the specificity of these antiganglioside antibodies is closely related to the nature of the infection preceding GBS. There is also a relationship between the specificity of the antibodies and the pattern of clinical features in these patients (Ang et al., 2004).

Molecular mimicry becomes more complex when T cells are involved. In order to serve as a molecular mimic for an autoreactive T cell, a microbial antigen must copy the shape of a self-antigenic epitope bound to an appropriate major histocompatibility complex (MHC) molecule. This type of T-cell mimicry has been documented in an experimental multiple sclerosis (MS) model in which a hepatitis-B virus polymerase peptide caused histologically proven autoimmune encephalitis in rabbits (Theophilopoulos et al., 2001). According to the mimicry hypothesis, it is possible that any microorganism that expresses an epitope which could serve as a molecular mimic for an autoantigen could induce autoimmune disease. In the model of the experimental antiphospholipid syndrome (APS), bacterial peptides homologous with beta-2 glycoprotein I (GPI) induced pathogenic anti beta-2 GPI antibodies along with APS manifestations in mice (Blank et al., 2002). Recent progress in our understanding of the interface between T-cell receptor (TCR) and peptide/MHC (pMHC) complexes has revealed the potential for degenerate recognition of numerous structurally similar pMHC complexes by each T lymphocyte. Experimental findings have shown that each TCR can recognize a broad range of epitopes, including peptides with totally different sequences (Hemmer et al., 2000). Due to this flexibility of the TCR moieties in contacting pMHC and the degeneracy of MHC binding motifs, very limited direct sequence identity between mimetic peptides is sufficient for T-cell cross-recognition. Thus, theoretically, the likelihood of T-cell cross-reactivity is very high, and induction of autoimmune disease by infection should not be an uncommon occurrence (Fourneau et al., 2004).

The second mechanism by which a microorganism might induce autoimmune disease involves bystander activation in a non-antigen-specific mechanism. In this case, microbial infection causes tissue damage, which leads to the release of self-antigens or stimulation of the innate immune response, thus activating the antigen presenting cell that expresses self-antigen (see Chapter 15). The engaging of Toll-like receptors on antigen-presenting cells by microbial molecules results in upregulation of MHC and costimulatory molecule expression, leading to secretion of various cytokines, local inflammation, and recruitment of additional autoreactive lymphocytes (Panoutsakopouilou et al., 2001). The high probability that microbial antigens can cross-react with self antigens together with the innate immune response to microorganisms increases the chance of provoking autoimmune disease following infection. The same mechanisms that act in infectious invasion of the host could apply equally to the host's response to vaccination (i.e., an antigen of a recombinant vaccine or of a live attenuated virus may resemble a host antigen and so trigger autoimmunity). Some of the molecules presumably involved in these processes are a group of proteins called "stress proteins" (Winfield, 1989). These substances, which have been well conserved among species in the course of evolution, are involved in reactions occurring in the body during stress. The stress proteins—one of which is the heat-shock protein 65 kDa of *Mycobacterium tuberculosis*, the organism that causes tuberculosis (TB)—have a remarkable structural similarity among different species: viruses, microbes, and mammals. As infections do not cause overt autoimmune disease in most individuals, the interplay of several factors is needed for the development of autoimmunity. Hence, patients with genetic predisposition for autoimmunity probably also have an increased risk for post-vaccination autoimmune disease. Theoretically, the more complex a vaccine and the more varied the array of its antigens, the more likely it would be to trigger an autoimmune response that may eventually turn into an established autoimmune disease.

AUTOIMMUNE DISEASES AND VACCINATION

Measurable laboratory signs of autoimmunity can appear following infection or vaccination. The medical literature is replete with claims and counter claims with respect to the risk of developing autoimmune disease as a consequence of vaccination (Wraith et al., 2003). The accumulating reports in the literature concerning neurologic manifestations, articular disease, and other autoimmune expressions following various vaccines have troubled both professional and lay communities, leading to the appointment of national investigational committees both in Europe and in the United States (Aharon-Maor and Shoenfeld, 2000). The wide spectrum of disorders claimed to be connected temporally and/or causally with vaccination, many of them autoimmune, are listed in Table 24.1.

GUILLAIN–BARRÉ SYNDROME

Guillain–Barré syndrome is a transient neurologic disorder characterized by a reflex motor paralysis, infrequent involvement of cranial nerves, with only mild sensory disturbances (see Chapter 47). Although its exact etiology is unclear, it is likely to be an immune-mediated disease affecting both the myelin sheath and the axons. Guillain–Barré syndrome is a monophasic disease with an acute course that can involve respiratory muscles, and spontaneous recovery occurs in most cases; the mortality in acute care units ranges between 3% and 7% (Hughes and Rees, 1997). Prior viral infections are often associated with GBS, particularly herpes virus, Epstein–Barr virus (EBV), cytomegalovirus, measles and others (Ropper, 1992). Furthermore, approximately 30% of GBS cases are preceded by *C. jejuni* infection. Autoantibodies to various myelin-associated glycoconjugates (gangliosides), which are a major constituent of the nerve cell membrane, have been detected (Willison and Yuki, 2002). The specificity of these antiganglioside antibodies is closely related to the nature of the preceding infection**.** The presence of microbe-specific antibodies and T cells with cross-reactivity to various nerve sheath components probably initiates a demyelinating process with shedding of peripheral-nerve autoantigens (Terryberry et al., 1995). Vaccines that contain live attenuated or killed microorganisms can presumably induce an autoimmune reaction. Although a temporal association of GBS with several vaccines has been reported, the firmest data are those for influenza vaccine.

The first report on the association of GBS with influenza vaccine was published in 1977 after a mass inoculation program in the United States in which 45 million adults received the "swine-flu" virus vaccine. While the estimated risk of GBS in the adult population was less than one case per 100,000 vaccinations, the occurrence of GBS in the "swine-flu" vaccinated population within 5 weeks after

TABLE 24.1 Status of autoimmune diseases reported after vaccination

Disease	Type of vaccine	References	Comments
Systemic lupus erythematosus	HBV, tetanus, anthrax	Tudela et al., 1992; Guiserix, 1996; Finierz et al., 1998	Scattered case reports
Rheumatoid arthritis	HBV, tetanus, typhoid/paratyphoid, MMR	Grasland & Vinceneux, 1994; Gross et al., 1995; Pope et al., 1998	Scattered case reports
Multiple sclerosis	HBV	Tourbah et al., 1999; Ascherio et al., 2001; Confavreux et al., 2001	Conflicting results
Reactive arthritis	BCG, typhoid, DPT, MMR, HBV, influenza	Benjamin et al., 1992; Grasland & Vinceneux, 1994; Gross et al., 1995; Mitchell et al., 1998; Aharon-Maor & Shoenfeld, 2000; Tishler, 2004	Scattered case reports
Polymyositis/dermatomyositis	BCG, smallpox, diphtheria, DPT	Aharon-Maor & Shoenfeld, 2000; Yanai-Berar et al., 2002	Scattered case reports
Polyarteritis nodosa	Influenza, pertussis, HBV	Bishop et al., 1966; LeHello et al., 1999; De Keyser et al., 2000; Yanai-Berar et al., 2002	Scattered case reports
Guillain–Barré syndrome	Influenza, polio, tetanus	Hugges et al., 1996; Shonberger et al., 1979; Lasky et al., 1998	Established connection (only for influenza)
Diabetes mellitus—type I	HiB	Classen & Classen, 1999b; Karvonen et al., 1999; Sipetic et al., 2003	Conflicting results
Autoimmune thrombocytopenia	MMR, HBV, influenza	Casoli et al., 1989; Miller et al., 2001	Scattered case reports

BCG, *Bacillus Calmette–Guerin*; DPT, Diphtheria-tetanus; HBV, Hepatitis B virus; HiB, *Hemophilus influenza* B; MMR, Measles-mumps-rubella.

vaccination was 7.6 times greater (Shonberger et al., 1979). Subsequent studies in the following years found only a slight increase in GBS incidence (1–2 additional cases per million vaccinated persons) after the 1990–1993 and 1993–1999 vaccination programs (Lasky et al., 1998). Later studies found that the influenza vaccines were safe and concluded that the risk of developing GBS after vaccination is lower than the risk for severe influenza and/or influenza-related complications (Chen et al., 2001). However, a recent study has again raised the issue of influenza vaccination and GBS. In this report, which analyzed the database of the Vaccine Adverse Events Reporting System (VAERS), GBS incidence following influenza vaccination was compared with that of an adult diphtheria-tetanus vaccine (DPT) control group (Geier et al., 2003). The authors found that in the years 1991–1999, a total of 382 cases of GBS were reported to the VAERS. The median onset of GBS following influenza vaccination was 12 days, and the relative risk for acute GBS was 4.3 compared with the adult DPT control group. They found that the maximal incidence of GBS following influenza vaccination occurred in the years 1993, 1996, and 1998, and that there were statistically significant variations in the incidence of GBS among influenza vaccines from different manufacturers. This important issue led the Center for Disease Control and Prevention to assess once again the trends of reports to VAERS of GBS following influenza vaccination in adults. The authors analyzed the reports to VAERS from 1990 to 2003 and conducted also an active follow-up from 1994 to verify the diagnosis of GBS. They concluded that that there is a possible causal association between GBS and influenza vaccine, although the annual reporting rate decreased four-fold from a high of 0.17 per 100,000 vaccinated in 1993–1994 to 0.04 in 2002–2003 (Haber et al. 2004). To date there are no studies that specifically address the question of the risk of relapse in GBS patients after influenza vaccination, although a threat of relapse has been reported following tetanus toxoid vaccination (Hugges et al., 1996).

These data suggest that, although in most cases influenza vaccination is safe, the chance still exists that in the future another serotype of influenza virus that becomes included in a vaccine might induce autoimmune expression as previously observed.

MULTIPLE SCLEROSIS

Multiple sclerosis is a disease characterized by a central nervous system (CNS) demyelinating process associated with clinical progressive paralysis (see Chapter 46). This disease is considered to be an autoimmune disorder, and autoantibodies specific for CNS myelin/oligodendrocyte glycoprotein have been identified (O'Connor et al., 2003). These autoantibodies were specifically bound to the disintegrating myelin around axons in lesions of acute MS. The possibility of an association between MS and hepatitis B virus (HBV) vaccination was first suggested in France following a report of 35 cases of demyelinating disease occurring within 8 weeks of recombinant hepatitis B vaccination (Tourbah et al., 1999). The neurologic manifestations were similar to those of MS. Inflammatory changes in the cerebrospinal fluid and specific lesions detected on magnetic resonance (MR) images were compatible with definite MS in 50% of affected patients. Most patients diagnosed as having MS were in a high-risk group, i.e. a preponderance of women, a higher presentation of HLA-DR2, and a family history for MS. Following this report, more than 600 cases of illness, most of them with MS-like symptoms, were identified in France from 1993 to 1999 after recombinant HBV vaccination. Subsequently, several large-scale studies tried to solve this enigma, but most did not have the statistical power to do so. However, two large-scale studies have shown no significant association between HBV vaccination and the occurrence of MS. The first study looked at patients included in the European Database for Multiple Sclerosis who had a relapse of their disease between 1993 and 1997; no increase in the specific short-term risk of relapse could be found in association with HBV vaccination (Confavreux et al., 2001). The second analyzed the Nurses' Health Study in the USA; which has followed up 121,700 nurses since 1976; in this study, the relative risk of MS associated with exposure to HBV vaccine at any time before the onset of the disease was 0.9 (Ascherio et al., 2001).

Due to the discrepancy in the results, two recent studies tried to solve this complicated issue. The first study was a nested case-control study using the General Practice Data Base in the United Kingdom, which used data of MS diagnosis recorded between 1993 and 2000. The analysis, which included 163 cases of MS and 1604 controls, revealed a 3.1-fold risk of MS following HBV vaccination while no increased risk of MS was recorded following tetanus or influenza vaccination (Hernan et al., 2004). Another study analyzing the VAERS found that HBV vaccination was associated with a number of serious conditions including arthritis, myelitis, systemic lupus erythematosus (SLE), GBS, glomerulonephritis, thrombocytopenia, and MS. In some of these cases a positive re-challenge was reported with an overall median onset of disease of 1 day (Geier and Geier, 2004). Nevertheless, it should be noted that some changes that had been made to the vaccines by the manufacturers in the mid 1990s could have changed their toxic effect. In contrast to several other viruses including Epstein–Barr virus that have been postulated to cause MS, HBV has not been discussed as causing MS, therefore, it is unclear how a vaccine that contains purified HbsAg, a portion of the HBV, could trigger an immunologic process leading to MS.

Although the data are conflicting, the large-scale studies support the safety of the vaccines against HBV used nowadays.

TYPE I DIABETES MELLITUS

The fact that the incidence of type I diabetes (T1D) is increasing rapidly in children in many developed countries throughout the world has raised a serious question about the role of vaccination as a factor contributing to this. A case-control study conducted in the mid-1980s in Sweden did not reveal any effect of vaccination against tuberculosis, pertussis, or rubella on the children's risk of developing diabetes mellitus (DM) (Blom et al., 1991). Another study that followed 100,000 Finnish children who received *Hemophilus influenza* type B (HiB) vaccine for 10 years also supported these findings (Karvonen et al., 1999). Results of this study showed no increased risk of diabetes when children who had received four doses of HiB vaccine at ages 3, 4, 6 and 18 months were compared with those receiving only one dose at the age of 2 years. On the other hand, there are several studies that show opposite results. A group from The Netherlands has shown that the timing of vaccination in childhood is an important factor in the development of T1D. While immunization in the first month of life was associated with a decline in the incidence of T1D, vaccination with HiB vaccine given at age 2 months or older can increase the risk (Classen and Classen, 1999a, 1999b). Another recently published study of a cohort of 4400 babies from southeast Sweden investigated the induction of diabetes-related autoantibodies following HiB vaccination, and found that HiB vaccination stimulated the immune system. Vaccination with HiB appeared to be a risk factor for the production of tyrosine phosphatase antibodies (1A-2A) and glutamic acid decarboxylase antibodies (GADA) which may be of importance under special circumstances when the pancreatic β cell-related immune response is activated by other mechanisms (Wahrberg et al., 2003). Conflicting results were found in a controlled study conducted in Belgrade between 1994 and 1997 wherein children with recent onset diabetes were compared with controls matched by age, sex, and place of residence. The results of this study showed that infections during the 6 months preceding the onset of T1D increased the risk of developing the disease 4.5-fold, while regular vaccination decreased that risk significantly (Sipetic et al., 2003). A recently published study evaluating the development of T1D in a cohort of all children born in Denmark from January 1, 1990, through December 31, 2000, supplies more solid evidence for this issue. The results of this comprehensive study disclosed that the rate ratio for T1D in children who received at least one dose of vaccine compared with unvaccinated children was 0.91. These results were also true for each of the specific vaccines evaluated in this study: HiB, DPT, inactivated polio virus, whole-cell pertussis, and measles, mumps, rubella (MMR) vaccines (Hviid et al., 2004).The advantage of this study is that it was able to evaluate the association of T1D and vaccination in a nationwide cohort with longitudinal information. The conclusion of the authors excludes any relation between childhood vaccination and T1D. Although there are conflicting results, most of the well-conducted studies do not support such a causal relationship.

VACCINATION AND AUTISM

Autism is a behavioral syndrome in children identified by neuropsychiatric manifestations, such as lack of imaginative and language skills; self-injurious behavior; and abnormal responses to people, sensations, or triggering. The etiology of this syndrome is obscure, yet an autoimmune hypothesis has been suggested as a possible pathogenic mechanism (Weizman et al., 1982). The findings of slightly higher titers of measles IgG in autistic children compared with normal controls, which were associated with brain autoantibodies, raised the hypothesis that the measles component of the MMR vaccine can be implicated in the etiology of this syndrome (Lee et al., 1998). In the late 1990s, it was suggested that there was a connection between gastrointestinal findings of lymphoid nodular hyperplasia, MMR vaccine and autism (Wakenfield et al., 1998), but other epidemiologic studies failed to demonstrate such a connection (Taylor et al., 1999). The lack of association between MMR vaccine and autism has lately received support from the authors of the original article connecting these two who retracted their initial interpretation of the data presented in 1998 (Murch et al., 2004).

To date we can be sure that no relationship exists between autism and MMR vaccine, and it can be used safely.

ARTHRITIS

Arthritis and the Measles, Mumps, Rubella Vaccines

Arthritis, a common complication of rubella virus infection in adults, occurs less often in children. In most cases, this type of arthritis resolves completely without any permanent sequelae; only in rare cases does a systemic chronic polyarthritis resembling rheumatoid arthritis (RA) develop. There is only one large study that assessed joint manifestations in children 6 weeks after MMR vaccination. In this study of 5000 children, there was an increased occurrence of joint symptoms—arthralgia and/or arthritis—in the immunized children, although the incidence of frank arthritis was lower than that after natural rubella infection (Benjamin et al., 1992). The risk of developing joint

manifestations was examined in 1998 in 283 women receiving rubella vaccination post partum in relation to HLA-DR (Mitchell et al., 1998). The conclusion of this study based on statistical analysis was that the risk for developing arthritis in this group of women was 1.9 times greater compared with placebo. The risk of arthropathy was also influenced by DR, since individuals with both DR1 and DR4 had an odds ratio of 8:1 of developing post-vaccination arthropathy.

Arthritis and Hepatitus B Virus Vaccine

Only a few cases of arthritis following HBV vaccination have been reported. One study describes three cases of seronegative arthritis showing a pattern resembling reactive arthritis with a slowly remitting course (Gross et al., 1995). An additional series of 11 otherwise healthy patients who developed polyarthritis after HBV inoculation is described (Pope et al., 1998). All of them developed persistent arthritis fulfilling the American College of Rheumatology criteria for RA, and five expressed the HLA-DR4 antigen.

Arthritis and *Bacillus Calmette–Guerin* Vaccine

Arthritis secondary to intravesical *Bacillus Calmette–Guerin* (BCG) administration for bladder cancer is a rare and poorly documented side effect. BCG-induced arthritis has been reported only as case reports, with no more than 30 cases published thus far. It is manifested by oligo- or polyarthritis affecting the large joints of the lower limbs, associated with low back pain. Approximately 50% of the cases are HLA-B27 positive and most cases resolve completely without sequelae with nonsteroidal anti-inflammatory drug (NSAID) treatment only (Tishler, 2004).

Arthritis and Other Vaccines

There are several case series of healthy people who had been immunized against a variety of microorganisms (tetanus, typhoid, paratyphoid, mumps, diphtheria, polio, smallpox) and developed either a transient elevation in rheumatoid factor or some form of arthritis (Grasland and Vinceneux, 1994).

The pattern of arthritis following various types of vaccination is not clear since the numbers involved are too small to reach a firm decision. There is no doubt that major investigations are required before conclusions can be drawn.

OTHER AUTOIMMUNE DISORDERS

A variety of additional autoimmune manifestations have been described in relation to vaccination. Systemic lupus erythematosus following HBV vaccination has been described in some case reports (Tudela et al., 1992; Guiserix, 1996; Finierz et al., 1998). Autoimmune thrombocytopenic purpura has been documented following either MMR vaccination or HBV vaccination (Finierz et al., 1998; Miller et al., 2001).There are also isolated reports linking influenza vaccine with both development and relapse of autoimmune thrombocytopenic purpura (Casoli and Tumiati, 1989). Systemic vasculitis resembling polyarthritis nodosa with gastrointestinal, respiratory and kidney involvement with histologic evidence of glomerulonephritis has been described in some case reports following vaccination against HBV (LeHello et al., 1999, De Keyser et al., 2000), pertussis (Bishop et al., 1966), and influenza (Yanai-Berar et al., 2002). Recently an immune form of perimyocarditis was documented in more than 20 patients following mass smallpox vaccination of USA military personnel before the second war in Iraq (Halsell et al., 2003). A recent report from Switzerland found a strong association between inactivated intranasal influenza vaccine used during the 2000–2001 season and Bell's palsy. In contrast to parenteral vaccine the intranasal vaccine increased the risk of Bell's palsy 19-fold (Mutsch et al., 2004).

VACCINATION OF PATIENTS WITH KNOWN AUTOIMMUNE DISEASE

The question of tolerance to vaccination by patients already suffering from autoimmune diseases was addressed in a number of studies over the years. Studies on influenza vaccination in patients with MS and in patients with SLE did not reveal any worsening of these diseases and the rates of systemic adverse effects were similar to those after vaccination of the general population (Williams et al., 1978; Salvetti et al., 1995). However, the immune response to influenza vaccine in SLE patients was lower than that seen in adults in the general population (Abu-Shaakra et al., 2002). A similar kind of reduced response to vaccination was observed in RA patients in whom influenza vaccination did not cause a flare in their disease (Cimmino et al., 1995). Furthermore, HBV vaccination in patients with RA was not associated with deterioration of any laboratory or clinical indicators of the disease and antibodies were produced by 68% of patients (Elkayam et al., 2002). It seems that vaccination in patients with autoimmune diseases is safe, and the infection against which the vaccine is given itself poses danger to these patients which is greater than that of vaccination.

EXPERIMENTAL MODELS

The first and, to our knowledge, only controlled experimental model to test the effects of vaccination on the

immune system was performed in dogs in 1999 (Hogenesch et al., 1999). The purpose of this study was to investigate the effect of vaccination in young dogs that had not been immunized previously. A marked increase in titers of autoantibodies directed against fibronectin and laminin was observed. Both these antibodies have been found in humans with a variety of autoimmune diseases: SLE, RA, and vasculitis. Although there was an elevation in autoantibody titers, no evidence of autoimmune disease was found in any of the immunized animals.

CONCLUSIONS

1. Vaccination has greatly improved the quality and length of life by reducing morbidity and mortality, especially in children but also in the adult population. The available epidemiologic data are reassuring although we must stay vigilant particularly with some of the newer vaccines to which there are no long-term post-marketing data.
2. There are clear data that vaccination can potentially induce autoimmune effects but, fortunately, most of them do not cause a frank autoimmune disease.
3. The only case in which there is almost a complete agreement that vaccination can cause an autoimmune disease is induction of GBS after influenza vaccination. Nevertheless, even in this case the incidence is low and has been declining in the last few years.
4. We are as yet unable to identify those who are prone to develop these complications. It is apparent that susceptibility to vaccine-induced autoimmunity would be determined by genetic predisposition, which further illustrates the concept of "the mosaic of autoimmunity."
5. Although surveillance systems in many countries watch carefully the data concerning the safety of vaccinations, investigations are required to reach firmer decisions, especially since it is surprising that simple subunit vaccines have been proposed as a cause for such autoimmune effects.

References

Abu-Shakra, M., Press, J., Varsano, N., Levy, V., Mendelson, E., Sukenik, S., and Bushila, D. (2002). Specific antibody response after influenza immunization in systemic lupus erythematosus. *J. Rheumatol. 29,* 2555–2557.

Aharon-Maor, A. and Shoenfeld, Y. (2000). The good, the bad and the ugly: vaccination. Isr. Med. Assoc. J. *2,* 225–227.

Albert, L.J. and Inman, R.D. (1999). Molecular mimicry and autoimmunity. *N. Engl. J. Med. 341,* 2068–2074.

Ang, C.W., Jacobs, B.C., and Laman, J.D. (2004). The Guillain-Barré syndrome: a true case of molecular mimicry. *Trends Immunol. 25,* 61–66.

Ascherio, A., Zhanj, S.M., Herman, M.A., Olek, M., Coplan, P., and Brodoricz, K. (2001). Hepatitis B vaccination and the risk of multiple sclerosis. *N. Engl. J. Med. 344,* 327–332.

Bach, J.F. (2002). The effect of infections on the susceptibility to autoimmune and allergic disorders. *N. Engl. J. Med. 347,* 911–920.

Benjamin, C.M., Chew, C.G., and Silman A.J. (1992). Joint and limb symptoms in children after immunization with measles, mumps and rubella vaccine. *Br. Med. J. 304,* 1075–1077.

Bishop, W.B., Carlton, R.F., and Fanders, L.L. (1966). Diffuse vasculitis and death after hyperimmunization with pertussis vaccine. *N. Engl. J. Med. 274,* 661–669.

Blank, M., Krause, I., Fridkin, M., Keller, N., Kopolovic, J., and Goldberg, J., (2002). Bacterial induction of autoantibodies to beta 2-glycoprotein I accounts for the infectious etiology of antiphospholipid syndrome. *J. Clin. Invest. 109,* 797–804.

Blom, L., Mystrom, L., and Dahlquist, G. (1991). The Swedish childhood diabetes study: vaccinations and infections as risk determinants for diabetes in childhood. *Diabetologia 34,* 176–181.

Carpo, M., Pedotti, R., Allaria, S., et al. (1999). Clinical presentation and outcome of Guillain-Barré and related syndromes in relation to antiganglioside antibodies. *J. Neurol. Sci. 168,* 78–84.

Casoli, P. and Tumiati, B. (1989). Acute idiopathic thrombocytopenic purpura after anti-influenza vaccination. *Medicina (Firenze). 9,* 417–418.

Chen, R.T., Pless, R., and Destefano, F. (2001). Epidemiology of autoimmune reactions induced by vaccination. *J. Autoimmun. 16,* 309–318.

Cimmino, M.A., Seriolo, B., and Accardo, S. (1995). Influenza vaccination in rheumatoid arthritis. *J. Rheumatol. 22,* 1802.

Classen, J.B. and Classen, D.C. (1999a). Association between type I diabetes and Hib vaccine: causal relation is likely. *Br. Med. J. 319,* 1133.

Classen, J.B. and Classen, D.C. (1999b). Immunization in the first month of life may explain decline in incidence of IDDM in The Netherlands. *Autoimmunity 31,* 43–45.

Cohen, A.D. and Shoenfeld Y. (1996). Vaccine induced autoimmunity. *J. Autoimmun. 9,* 699–703.

Confavreux, C., Suissa, S., Saddier, P., Bourdes, V., and Vukusic, S. (2001). Vaccination and the risk of relapse in multiple sclerosis. *N. Engl. J. Med. 344,* 319–326.

De Keyser, F., Naryaert, J.M., Hindryckx, P., et al. (2000). Immune mediated pathology following hepatitis B vaccination. Two cases of polyarteritis nodosa and one case of pityriasis rosea-like drug eruption. *Clin. Exp. Rheumatol. 18,* 81–85.

Elkayam, O., Yaron, M., and Caspi, D. (2002). Safety and efficacy of vaccination against hepatitis B in patients with rheumatoid arthritis. *Ann. Rheum. Dis. 61,* 623–625.

EURODIAB ACE Study Group (2000). Variation and trends in incidence of childhood diabetes in Europe. *Lancet 355,* 873–876.

Finierz, P., Lam-Kam-Sang, L.F., and Guiserix, J. (1998). Systemic lupus erythematosus and thrombocytopenic purpura in two members of the same family following hepatitis B vaccine. *Nephrol. Dial. Transplant. 13,* 2420–2421.

Fourneau, J.M., Bach, J.M., van Endert, P.M., and Bach J.F. (2004). The elusive case for a role of mimicry in autoimmune diseases. *Molec. Immunol. 40,* 1095–1102.

Geier, M.R. and Geier, D.A., and Zahalsky, A.C. (2003). Influenza vaccination and Guillain-Barré syndrome. *Clin. Immunol. 107,* 116–121.

Geier, M.R. and Geier, D.A. (2004). A case-series of adverse events, positive re-challenge of symptoms, and events in identical twins following hepatitis B vaccination: analysis of the Vaccine Adverse Event Reporting System (VAERS) database and literature review. *Clin Exp Rheumatol. 22,* 749–755.

Grasland, A. and Vinceneux, P. (1994). Rheumatic manifestations of vaccination. *Rheumatol. Eur. 28,* 25–27.

Gross, K., Combe, C., Kruger, K., and Schattenkkirschner, M. (1995). Arthritis after hepatitis B vaccination. *Scand. J. Rheumatol. 24,* 50–52.

Guiserix, J. (1996). Systemic lupus erythematosus following hepatitis B vaccine. *Nephron 74,* 441.

Haber, P., DeStefano, F., Angulo, F.J., Iskander, J., Shadomy, S.V., Weintrab, E., and Chen, R.T. (2004). Guillan-Barré syndrome following influenza vaccination. *J.A.M.A. 292,* 2478–2481.

Halsell, J.S., Riddle, J.P., Arwood, J.E., Gardner, P., Shape, R., Poland, G.A., Gray, G.C., Ostroff, S., Eckart, R.E., Hospenthal, D.R., Gibson, R.L., and Tomberg, D.N. (2003). Myopericarditis following smallpox vaccination among US military personnel. *J.A.M.A. 289,* 671–672.

Hemmer, B., Jacobsen, M., and Sommer, N. (2000). Degeneracy in T-cell antigen recognition: implication for the pathogenesis of autoimmune diseases. *Neuroimmunology 107,* 148–153.

Hernan, M.A., Jick, S.S., Olek, M.J., and Jick, H. (2004). Recombinant hepatitis B vaccine and the risk of multiple sclerosis. *Neurology 63,* 838–842.

Hogenesch, H., Azcona-Olivera, J., Scott-Montcrieff, C., et al. (1999). Vaccine induced autoimmunity in the dog. *Adv. Vet. Med. 41,* 733–747.

Hugges, R.A., Chondhary, P.P., Osborn, M., et al. (1996). Immunization and risk of relapse of Guillain-Barré syndrome or chronic inflammatory demyelinating polyradiculoneuropathy. *Muscle Nerve 19,* 1230–1231.

Hughes, R.A. and Rees, J.H. (1997). Clinical and epidemiological features of Guillain-Barré syndrome. *J. Infect. Dis. 176,* S92–S98.

Hviid, A., Stellfeld, M., Wohlfahrt, J., and Melbye, M. (2004). Childhood vaccination and type 1 diabetes. *N. Engl. J. Med. 350,* 1398–1404.

Karvonen, M., Cepaitis, Z., and Tuomilehto, J. (1999). Association between type I diabetes and Hib vaccination: birth cohort study. *Br. Med. J. 318,* 1169–1172.

Lasky, T., Terracciano, G.J., Magder, L., Koski, C.L., Ballesteros, M., Nash, D., Clark, S., Haber, P., Stolley, P.D., Slonberger L.B., and Chen, R.T. (1998). The Guillain-Barré syndrome and the 1992–1993 and 1993–1999 influenza vaccines. *N. Engl. J. Med. 339,* 1797–1802.

Lee, J.W., Melgaard, B., and Clementa, C.J. (1998). Autism, inflammatory bowel disease and MMR vaccine. *Lancet 355,* 905–909.

LeHello, C., Cohen, P., Bousser, M.G., and Lethellier, P. (1999). Suspected hepatitis B vaccination related vasculitis. *J. Rheumatol. 26,* 191–192.

Miller, E., Waight, P., Farrington, C.P., et al. (2001). Idiopathic thrombocytopenic purpura and MMR vaccine. *Arch. Dis. Child. 84,* 227–229.

Mitchell, L.A., Tingle, A.J., MacWilliam, L., et al. (1998). HLA-DR class II associations with rubella vaccine-induced joints manifestations. *J. Infect. Dis. 177,* 5–12.

Murch, S.H., Anthony, A., Casson, D.H., et al. (2004). Retraction of an interpretation. *Lancet 363,* 750.

Mutsch, M., Weigong, Z., Rhodes, P., et al. (2004). Use of inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *N. Engl. J. Med. 350,* 896–903.

OConnor, K.C., Chitnis, T., Griffen, D.E., et al. (2003). Myelin basic protein-reactive autoantibodies in the serum and cerebrospinal fluid of multiple sclerosis patients are characterized by low-affinity interactions. *J. Neuroimmunol. 136,* 140–148.

Panoutsakopouilou, V., Sanchirico, M.E., Huster, K.M., et al. (2001). Analysis of the relationship between viral infection and autoimmune disease. *Immunity 15,* 137–147.

Pope, J.E., Stevens, A., Howson, W., and Bell, D.A. (1998). The development of rheumatoid arthritis after recombinant hepatitis B vaccine. *J. Rheumatol. 25,* 1687–1693.

Ropper, A.H. (1992). The Guillain Barré syndrome. *N. Engl. J. Med. 326,* 1130–1136.

Salvetti, M., Pisani, A., Bastianello, S., Millefiorini, E., Buttinelli, C., and Pozzilli, C. (1995). Clinical and MRI assessment of disease activity in patients with multiple sclerosis after influenza vaccination. *J. Neurol. 242,* 143–146.

Shoenfeld, Y. and Aharon-Maor, A. (2000). Vaccination and autoimmunity —vaccinosis, a dangerous liaison? *J. Autoimmun. 14,* 1–10.

Shoenfeld, Y., Aharon-Maor, A., and Sherer, Y. (2000). Vaccination as an additional player in the mosaic of autoimmunity. *Clin. Exp. Rheumatol. 18,* 181–184.

Shoenfeld, Y. and Isenberg, D.A. (1989). The mosaic of autoimmunity. *Immunol. Today 10,* 123–126.

Shoenfeld, Y. and Rose, N. (eds.). *Infections and Autoimmunity.* Amsterdam: Elsevier. 2004.

Shonberger, L.B., Bregman, D.J., Sullivan-Balyni, J.Z., et al. (1979). Guillain-Barré syndrome following vaccination in the National Influenza Immunity Program, United States, 1976–1977. *Am. J. Epidemiol. 110,* 105–123.

Sipetic, S., Vlajinac, H., Kocev, N., and Radnanovic, S. (2003). The Belgrade childhood diabetes study: association of infections and vaccinations on diabetes in childhood. *Ann. Epidemiol. 13,* 645–651.

Taylor, B., Miller, E., Farrington, C.P., Petropoulos, M.C., Favot-Mayaud, I., Li, J., and Wright, P.A. (1999). Autism and measles, mumps and rubella vaccine: no epidemiological evidence for a causal association. *Lancet 353,* 2026–2029.

Terryberry, J., Sutjita, M., Shoenfeld, Y., et al. (1995). Myelin and microbe specific antibodies in Guillain-Barré syndrome. *J. Clin. Lab. Analysis 9,* 308–319.

Theophilopoulos, A.N., Dummer, W., and Kono, D.W. (2001). T-cell homeostasis and systemic autoimmunity. *J. Clin. Invest. 108,* 335–340.

Tishler, M. (2004). BCG vaccination. In Y. Shoenfeld and N. Rose (eds), *Infections and Autoimmunity.* (pp. 119–121). Amsterdam: Elsevier.

Tourbah, A., Gout, O., Liblau, R., Lyon-Caen, O., Bougniot, C., Iba-Zizen, M.T., and Cabanis, E.A. (1999). Encephalitis after hepatitis B vaccination: recurrent disseminated encephalitis or MS? *Neurology 53,* 396–401.

Tudela, P., Marti, S., and Bonal, J. (1992). Systemic lupus erythematosus and vaccination against hepatitis B. *Nephron 62,* 236.

Wahrberg, J., Fredriksson, J., Vaarala, Q., and Ludvigsson, J., for ABIS Study Group. (2003). Vaccination may induce diabetes related autoantibodies in one-year-old children. *Ann. N.Y. Acad. Sci. 1005,* 405–408.

Wakenfield, A.J., Murch, S.H., Anthony, A., et al. (1998). Ileal-lymphoid-nodular hyperplasia, non-specific colitis and pervasive developmental disorder in children. *Lancet 351,* 633–641.

Weizman, A., Weizman, R., Szekely, G.A., Wijsenbech, H., and Livni, E. (1982). Abnormal immune response to brain tissue antigaen in the syndrome of autism. *Am. J. Psychiat. 7,* 1462–1465.

Williams, G.W., Steinberg, A.D., and Reinertsen, J.L. (1978). Influenza vaccination in systemic lupus erythematosus. A double blind trial. *Ann. Intern. Med. 88,* 729–734.

Willison, H.J. and Yuki, N. (2002). Peripheral neuropathies and anti-glycolipids antibodies. *Brain 125,* 2591–2625.

Winfield, J.B. (1989). Stress proteins, arthritis and autoimmunity. *Arthritis Rheum 32,* 1497–1504.

Wraith, D.C., Goldman, M., and Lambert, P.H. (2003). Vaccination and autoimmune disease: what is the evidence? *Lancet 362,* 1659–1666.

Wucherpfennig, K.W. (2001). Mechanisms for the induction of autoimmunity by infectious agents. *J. Clin. Invest. 108,* 1097–1104.

Wynn, D.R., Rodriguez, M., OFallon, W.M., and Kurland, L.T. (1990). A reappraisal of the epidemiology of multiple sclerosis in Olmsted County, Minnesota. *Neurology 40,* 780–786.

Yanai-Berar, N., Ben-Izhak, O., Gree, J., and Nakhoul, F. (2002). Influenza vaccination induced leukocytoclastic vasculitis and pauci-immune crescentic glomerulonephritis. *Clin. Nephrol. 58,* 220–223.

CHAPTER

25

Sex, Pregnancy, and Autoimmunity

ROBERT G. LAHITA

Professor of Medicine Mount Sinai Medical School, New York, N. Y. Chairman of Medicine and Vice President, Liberty Health System, Jersey City Medical Center

Sex hormones have a major role in the manifestation of autoimmune disease. Sex steroids affect the phenotype of the individual and have a major role to play in biological development of the brain and the immune system. In the early part of this chapter, the role of sex steroids on immune function in general will be detailed, and the role such hormones have on the expression of human disease will be discussed. The role of sex steroids in the pregnant patient with autoimmune disease will then be explored.

SEX HORMONES AND AUTOIMMUNITY

Androgen Effects on Immune Function

Androgens have many effects on immune function (Grossman, 1984). They are considered immunosuppressive hormones because of *in vitro* observations of their effects on normal lymphocytes and because of their effects on the disease manifestations of inbred autoimmune mice (Ahmed et al., 1985).

Androgens are more suppressive than estrogens—and this may be one reason why females have an increased incidence of autoimmunity.

Testosterone suppresses DNA antibody production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus (SLE) (Blank et al., 1990) and also inhibits pokeweed mitogen stimulation of B-cell differentiation (Sthoeger et al., 1988). This suppression involves downregulation of interleukin 6 (IL-6) and the inhibition of B-cell activity. Graft rejection in rodents is delayed by the injection of testosterone (Weinstein and Berkovich, 1981). Resistance to certain viral infections can be reduced but in some cases enhanced when androgens are given at certain doses (Yohn 1973; Jungers et al., 1982; Lahita et al., 1987). A consistent effect of androgens in the chicken is immunosuppression through retardation of the function and development of the bursa of fabricius (Meyer et al 1959; Hirota et al., 1976; Hirota, Suzuki and Bito, 1980; Verheul et al., 1986). Androgens accelerate the proliferation and differentiation of pluripotent stem cells from bone marrow into compartments of cells that include lymphoid elements (Dunkel et al., 1985).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Importantly, receptors for estrogen and dihydrotestosterone, a 5α-reduced metabolite of testosterone, exist in the thymus (Sholitan et al., 1980; Grossman et al., 1982; Grossman et al., 1989) and these receptors are functional and present on thymocytes (Grossman, 1989). Lymphocytes, specifically $CD8^+$ T cells, have androgen receptors as well, but the data are inconsistent (Raveche et al., 1980; Cohen et al., 1983). Androgens inhibit B- and T-cell maturation, reduce B-cell synthesis of immunoglobulins, and suppress the phytohemagglutinin-induced blast transformation of lymphocytes (Weinstein and Isakov, 1983; Dunkel et al., 1985). These male hormones are also implicated as modifiers of regulatory genes that influence the function of structural genes (Lubahn et al., 1988), and this may be their principal mode of action. Inhibition of thymocyte function depends on androgen receptors on thymic stroma, and such receptors need to be expressed for atrophy to occur. Androgens induce apoptosis via the tumor necrosis factor receptor (TNFR). Of interest, guinea pig mammary epithelial major histocompatibility (MHC) antigens are increased in number through the effects of estrogens and prolactin, and these MHC antigens are decreased by testosterone (Klareskog et al., 1980). Yet, despite these findings, male and female mice have equivalent numbers of MHC antigens. The wide range of effects that testosterone has on immune function might be due to variable androgen sensitivity of certain cell groups and the *in vivo* conversion of androgens to estrogens through well-recognized pathways.

In the presence of estrogen, the thymus gland does not atrophy. On the contrary, it enlarges, whereas androgens cause apoptosis of thymic cells (Olsen et al., 2001). Some investigators have described an age-dependent change in thymic maturation in the presence of estrogens (Forsberg, 1996).

Androgen Metabolism and Autoimmune Disease

Studies of the levels and metabolism of androgens in certain autoimmune diseases are of interest (Folomeev et al., 1992; Lahita 1992). The occasional male with autoimmune disease defies explanation when sex hormone levels are examined. For example, SLE in males is not merely the result of too little androgen, although there are recent data that might support this; thus men with hypogonadism and testicular dysfunction have a higher incidence of autoimmune disease (Lahita, unpublished observation). An early reason proposed for SLE in the male was the result of too little androgen and too much estrogen (Inman et al., 1982; Bhardwaj et al., 1989). Actual studies have shown no hormonal differences between men with and men without SLE (Chang et al., 1999). Men with SLE have normal hormone levels and estrogen: androgen ratios are not altered in the majority of patients. None of the hormonal data from the studies of patients with SLE explain the large number of females who predominate with the disease. Females with SLE differ from males in the overall metabolism of androgens, but it is unclear whether these findings are reason enough for the female predilection for SLE. The oxidation of testosterone at C17 in females with SLE is increased in comparison with males, who have both normal oxidations of testosterone and normal plasma androgen levels (Lahita et al., 1983). Females with active SLE had decreased plasma levels of androgen (Folomeev et al., 1992; Lahita, 1992) and these values were from patients who did not take corticosteroid, an independent reason for low levels (Jungers et al., 1980). This observation is also found in Klinefelter patients (XXY) but not in men (Lahita and Bradlow, 1987). Low plasma androgens in women with SLE form the basis for androgen replacement therapy in this disease (Lahita et al., 1983). Clinical studies involving the use of dehydroepiandrosterone (DHEA) as a therapy (Van et al., 1995) for SLE are also a result of this observation (Van and McGuire, 1996).

The end products of androgen metabolism in a normal male or female are the same, but the total amount of testosterone in the male is very high compared with the female. Testosterone from either sex is oxidized to androstenedione, converted to DHEA and then sulfated. Both testosterone and androstenedione can be metabolized to estrone and estradiol. After the male hormones are aromatized to estrogens they can then enter the pathways for estrone hydroxylation. Specific cytokine levels change with levels of the sex steroids (van Vollenhoven et al., 1994; Soffer et al., 1982; McMurray et al., 1997). By this mechanism, certain sex steroid levels affect the T-cell populations and may determine anti-inflammatory versus proinflammatory cytokine profiles.

Estrogens and their Effects on Immune Mechanisms

The early work on the effects of sex hormones on autoimmune diseases involved careful studies with murine strains that were genetically prone to the acquisition of SLE. In some strains females were more likely to die sooner than males, with this effect being linked to estrogen (Roubinian et al., 1977; Roubinian et al., 1979; Siiteri et al., 1980; Talal, 1981; Ahmed et al., 1985). Furthermore, female mice made more antibodies to foreign antigens than males (Terres et al., 1968). In another study, after injection of humans with the antigen flagellin, men produced significantly lower titers of antibody than women. This may be due to the fact that women have higher levels of serum IgM than men, but the men produced less IgG and IgM antibody (Rowley and Mackay, 1969). Paradoxically, however, estrogens, depending on dose and condition, were 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 (Thompson et al., 1957).

The overall effects of estrogen on various immune parameters begin early in the life of the animal. Skin allograft rejection is naturally more likely in females than in males (Graff et al., 1969; Waltman et al., 1971), and estrogens regulate immunity by causing thymic atrophy in rodents (Golsteyn and Fritzler, 1987) and decreasing the overall thymic population. Castrated male and certain female mice display enlarged thymus and hyperplastic spleens after challenge with thymic-dependent antigens, indicating an effect of the estrogens on T-cell activity (Shirai et al., 1972; Mc Cruden and Stimson, 1980; Smith et al., 1989). Estradiol and diethylstilbestrol (in concentrations of 10–50 mg/mL) are known to reduce the phytohemagglutinin and concanavalin-A response of lymphocytes *in vitro* (Wyle and Kent, 1977), although the mixed lymphocyte reaction (MLR) may be enhanced by estradiol. For example, during normal menses (Bjune, 1979), pregnancy, and during the use of oral contraceptives (Satoh et al., 1977), the immune response may be heightened. Moreover, castration of males leads to accelerated allograft rejection through a mechanism that involves an increase in the levels of T cells. T cells from such mice proliferate more vigorously to T-cell receptor (TCR)- and CD28-mediated costimulation, as well as antigen-specific activation (Roden et al., 2004). Syngeneic grafts of ovaries in males and grafts of testes in females have no significant effect on allograft rejection (Graff et al., 1969; Sasson and Mayer, 1981). Estrogen given prior to bone marrow transplantation results in increased graft failure; however, with regard to renal transplants, there is essentially no difference in graft survival with regard to gender over a long period of time. Females have a higher incidence of acute rejection while chronic allograft failure in the female is decreased (Meier-Kriesche et al., 2001). Sustained levels of estrogens in mice lead to a marked reduction in natural killer cell activity (Seaman et al., 1978; Tuo et al., 1993). In other studies, estrogens depress cell-mediated immunity, natural killer cell function, and cancer cell immune surveillance. Estrogens prevent monocytes from responding to various chemokines and also prevent the secretion of specific chemokines like IP-10, CXCR3 and CCR5 (Aronica et al., 2004). This suppression of the monocytes may be interpreted as a decrease in the overall process of immune surveillance associated with estrogens (Janis et al., 2004). This effect can be specific to $CD8^+$ T cells (Effros, 2004). When given in excessive amounts, estrogens also deplete thymic hormones and are known to produce a relative lymphopenia (Olsen et al., 1991; Olsen et al., 1993).

The effects of estrogens on disease depend on the specific diagnosis. Estrogens increase the severity of SLE but appear to have no effect or a salutary effect on rheumatoid arthritis (RA) by different mechanisms (Lahita, 1985). Tamoxifen and antiestradiol antibody have beneficial effects on experimental SLE, and the mechanism may be via cytokine regulation (Dayan et al., 1997). Men with prostate cancer who are given diethylstilbestrol have markedly depressed cell-mediated immunity. The use of gonadotropin-releasing hormone (GnRH) to suppress prostate cancer is also based in part on immunosuppression (Hurwitz et al., 2003; Roden et al., 2004). Using normal lymphocytes, estradiol treatment of pokeweed mitogen-treated B cells shows an increase in plaque-forming cells (*in vitro*) (Paavonen et al., 1981; Paavonen et al., 1991; Dayan et al., 1997). Estrogen receptors are found on $CD8^+$ and $CD4^+$ T lymphocytes in some studies from both mice and men (Cohen et al., 1983; Danel et al., 1983; Arthreya et al., 1989; Kassi et al., 2001). In that regard, there are studies to show that $CD4^+$ helper T cells increase after estrogen therapy (Stimson, 1988), while other studies show estrogen as an inhibitor of $CD8^+$ suppressor T cells. Consequently, estrogen would increase helper T cells, which would result in enhanced polyclonal B-cell immunoglobulin production. Estradiol inhibits apoptosis *in vitro*, using peripheral blood mononuclear cells from women with normal menses (Evans et al., 1997). Estradiol decreases production of TNF-α by blood mononuclear cells in SLE but not those from unaffected people (Evans et al., 1997). This would suggest that flares of SLE might occur through the inhibition of TNF-α in the SLE patient (Gomez et al., 2004).

Estrogen Metabolism and Autoimmunity

Estradiol is quickly converted to estrone, which can be metabolized in one of two ways. Estrone can enter one pathway, a very feminizing pathway, by being hydroxylated to 16α-hydroxyestrone or estriol. Alternatively, the estrone can be metabolized to one of the catechol estrogens, 2-hydroxy or 2-methoxy estrone, which are less feminizing hormones (Lahita et al., 1981; Lahita, 1986). The change appears to have some importance for the normal physiology of the individual, and the amount of the products depends on the substrate amounts of estradiol. In the female the metabolism is likely to have profound effects, whereas in the male the overall effects of the lesser amounts of estradiol are unknown. An elevation of the 16-hydroxylated estrogens in the male results in adverse effects such as gynecomastia or decreased libido. Any significant change of estrone hydroxylation toward the catechol estrogens in the female will result in oligomenorrhea or osteoporosis. Factors such as smoking, diet, and the normal changes of pregnancy affect the direction of estrone metabolism (Fishman and Martucci, 1980; Michnovicz and Bradlow, 1991). Whereas smoking and dietary change might increase the metabolism of estrone toward the 2-hydroxylated compounds, pregnancy shifts the hydroxylation of estrone toward the very feminizing compounds.

One estrogen, 16α-hydroxyestrone, is feminizing, highly uterotropic, and modestly bound to cytosol receptors and testosterone/estradiol-binding globulin (TEBG) in contradistinction to compounds like 17β-estradiol (Fishman and Martucci, 1980). A radioimmunoassay, however, failed to show uniformly elevated levels of 16α-hydroxyestrone in all active SLE patients (Ikegawa et al., 1983). This suggested either that a conjugated form of this steroid was active or that there are other metabolites of importance to SLE that are not apparent. Enzymatic systems in certain animals might favor the formation of such compounds, although nothing is known about SLE mice with regard to the metabolism of estrone.

Clinical studies on the steroid 16α-hydroxyestrone show interesting properties *in vivo* that might explain its possible role in disease; these include covalent binding of this steroid to erythrocytes and lymphocytes via a Heyn's rearrangement *in vivo*, and the possibility that this covalent binding might occur at the level of the estrogen receptor or the TCR and result in an alteration of immune function (Bucala et al., 1982, 1984a, 1984b, 1987). Studies of family members of SLE patients indicated that elevated hydroxylation of estradiol was commonly observed in non-affected first-degree relatives as well as patients (Lahita et al., 1982) suggesting a genetic basis for this metabolic pathway.

Unaffected women who ingest oral contraceptives have enhanced binding of l6α-hydroxyestrone to various cell proteins (Bucala et al., 1987). Specific antiestrogen–protein adduct immunoglobulins are isolated from unaffected women 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 high estrogen level (Bucala et al., 1987). Males with SLE were also reported to have hormone–protein adduct-specific IgG in their sera (Bucala et al., 1982)

Progestogens

Progesterone, like other steroids, has been considered as a therapeutic agent in diseases like SLE because it plays such a prominent role during pregnancy, a time of careful immunosuppression. This is because progesterone is an immunosuppressive agent (Clemens et al., 1979; Holdstock et al., 1982; Ashworth et al., 1990; Keisler et al., 1990), and its levels rise during pregnancy when the placenta assumes an active role in its synthesis and secretion. This steroid at concentrations of 10–15 mg/mL reduces lymphocyte responses to phytohemagglutinin and to concanavalin A *in vitro*. Other analogs such as 20α-hydroxyprogesterone have similar effects (Mori et al., 1977). Progesterone has been known to increase the relative amounts of $CD8^+$ suppressor T cells in humans 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 (Van Vollenhoven and McGuire, 1994). A logical consequence of all these suppressive effects would be to facilitate acceptance of the fetal "graft."

Gonadotrophins

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 CNS manifestations (El-Garf et al., 1996). 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 (Gutierrez et al., 1996). One study correlates elevated prolactin levels with elevated cortisol levels (Neidhart, 1997; Dostal et al., 2002).

Women who are pregnant have higher serum prolactin levels if they have SLE (Jara-Quezada et al., 1991). Moreover, some investigators have associated the decline in serum testosterone during pregnancy in SLE patients with hyperprolactinemia. Perhaps, however, the most significant descriptions of hyperprolactinemia have been in men with SLE (Lavalle et al., 1987). This is of particular interest since hyperprolactinemia is readily treated with bromocriptine; however, studies using bromocriptine to treat SLE are not routine and data have been inconclusive (McMurray et al., 1995).

Many studies refute the significance of prolactin in human SLE (Buskila et al., 1996; Mok and Lau, 1996; Ostendorf et al., 1996). In a detailed study of a Chinese cohort of patients with SLE, Mok et al. (1997) found no correlation of clinical activity with levels of prolactin in 72 SLE patients. A look at autoantibodies in patients with SLE found no association of prolactin levels with specific SLE autoantibodies (Kozakova et al., 2000). One particular study suggests that prolactin is complexed with IgG but remains biologically active in the SLE patient (Leanos-Miranda et al., 2001a, 2001b). This binding of prolactin (a specific 23-kDa 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 SLE.

Many investigators associate prolactin with disease activity (Pacilio et al., 2001). In one study, 61.9% of patients with hyperprolactinemia had active disease. However, elevated prolactin levels are found in only 20–30% of patients with SLE in most clinics (Jara et al., 2001). The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) correlated with the levels of prolactin in these patients as well when

prolactin levels were measured whether by tests of immunoreactivity or biological activity. Another series noted an association of hyperprolactinemia in the serum and urine of patients with severe renal disease (Miranda et al., 1998). The weak statistical power of most of these prolactin studies could explain the lack of consistency between research laboratories. One study suggests that the published results are contradictory due to the weakness of 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 SLE activity and prolactin levels, while the other three studies did suggest an association (Blanco-Favela et al., 1999). In addition to the suggestion that prolactin is itself a cytokine that provokes the synthesis of immunoglobulins by lymphocytes (Jacobi et al., 2001b), German investigators found using lymphocytes from SLE patients and measuring clinical activity with the European activity measure (ECLAM), that SLE patients have lymphocytes that are sensitive to normal levels of prolactin and likely to be activated by normal plasma concentrations (Jacobi et al., 2001a). These data are supported by the findings that T lymphocytes from SLE patients secrete more prolactin than controls, suggesting a difference in 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 could have affected prolactin transcription and possibly disease association in a cohort of SLE patients; this was the case in a study where patients had an increased frequency of the prolactin-1149G allele compared with control subjects (Stevens et al., 2001). Whether prolactin is a cytokine or is itself increased by cytokines common in certain active SLE patients requires major investigation.

Finally, prolactin levels of women who were pregnant or breastfeeding and had SLE were studied as reproductive risk factors for the development of disease (Cooper et al., 2002). Surprisingly, breastfeeding was associated with a decreasing risk of developing SLE. In addition, the numbers of pregnancies or live births with SLE activity showed no relationship to levels of prolactin. These authors found no association of elevated prolactin levels with an increased risk of SLE.

Sex Chromosomal Abnormalities and Autoimmunity

Autoimmunity is not common in patients with Klinefelter syndrome. In fact it is not correct to say that autoimmunity is more common in men who have the extra X chromosome (Fam et al., 1980; Lahita and Bradlow 1987; Gilliland and Stashower, 2000; Oktenli et al., 2002). There is a report of monosomy X in patients with primary biliary cirrhosis, and it is suggested that haploinsufficiency of specific X-linked genes leads to female susceptibility for primary biliary cirrhosis (Invernizzi et al., 2004).

SPECIFIC DISEASES AND THE EFFECTS OF SEX STEROIDS

Systemic Lupus Erythematosus

Sex steroids affect the severity of SLE (but they are also important in fibromyalgia or Hashimoto's thyroiditis), but a direct relationship between both androgen and estrogen metabolites with the severity of the disease, the production of antibodies, or the appearance of certain clinical signs in SLE has never been established. It is widely accepted, however, that estrogen does increase the severity of this disease (Petri et al., 1991). The basic observations found in SLE patients involve the hydroxylation of estrone and the oxidation of testosterone as mentioned above.

Rheumatoid Arthritis

Rheumatoid arthritis is also associated with hormone changes. As is the case with SLE, RA patients have low androgen levels (Cutolo et al., 1988). In many cases of RA, the signs and symptoms of disease coincide with the menstrual cycle (Latman, 1983), an observation that has also been made anecdotally in SLE patients. Exacerbations of RA occur during certain times in the menstrual cycle. This disease, multiple sclerosis, and thyroiditis are illnesses where this observation is made (Kim et al., 2004). Several observations about RA are not clear, namely the remission of RA in the pregnant woman and also the response of many patients and animal models to estrogen (Bijllsma et al., 1987; Spector et al., 1991).

Sjögren Syndrome, Antiphospholipid Syndrome, and Other Conditions

There are data from Sjögren syndrome to show that the levels of androgens in the tears of patients with the disease are low (Sullivan et al., 1990). The significance of this finding is not known.

New data from animals indicates that the withdrawal of estrogen promotes the infiltration of both kidney and salivary glands in mouse models and the development of a Sjögren-like syndrome. Experiments with aromatase-knockout mice show that those exposed to an estrogen-containing diet have no Sjögren-like changes or anti-fodrin antibodies (Shim et al., 2004).

The antiphospholipid syndrome, an autoimmune disease that produces a procoagulant condition, has no apparent

predilection for sex, although the secondary form of the disease affects more women than men.

AUTOIMMUNE DISEASE AND PREGNANCY

In pregnancy a semi-allograft is allowed to flourish for 9 months because of certain events that involve the immune system. Some aspects of pregnancy, fertility, and fetal health are dependent on normal immune function. This section will examine the role of pregnancy and autoimmunity. The immunology of pregnancy will be considered, followed by a review of the effects that the mother's immune system has on the fetus and the effects that the pregnancy has on the mother's autoimmune disease.

Immunology and Pregnancy

Neither spermatozoa nor the embryonic trophoblast express MHC class I or class II molecules (Johnson, 1993). This allows the trophoblast to avoid the immune system in the development of the fetus and also allows spermatozoa to gain easy entry into a charged ovarian milieu of cytokines and protective antibodies. Certain seminal proteins including transforming growth factor-β (TGF-β) act to suppress the immune response locally, while animal studies indicate that this cytokine increases the endometrial inflammatory response (Robertson et al., 1997). This inflammatory response could be responsible for the shift of cytokines from Th1 to Th2 in the pregnant state. This shift has theoretical and practical implications for both the mother and the fetus, since many of the autoimmune diseases like SLE are of Th2 cytokine character and RA or MS are predominantly Th1 illnesses (Raghupathy, 1997; Lim et al., 1998). The trophoblast is not without genetic identity since it expresses a unique MHC marker called HLA-G. This antigen is responsible for the protection of the trophoblast (VanVoorhis and Stovall, 1997). There is a population of natural killer cells within the reproductive tract that would easily destroy the trophoblast, were it not for the HLA-G MHC as well as the new cytokine milieu induced by TGF-β (Kovats et al., 1990; Carosella et al., 1996).

Other relevant immune functions such as apoptosis are essential to the normal pregnancy. The Fas ligand (FAS-L) is a part of the protective network for the fetus. The expression of this ligand allows for apoptosis of the mother's Fas-positive T cells, which in turn protects the fetus from total destruction by the mother's immune system (Makrigiannakis et al., 2001).

The role of complement in the pregnancy process is very important. There are complement regulation proteins that are expressed by the trophoblast that limit the activation of complement (Holmes and Simpson, 1992). In animals that lack these proteins, there is enhanced fetal loss. In animal models of the antiphospholipid syndrome (APS), complement is essential for fetal loss. In recent work, C3 is deposited in the placentas of affected APS animals whereas mice deficient in C3 were resistant to the lethal effects of APS (Xu et al., 2000).

As one might suspect, the Th1 cytokines are lethal to the pregnant female since Th2 cytokine profiles are the natural milieu for a normal pregnancy; for example, TNF-α induces apoptosis of the cells of the trophoblast (Yui et al., 1994). Besides the previously mentioned effects of TGF-β on induction of Th2 cytokines, both progesterone and PGE2 suppress Th1 cytokine production. This may be the reason for remission of disease in patients with RA (Hench, 1938). The role of the adhesins, such as annexin, are also important during pregnancy (Rand et al., 1998; Rand 2000). Specifically, annexin V, thought to be another target antigen for the antiphospholipid antibodies, shields the trophoblast from harm in the normal state. There are doubtless other glycoproteins within this family of adhesins that have importance (Aarli and Matre, 1998).

Infertility and Fetal Loss

Autoimmunity is intimately tied together with fetal loss and infertility (Wilson et al., 1975; Taylor et al., 1989). In fact, there is evidence that infertility is common in patients with autoimmune diseases (Roussex et al., 1996). One disease that is common to patients with infertility and is a harbinger of autoimmune disease is thyroiditis. Studies have shown that the thyroid peroxidase antibodies are commonly found in infertile women, and this may be the way that autoimmunity is defined in this population. Endometriosis and polycystic ovarian disease are two diseases of the gonads that have been associated with autoimmune phenomena (Gleicher et al., 1987; Gleicher and El-Roeiy, 1988; Gleicher et al., 1989; Gleicher, 1992). Endometriosis has been thought to be itself an autoimmune disease (Odukoya et al., 1995). Both of these illnesses are associated with autoimmune disorders. Interestingly, infertility is more common with organ-specific diseases like thyroiditis and less common in patients with diseases like SLE and RA, which are more systemic (Gerhard et al., 1991; Krassas, 2000).

Fetal loss is a common feature of both systemic and organ-specific autoimmune disease. However, RA, one of the most common autoimmune disease, is not associated with fetal loss. Patients with APS and SLE have more fetal loss than any other subgroup. Much of it depends on the activity of the disease in the mother at the time of conception. Growth retardation, pre-eclampsia, and multiple miscarriages are common with these two entities. The presence of antiphospholipid antibody leads to placental infarction, the suppression of annexin V, and has other direct effects on

the placental health; it is largely responsible for the fetal demise syndrome. Much of the latter pathology related to infarction has been obviated with anticoagulation during gestation; however, there are still other reasons for this increased fetal loss that await elucidation.

Disease activity in SLE is the principal cause of fetal demise in this group if they do not have secondary APS (Fraga et al., 1974; Petri et al., 1992; Petri, 1997). Parke suggests 6 months of disease remission prior to attempting conception (Parke, 1992).

Among those who have SLE and Sjogren syndrome, there are patients who have anti-Ro and anti-La antibodies that can be passed transplacentally to the fetus. Such antibodies can result in conditions such as neonatal heart block and transient neonatal lupus syndrome in offspring of mothers with these antibodies (Reed et al., 1983; Alexander et al., 1992; Buyon, 1992; Buyon et al., 1995) (See Chapter 71). In other conditions such as scleroderma, Graves' disease, and polymyositis in the mother, there will be a slight increase of infertility and fetal loss.

Effect of Pregnancy on the Mother's Disease

One of the most interesting aspects of autoimmune disease is the effect of pregnancy on conditions like multiple sclerosis and RA, where patients achieve a complete remission. Some 80% of patients who have RA and are pregnant have remission of their illness (Allebeck et al., 1984; Del Junco et al., 1985; Bijllsma et al., 1987; Berczi et al., 1989; Cutolo and Accardo, 1991). Early explanations for this remission suggested that estrogens like estriol were responsible for this remission. This was especially suggested by the fact that RA seemed to decrease in incidence with the widespread use of estrogen-containing oral contraceptives (Allebeck et al., 1984; Rook et al., 1990). This was not a long-lived hypothesis, however, and most experts note that the remission continues into the postpartum period, when the hormone surges observed during the pregnancy are no longer applicable. The epidemiologic effects of this protective effect are difficult to prove. Exposure to oral contraceptives but not hormone replacement therapy reduces the risk of developing RA. This is particularly true if the exposure to the hormones occurs in a woman's early years (Doran et al., 2004). The Th1 cytokines have been suggested as reasons for this remission; however, no definitive data on this exist (Wingrave and Kay, 1978; Kanik and Wilder, 2000; Weidler et al., 2004).

Another condition that remits during pregnancy is Graves' disease which, as with all other autoimmune diseases, can return with a vengeance after parturition (Kung and Jones, 1998; Badenhoop, 2004). Moreover, thyroiditis is common in women with autoimmune disease and can occur after parturition and cause hypothyroidism. This hypothyroid state can become permanent. Multiple sclerosis might also remit with pregnancy, but like RA can recur after parturition. Interestingly, there are new data to suggest that in mice estradiol might cause remission of the model disease experimental autoimmune encephalomyelitis (Polanczyk et al., 2003). This remission is dependent on a form of the estrogen receptor, estrogen receptor-α, and indicates a real role for sex steroids. The suggested reason for this improvement is the effect of the estriol on the down-regulatory cytokine IL-10.

There is much controversy over the effects of pregnancy on SLE. Petri and Ruiz-Iraslorza (Ruiz-Irastorza et al., 1996) indicate that the flare rates of the disease in patients who are pregnant are significantly higher than control patients with SLE who are not pregnant. This is supported by data from others as well (Khamashta et al., 1997; Cooper et al., 2002). Patients who have SLE require immunosuppression if their disease becomes active during gestation. Patients who have secondary APS associated with SLE must also be considered for anticoagulation to prevent fetal loss, as well as continued immunosuppression.

Microchimerism and Autoimmune Disease

There is a phenomenon called microchimerism, which is associated with the onset of SLE, scleroderma, and thyroid disease (Nelson, 1996). It is included here under pregnancy and autoimmune disease because it has relevance to the mother, and includes the fetus and gestation. This is essentially the presence in the circulation of women who have autoimmune disease of cells that belong to an offspring (Galliard et al., 1997). The interesting aspect of this condition is that the offspring could have been born many years before the onset of the mother's illness, meaning that the fetal cells persist for many years in the mother's circulation. Similar conditions have been proposed for host-versus-graft disease in both animals (Feikje et al., 1982; Kuppers et al., 1988) and recipients of allogeneic bone marrow transplants.

Chimerism is normally linked to host-graft tolerance induction. It can induce autoimmunity, however, as in early mouse models of the disease. There is now evidence that the presence of fetal cells in the mother induces a host-versus-graft, and this in turn induces autoimmune disease (Johnson et al., 2001).

SUMMARY AND CONCLUSION

Both sex hormones and pregnancy greatly affect the immune system in many ways. Androgens, estrogens, and gonadotropins have effects on antigen presentation, the synthesis of cytokines and chemokines, and, consequently, the function of various cell types within the immune system.

The fundamental answers about why women predominate with diseases of the immune system are not known. However, much insight comes from both animal and clinical models of disease.

References

Aarli, A. and Matre, R. (1998). Suppression of immunoglobulin secretion by soluble annexin II. *Scand. J. Immunol. 48,* 522–526.

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.

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.

Allebeck, P., Ahlboom, A., Ljungstrom, K., and Allander, E. (1984). Do oral contraceptives reduce the incidence of rheumatoid arthritis? *Scand. J. Rheumatol. 13,* 140–146.

Aronica, S.M., Fanti, P., Kaminskaya, K., Gibbs, K., Raiber, L., Nazareth, M., Bucelli, R., Mineo, M., Gryzbek, K., Kumin, M., Poppenberg, K., Schwach, C., and Janis, K. (2004). Estrogen disrupts chemokine mediated chemokine release from mammary cells: implications for the interplay between estrogen and IP-10 in the regulation of mammary tumor formation. *Breast Cancer Res. Treat. 84*, 235–245.

Arthreya, B., Moore, W.C., Wadsworth, S.A., Gupta, C., and Goldman, S. (1989) Estrogen receptor levels in a murine model of systemic lupus erythematosus. *Clin. Exp. Rheumatol. 7,* 589–593.

Ashworth, C.J., Fliss, M.F., and Bazer, F.W. (1990). Evidence for steroid control of a putative angiogenic factor in the porcine uterus. *J. Endocrinol. 125,* 15–19.

Badenhoop, K. (2004). Intrathyroidal microchimerism in Graves' disease or Hashimoto's thyroiditis: regulation of tolerance or alloimmunity by fetal-maternal immune interactions? *Eur. J. Endocrinol. 150*(4), 421–423.

Berczi, I., Cosby, H., Hunter, T., Baragar, F., McNeilly, A.S., and Friesen, H.G. (1989). Decreased bioactivity of circulating prolactin in patients with rheumatoid arthritis. Unpublished work.

Bhardwaj, N., Santhanam, U., Lau, L.L., Tatter, S.B., Ghrayeb, J., Rivelis, M., Steinman, R. M., Sehgal, P.R., and May, L.T. (1989). Il-6/IFN-β_2 in synovial effusions of patients with rheumatoid arthritis and other arthritides. *J. Immunol. 143,* 2153–2159.

Bijllsma, J.W.J., Huber-Bruning, O., and Thijssen, J.H.H. (1987). Effect of estrogen treatment on clinical and laboratory manifestation of rheumatoid arthritis. *Ann Rheum Disease*, 46, 777–779.

Bjune, G. (1979). *In vitro* lymphocyte responses to PHA show co-variation with the menstrual cycle. *Tenth Scandinavian congress, Immunol. Abstr. 51*.

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*, 55–59.

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.

Bucala, R., Fishman, J., and Cerami, A. (1982). Formation of covalent adducts between cortisol and 16-alpha-hydroxyestrone and protein, possible role in pathogenesis of cortisol toxicity and SLE. *Proc. Natl Acad. Sci. U.S.A. 79,* 3320.

Bucala, R., Fishman, J., and Cerami, A. (1984a). The reaction of 16-α hydroxyestrone with erythrocytes *in vitro* and *in vivo*. *Eur. J. Biochem. 140,* 593–598.

Bucala, R., Lahita, R.G., Fishman, J., and Cerami, A. (1984b). Nonenzymatic modification of red cell and lymphocyte membrane proteins by 16-alpha hydroxyestrone in patients with systemic lupus erythematosus. *Arthritis Rheum. 27,* s40.

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 [abstract]. *Clin. Exp. Immunol. 67,* 167–175.

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 [see comments]. *J. Rheumatol. 23*, 629–632.

Buyon, J., Waltuck, J., and Klein, C. (1995). In utero identification and therapy of congenital heart block. *Lupus 4,* 116.

Buyon, J.P. (1992) Neonatal lupus syndromes. In R.G. Lahita (ed.), *Systemic Lupus Erythematosus*, 2nd ed. (p. 419). New York: Churchill Livingstone.

Carosella, E.D., Dausset, J., and Kirzem, B. (1996). HLA-G revisited. *Immunol. Today 17,* 407–409.

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*, 158–162.

Clemens, L.E., Siiteri, P.K., and Stites, D.P. (1979). Mechanisms of immunosuppression of progesterone on maternal lymphocyte activation during pregnancy. *J. Immunol.122,* 1978–1985.

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 [abstract]. *J. Immunol. 131,* 2767–2771.

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*, 1830–1839.

Cutolo, M. and Accardo, S. (1991). Sex hormones and rheumatoid arthritis. *Clin. Exp. Rheumatol. 9,* 641–646.

Cutolo, M., Balleari, E., Giusti, M., Monachesi, M., and Accardo, S. (1988). Sex hormone status of male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gonadotropin stimulation. *Arthritis Rheum. 31,* 1314–1317.

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.

Dayan, M., Zinger, H., Kalush, F., Mor, G., Amir-Zaltzman, Y., Kohen, F., Sthoeger, Z., and Mozes, E. (1997). The beneficial effects of treatment with tamoxifen and anti-oestradiol antibody on experimental systemic lupus erythematosus are associated with cytokine modulations. *Immunology 90*, 101–108.

Del Junco, D.J., Annegers, J.F., and Luthra, H.S. (1985). Do oral contraceptives prevent rheumatoid arthritis? *J. Am. Med. Assoc. 254,* 1938–1941.

Doran, M.F., Crowson, C.S., O'Fallon, W.M., and Gabriel, S. (2004). The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. *J. Rheumatol. 31*, 207–213.

Dostal, C., Marek, J., Moszkorzova, L., Lacinova, Z., Musilova, L., and Zvarova, J. (2002). Effects of stress on serum prolactin levels in patients with systemic lupus erythematosus. *Ann. N.Y. Acad. Sci. 966,* 247–251.

Dunkel, L., Taino, V.M., Savilahti, E., and Eskola, J. (1985). Effect of endogenous androgens on lymphocyte subpopulations. *Lancet* 440–441.

Effros, R.B. (2004). Replicative senescence of CD8 T cells: potential effects on cancer immune surveillance and immunotherapy. *Cancer Immunol. Immunother. 53*, 925–933.

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*, 374–377.

Evans, M.J., MacLaughlin, S., Marvin, R.D., and Abdou, N.I. (1997). Estrogen decreases *in vitro* apoptosis of peripheral blood mononuclear cells from women with normal menstrual cycles and decreases TNF-alpha production in SLE but not in normal cultures. *Clin. Immunol. Immunopathol. 82*, 258–262.

Fam, A., Izsak, M., and Saiphoo, C. (1980) SLE and Klinefelter's syndrome. *Arthritis Rheum. 23,* 124.

Feikje, M., van der Veen, R., Rolink, A.G., and Gleichmann, E. (1982). Diseases caused by reactions of T lymphocytes towards incompatible structures of the major histocompatibility complex. *J. Exp. Med. 155,* 1555.

Fishman, J. and Martucci, C. (1980). Biological properties of 16-alpha hydroxyestrone: implicationso in estrogen physiology and pathophysiology. *J. Clin. Endocrinol. Metab. 51,* 611–615.

Folomeev, M., Dougados, M., Beaune, J., Kouyoumdjian, J.C., Nahoul, K., Amor, B., and Alekberova, Z. (1992). Plasma sex hormones and aromatase activity in tissues of patients with systemic lupus erythematosus. *Lupus 1*, 191–195.

Forsberg, J.G. (1996). The different responses of the female mouse thymus to estrogen after treatment of neonatal, prepubertal, and adult animals. *Acta Anat. (Basel)*, *157*, 275–290.

Fraga, A., Mintz, G., and Orozco, J. (1974). Sterility and fertility rates, fetal wastage and maternal morbidity in SLE. *J. Rheumatol. 1,* 293–298.

Galliard, M.C., Ouvre, E., and Liegeois, A. (1997). The concentration of fetal cells in maternal hematopoetic organs during pregnancy, an experimental study in mice. *J. Gynecol. Obstet. Biol. Reprod. (Paris) 7,* 1043–1050.

Gerhard, I., Becker, T., and Eggert-Kruse, W. (1991). Thyroid and ovarian function in infertile women. *Hum. Reprod. 6,* 338–345.

Gilliland, W.R. and Stashower, M.E. (2000). Klinefelter's syndrome and systemic lupus erythematosus. *Clin. Exp. Rheumatol. 18*(1), 107–109.

Gleicher, N. (1992) Autoantibodies in normal and abnormal pregnancy. *Am. J. Reprod. Immunol. 28,* 269–273.

Gleicher, N. and El-Roeiy, A. (1988). The reproductive autoimmune failure syndrome. *American J. Obstet. Gynecol. 159,* 223–227.

Gleicher, N., El-Roeiy, A., and Confino, E. (1989). Reproductive failure because of autoantibodies: unexplained infertility and pregnancy wastage. *Am. J. Obstet. Gynecol. 160,* 1376–1385.

Gleicher, N., El-Roeiy, A., and Confino, E. (1987). Abnormal antibodies in endometriosis: Is endometriosis an autoimmune disease? *Obstet. Gynecol. 70,* 115–122.

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.

Gomez, D., Correa, P.A., Gomez, L.M., Cadena, J., Molina, J.F., and Anaya, J.M. (2004). TH1/TH2 cytokines in patients with systemic lupus erythematosus: is tumor necrosis factor alpha protective? *Semin. Arthritis Rheum. 33*, 404–413.

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.

Grossman, C. (1989). Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *J. Steroid Biochem. 34,* 241–251.

Grossman, C.J. (1984) Regulation of the immune system by sex steroids. *Endocr. Rev. 5,* 435–455.

Grossman, C.J., Sholitan, L.J., Blaha, G.C., and Nathan, P. (1989). Rat thymic estrogen receptor II. Physiologic properties. *J. Steroid Biochem. 11,* 1241.

Grossman, C.J., Sholitan, L.J., and Roselle, G. (1982). Estradiol regulation of thymic lymphocyte function in the rat: mediation by serum thymic factors. *J. Steroid Biochem. 16,* 683–690.

Gutierrez, M.A., Molina, J.F., Jara, L.J., Garcia, C., Gutierrez-Urena, S., Cuellar, M.L., Gharavi, A., and Espinoza, L.R. (1996). Prolactin-induced immunoglobulin and autoantibody production by peripheral blood mononuclear cells from systemic lupus erythematosus and normal individuals. *Int. Arch. Allergy Immunol. 109*, 229–235.

Hench, P.S. (1938). The ameliorating effect of pregnancy on chronic atrophic (infectious rheumatoid) arthritis, fibrositis, and intermittent hydrarthrosis. *Mayo Clinic Proc. 13,* 161–167.

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.

Hirota, Y., Suzuki, T., Chazono, Y., and Bito, Y. (1976). Humoral immune responses characteristics of testosterone propionate treated chickens. *Immunology 30,* 341.

Holdstock, G., Chastenay, B.F., and Krawitt, E.L. (1982). Effects of testosterone, estradiol and progesterone on immune regulation. *Clin. Exp. Immunol. 47,* 449.

Holmes, C.H. and Simpson, K.L. (1992). Complement and pregnancy. New insights into the immunobiology of the feto maternal relationship. *Ballières Clin. Obstet. Gynecol. 6,* 439–460.

Hurwitz, A.A., Yanover, P., Markowitz, M., Allison, J.P., and Kwon, E.D. (2003). Prostate cancer: advances in immunotherapy. *BioDrugs*, *17*, 131–138.

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.

Inman, R.D., Jovanovic, L., and Markenson, J.A. (1982). Systemic lupus erythematosus in men: genetic and endocrine features. *Arch. Intern. Med. 142,* 1813–1815.

Invernizzi, P., Miozzo, M., Battezzati, P.M., Bianchi, I., Grati, F., Simoni, G., Selmi, G., Watnick, M., Gershwin, M.E., and Podda, M. (2004). Frequency of monosomy X in women with primary biliary cirrhosis. *Lancet 363,* 533–534.

Jacobi, A.M., Rohde, W., Ventz, M., Riemekasten, G., Burmester, G.R., and Hiepe, F. (2001a). Enhanced serum prolactin (PRL) in patients with systemic lupus erythematosus: PRL levels are related to the disease activity. *Lupus 10*, 554–561.

Jacobi, A.M., Rohde, W., Volk, H.D., Dorner, T., Burmester, G.R., and Hiepe, F. (2001b). 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*, 242–247.

Janis, K., Hoeltke, J., Nazareth, M., Fanti, P., Poppenberg, K., and Aronica, S.M. (2004). Estrogen decreases expression of chemokine receptors and suppresses chemokine bioactivity in murine monocytes. *Am. J. Reprod. Immunol. 51*, 22–31.

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.

Jara-Quezada, L., Graef, A., and Lavalle, C. (1991). Prolactin and gonadal hormones during pregnancy in systemic lupus erythematosus. *J. Rheum. 18,* 349–353.

Johnson, K.L., McAlindon, T.E., Mulcahy, E., and Bianchi, D.W. (2001). Microchimerism in a female patient with systemic lupus erythematosus. *Arthritis Rheum.* 44, 2107–2111.

Johnson, P.M. (1993). Reproductive and maternofetal relations. In P.G.H. Gell and R.R.A. Coombs (eds) *Clinical Aspects of Immunology*. (pp. 755–767). Oxford: Blackwell.

Jungers, P., Nahoul, K., and Pelissier, C. (1982). Low plasma androgens in women with active or quiescent SLE. *Arthritis Rheum. 25,* 454–457.

Jungers, P., Pelissier, C., Bach, J.F., and Nahoul, K. (1980). Les androgenes plasmatiques chez les femmes atteintes de lupus erythemateux dissemine (LED). *Pathologie Biologie 28,* 391–392.

Kanik, K.S. and Wilder, R.L. (2000). Hormonal alterations in rheumatoid arthritis. *Rheum. Dis. Clin. N. Am. 26*, 805–823.

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.

Keisler, L.W., Kier, A.B., and Walker, S. (1990). Therapeutic benefits of the synthetic progestogens, norethindrone and norgestrel, in long term therapy of autoimmune disease in NZB/NZW mice: comparison with medroxyprogesterone and ethinyl estradiol. Unpublished Work.

Khamashta, M.A., Ruiz-Irastorza, G., and Hughes, G.R. (1997). Systemic lupus erythematosus flares during pregnancy. *Rheum. Dis. Clin. N. Am. 23*, 15–30.

Kim, S., Liva, S.M., and Dalal, M.A. (2004). Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. *Neurology 52*, 1230–1238.

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.

Kovats, S., Main, E.K., and Librack, C. (1990). Class I antigen HLA-γ expressed in human trophoblasts. *Science 248,* 220–223.

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, II/80–II/84.

Krassas, G.E. (2000). Thyroid disease and female reproduction. *Fertility and Sterility*, 74, 1063–1070.

Kung, A.W.C. and Jones, B.M. (1998). A change from stimulatory to blocking antibody activity in Graves disease during pregnancy. *J. Clin. Endocrinol. Metab. 83,* 514–518.

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.

Lahita, R.G. (1985). Sex steroids and the rheumatic diseases. *Arthritis Rheum*. *28,* 121–126.

Lahita, R.G. (1986). The influence of sex hormones on the disease systemic lupus erythematosus. *Springer Semin. Immunopathol. 9,* 305–314.

Lahita, R.G. (1992). Sex steroids and SLE: metabolism of androgens to estrogens [editorial]. *Lupus 1,* 125–127.

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.

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.

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.

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.

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.

Latman, N. (1983). Relation of menstrual cycle to the symptoms of rheumatoid arthritis. *Am. J. Med. 74,* 957–960.

Lavalle, C., Loyo, E., Paniagua, R., Bermudez, J.A., Herrera, J., Graef, A., Gonzalez-Barcena, D., and Fraga, A. (1987). Correlation study between prolactin and androgens in male patients with systemic lupus erythematosus. *J. Rheumatol. 14,* 268–272.

Leanos-Miranda, A., Chavez-Rueda, K.A., and Blanco-Favela, F. (2001a). Biologic activity and plasma clearance of prolactin-IgG complex in patients with systemic lupus erythematosus. *Arthritis Rheum. 44*, 866–875.

Leanos-Miranda, A., Pascoe-Lira, D., Chavez-Rueda, K.A., and Blanco-Favela, F. (2001b). Antiprolactin autoantibodies in systemic lupus erythematosus: frequency and correlation with prolactinemia and disease activity. *J. Rheumatol. 28*, 1546–1553.

Lim, K.J.H., Odukoya, O.A., and Ajjan, R.A. (1998). Profile of cytokine mRNA expression in peri-implantation human endometrium. *Mol. Hum. Reprod. 4,* 77–81.

Lubahn, D.B., Joseph, D.R., Sar, M., Tan, J., Higgs, H.N., Larson, R.E., French, F.S., and Wilson, E. M. (1988). The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. *Mol. Endocrinol. 2,* 1265–1275.

Makrigiannakis, A., Zoumakis, E., and Kalantaridou, S. (2001). Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance. *Natl Immunol*. *2,* 1018–1024.

Mc Cruden, A.B. and Stimson, W.H. (1980). Androgen and other sex steroids cytosol receptors in the rat thymus. *J. Endocrinol. 85,* P47.

McMurray, R.W., Hoffman, R.W., Nelson, W., and Walker, S.E. (1997). Cytokine mRNA expression in the B/W mouse model of systemic lupus erythematosus—analyses of strain, gender, and age effects. *Clin. Immunol. Immunopathol. 84,* 260–268.

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.

Meier-Kriesche, H.U., Ojo, A.O., Leavey, S.F., Hanson, J.A., Leichtman, A.B., Magee, J.C., Cibrik, D.M., and Kaplan, B. (2001). Gender differences in the risk for chronic renal allograft failure. *Transplantation 71*, 429–432.

Meyer, R.K., Rao, M.A., and Aspinall, R.L. (1959). Inhibition of the development of the bursa of Fabricius in the embryos of the common fowl by 19-nortestosterone. *Endocrinology*, *64,* 890.

Michnovicz, J.J. and Bradlow, H.L. (1991). Altered estrogen metabolism and excretion in humans following consumption of indole-3-carbinol. *Nutr. Cancer 16,* 59–66.

Miranda, J.M., Prieto, R.E., Paniagua, R., Garcia, G., Amato, D., Barile, L., and Jara, L.J. (1998). Clinical significance of serum and urine prolactin levels in lupus glomerulonephritis. *Lupus 7*, 387–391.

Mok, C.C. and Lau, C.S. (1996). Lack of association between prolactin levels and clinical activity in patients with systemic lupus erythematosus [letter; comment]. *J. Rheumatol. 23*, 2185–2186.

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*, 986–989.

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.

Neidhart, M. (1997). Bromocriptine has little direct effect on murine lymphocytes, the immunomodulatory effect being mediated by the suppression of prolactin secretion. *Biomed. Pharmacother. 51*, 118–125.

Nelson, J.L. (1996). Maternal fetal immunology and autoimmune disease, is some autoimmune disease auto-alloimmune or alloautoautoimmune? *Arthritis Rheum. 39,* 191–194.

Odukoya, O.A., Wheatcroft, N., and Weetman, A.P. (1995). The prevalence of endometrial immunoglobulin G antiobdies in patients with endometriosis. *Hum. Reprod. 10,* 1214–1219.

Oktenli, C., Yesilova, Z., Kocar, I.H., Musabak, U., Ozata, M., Inai, A., Gul, D., and Sanisoglu, Y. (2002). Study of autoimmunity in Klinefelter's syndrome and idiopathic hypogonadotrpohic hypogonadism. *J. Clin. Immunol*. *22*, 137–143.

Olsen, N.J., Olson, G., Viselli, S.M., Gu, X., and Kovacs, W.J. (2001). Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. *Endocrinology 142*, 1278–1283.

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. *Endocrinology 129,* 2471–2476.

Olsen, N.J., Zhou, P., Ong, H., and Kovacs, W.J. (1993). Testosterone induces expression of transforming growth factor-beta in the murine thymus. *J. Steroid Biochem. Mol. Biol. 45*, 327–332.

Ostendorf, B., Fischer, R., Santen, R., Schmitz-Linneweber, B., Specker, C., and Schneider, M. (1996). Hyperprolactinemia in systemic lupus erythematosus? *Scand. J. Rheumatol. 25*, 97–102.

Paavonen, T., Anderson, L.C., and Adlercreutz, H. (1981). Sex hormone regulation of *in vitro* immune response. Estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures. *J. Exp. Med. 154,* 1935–1945.

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. *Acta Pathol. Microbiol. Immunol. Scand. 99,* 163–170.

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*, 2216–2221.

Parke, A.L. (1992). Pregnancy and systemic lupus erythematosus. In R. G. Lahita (ed.), *Systemic Lupus Erythematosus*, 2nd ed. (p. 543). New York: Churchill Livingstone.

Petri, M. (1997). Hopkins Lupus Pregnancy Center 1987–1996. *Rheum. Dis. Clin. N.h Am. 23*, 1–13.

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.

Petri, M., Howard, D., and Repke, J. (1992). The Hopkins Lupus Pregnancy Center 1987–1991. update. *Am. J. Reprod. Immunol. 28,* 188–191.

Polanczyk, M., Zamora, A., Subramanian, S., Matejuk, A., Hess, D.L., Blakenhorn, E.P., Teuscher, C., Vandenbark, A. A., and Offner, H. (2003). The protective effect of 17β-estradiol on experimental autoimmune encephalomyelitis is mediated through estrogen receptor-α. *Am. J. Pathol. 163*, 1599–1605.

Raghupathy, R. (1997). Th–1 type immunity is incompatible with successful pregnancy. *Immunol. Today*, *18,* 478–482.

Rand, J.H. (2000). Antiphospholipid antibody mediated disruption of the annexin V antithrombotic shield: A thrombogenic mechanism for the aPL syndrome. *J Autoimmun*. *15,* 111.

Rand, J.H., Wu, X.X., and Andree, H.A.M. (1998). Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin V binding to phospholipids: A lupus procoagulant phenomenon. *Blood 92,* 1652–1660.

Raveche, E.S., Vigersky, R.A., Rice, M.K., and Steinberg, A.D. (1980). Murine thymic androgen receptors. *J. Immunopharmacol. 2,* 425.

Reed, B.R., Lee, L.A., and Harmon, C. (1983). Autoantibodies to SSA/Ro in infants with congenital heart block. *J. Pedia. 103*, 889–891.

Robertson, S.A., Mau, J.A., and Hudson, S.N. (1997). Cytokine-leukocyte networks and the establishment of pregnancy. *Am. J. Reprod. Immunol. 37,* 438–442.

Roden, A.C., Moser, M.T., Tri, S.D., Mercader, M., Kuntz, S.M., Dong, H., Hurwitz, A.A., McKean, D.J., Celis, E., Leibovich, B.C., Allison, J.P., and Kwon, E.D. (2004). Augmentation of T cell levels and responses induced by androgen deprivation. *J. Immunol*. *173*, 6098–6108.

Rook, G.A.W., Steele, J., Brealey, R., Whyte, A., Isenberg, D., Sumar, N., Nelson, J.L., Bodhan, K.B., Young, A., and Roitt, I.M. (1991). Changes in IgG glycoform levels may be relevant to remission of arthritis during pregnancy. *J. Autoimmun. 4*, 779–794.

Roubinian, J.R., Papoian, R., and Talal, N. (1977). Androgenic hormones modulate autoantibody reponses and improve survival in murine lupus. *J. Clin. Invest. 59,* 1066–1070.

Roubinian, J.R., Talal, N., and Greenspan, J.S. (1979). Sex hormone modulation of autoimmunity in NZB/NZW mice. *Arthritis Rheum. 22,* 1162–1169.

Roussex, R.G., Kaider, B.D., and Price, D.E. (1996). Laboratory evaluation of women experiencing reproductive failure. *Am. J. Reprod. Immunol*. *35,* 415–420.

Rowley, M.J. and Mackay, I.R. (1969). Measurement of antibody producing capacity in man. *Clin. Exp. Immunol. 5,* 407.

Ruiz-Irastorza, G., Lima, F., and Alves, J. (1996). Increased rate of lupus flare during pregnancy and the puerperium. *Br. J. Rheumatol. 35,* 133–138.

Sasson, S. and Mayer, M. (1981). Effect of androgenic steroids on rat thymus and thymocytes in suspension. *J. Steroid Biochem. 14,* 509–518.

Satoh, P.S., Fleming, W.E., Johnstone, K.A., and Ozmun, J.M. (1977). Active E rosette formation in women taking oral contraceptives. *N. Engl. J. Med. 296,* 54.

Seaman, W.E., Blackman, M.A., and Gindlast, T.D. (1978). Beta-estradiol reduced natural killer cells in mice. *J. Immunol. 121,* 2193.

Shim, G.J., Warner, M., Kim, H.J., Andersson, S., Liu, L., Ekman, J., Imamov, O., Jones, M.E., Simpson, E.R., and Gustafsson, J.A. (2004). Aromatase-deficient mice spontaneously develop lymphoproliferative autoimmune disease resembling Sjogren's syndrome. *Proc. National Acad. Sci. U.S.A. 101*, 12628–12633.

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.

Sholitan, L.J., Grossman, C.J., and Taylor, B.B. (1980). Rat thymic homogenates convert testosterone to androgenic metabolites. *J. Steroid Biochem. 13,* 1365.

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.

Smith, H., Chen, I.M., Kubo, R., and Tung, K. (1989). Neonatal thymectomy results in a repertoire enriched in T cells deleted in adult thymus. *Science 245,* 749–752.

Soffer, Y., Caspi, E., Peller, S., and Weinstein, Y. (1982) Modulation of endometrial lymphocyte response in menstrual cycle of fertile women. *Am. J. Reprod. Immunol. 2,* 18–22.

Spector, T.D., Brennan, P., Harris, P., Studd, J.W.W., and Silman, A.J. (1991). Does estrogen replacement therapy protect against rheumatoid arthritis? *J. Rheumatol. 18,* 1473–1476.

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*, 676–683.

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.

Stimson, W.H. (1988). Estrogen and human T lymphocytes: presence of specific receptors in the T-suppressor/cytotoxic subset. *Scand. J. Immunol. 28,* 345–350.

Sullivan, D.A., Kelleher, R.S., Vaerman, J.P., and Hann, L.E. (1990). Androgen regulation of secretory component synthesis by lachrymal gland acinar cells *in vitro*. *J. Immunol. 145*, 4238–4244.

Talal, N. (1981). Sex steroid hormones and systemic lupus erythematosus. *Arthritis Rheum. 24,* 1054.

Taylor, P.V., Campbell, J.M., and Scott, J.S. (1989). Presence of autoantibodies in women with unexplained infertility. *Am. J. Obstet. Gynecol. 161,* 377–379.

Terres, G., Morrison, S.L., and Habicht, G.L. (1968). A quantitative difference in the immune response between male and female mice. *Proc. Soc. Ex. Biol. Me. 27,* 664.

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.

Tuo, W., Ott, T.L., and Bazer, F.W. (1993). Natural killer cell activity of lymphocytes exposed to ovine, type I, trophoblast interferon. *Am. J. Reprod. Immunol. 29,* 26–34.

Van Vollenhoven, R.F. and McGuire, J.L. (1994). Estrogen, progesterone, and testosterone: can they be used to treat autoimmune diseases? *Cleve. Clin. J. Med. 61*, 276–284.

Van Vollenhoven, 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.

Van Vollenhoven, and McGuire, J.L. (1996). Studies of dehydroepiandrosterone (DHEA) as a therapeutic agent in systemic lupus erythematosus. *Ann. Med. Interne (Paris.) 147*, 290–296.

vanVollenhoven, R., Engleman, E.G., and McGuire, J.L. (1994). An open study of dehydroepiandrosterone in systemic lupus erythematosus. *Arthritis Rheum. 37,* 1305–1310.

VanVoorhis, B.J. and Stovall, D.W. (1997). Autoantibodies and fertility: a review of the literature. *J. Reprod. Immunol. 33,* 239–256.

Verheul, H.A., Tittes, E.V., Kelder, J., and Schuurs, A.H. (1986). Effects of steroids with different endocrine profiles on the development, morphology and function of the bursa of Fabricius in chickens. *J. Steroid Biochem. 25,* 665–675.

Waltman, S.R., Brude, R.M., and Benios, J. (1971). Prevention of corneal rejection by estrogens. *Transplantation 11,* 194.

Weidler, C., Harle, P., Schedel, J., Schmidt, M., Scholmerich, J., and Straub, R. (2004). Patients with rheumatoid arthritis and systemic lupus erythematosus have increased renal excretin of mitogenic estrogens in relation to endogenous antiestrogens. *J. Rheumatol. 31,* 489–494.

Weinstein, Y. and Berkovich, Z. (1981). Testosterone effect on bone marrow, thymus and suppressor T cells in NZB × NZW F1 mice: its relevance to autoimmunity. *J. Immunol. 126,* 998–1002.

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.

Wilson, C., Eade, O.E., and Elstein, M. (1975). Smooth muscle antibodies in infertility. *Lancet 1,* 238–239.

Wingrave, S.J. and Kay, S.R. (1978). Reduction in incidence of rheumatoid arthritis associated with oral contraceptives. *Lancet 1,* 569–571.

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.

Xu, C., Mao, D., and Holers, V.M. (2000). A critical role for the murine complement regulator Crry in feto maternal tolerance. *Science 287,* 498–501.

Yohn, D.S. (1973). Sex related resistance in hamsters to adenovirus oncogenesis. *Prog. Exp. Tumor Res. 18,* 138.

Yui, J., Garcia Lloret, M., and Wegmann, T.G. (1994). Cytotoxicity of tumor necrosis factor alpha and γ-interferon against primary human placental trophoblasts. *Placenta 15,* 819–835.

CHAPTER

26

Animal Models of Autoimmune Disease

MATTHIAS VON HERRATH*

**Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, CA 92121 USA*

GEORGE C. TSOKOS†

†Department of Cellular Injury, Walter Reed Institute of Research, Bldg 503, Rm 1A32, Silver Spring, MD USA 20910

BACKGROUND

Emerging Knowledge Gaps in Understanding the Pathogenesis of Autoimmunity

Despite intensive research over the past decades, there are some crucial elements in human and animal autoimmunity that we do not comprehend yet. One major one is the nature of the initiating autoantigens and precipitating events (Bach et al., 1998) and for most major autoimmune diseases we cannot precisely define the targets of the initial autoimmune response, exceptions being gluten hypersensitivity (Lundin et al., 1993) and pemphigus vulgaris (Veldman et al., 2004). However, difficulties in defining the primary initiating autoantigen(s) pertain not only to human disease but also to animal models of autoimmunity, such as the nonobese diabetic (NOD) mouse where we can readily access the target organs (Bach et al., 1998, Horwitz et al., 2002). Novel approaches to identify instigating autoantigens and the nature of the early autoimmune response may require novel high-throughput technologies. Identifying autoaggressive T-cell specificities on an individual basis will be crucial. The heterogeneity in individual T- and B-cell repertoires even in inbred mice is likely responsible for the fact that only very rarely do all individuals of a given inbred mouse strain exhibit the same course and severity of disease (Sercarz, 2000). Deciphering of underlying heterogeneity in autoreactive repertoires will be key to understanding and predicting development of autoimmune diseases. Real-time imaging and selective detection technologies, potentially relying on high-throughput proteomic approaches, are being developed to overcome this impasse (Robinson et al., 2003). Thus, heterogeneity in individual repertoires might result in differential recognition patterns of autoantigens, which impede the recognition of autoantigens that are always targeted. However, we have defined a relatively large number of self-antigens for many diseases, which has been possible by analyzing specificities of autoantibodies and, more recently, by using knockout technology (Eisenbarth et al., 2002; Greeley et al., 2002; Moriyama et al., 2003; Robinson et al., 2003; Mantegazza et al., 2004).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

An additional factor that interferes with the identification of the initiating autoantigens is antigenic spreading that occurs during disease progression. Both human and animal studies indicate that once an autoimmune process has been initiated, there is a rather rapid increase in the number of autoantigenic targets (Katz-Levy et al., 1999; Croxford et al., 2002). It is unclear whether tolerizing "driver" clones would be sufficient to reverse an ongoing autoimmune process and have therapeutic value. Restricted T-cell receptor (TCR) usage in pancreatic-draining lymph nodes of diabetic individuals must be investigated to understand whether a few clonally expanded T cells can cause destruction of the pancreatic islets. Recent investigations suggest a striking oligoclonality, which might make deletional immunotherapy using one or a few autoantigenic determinants feasible (Hafler et al., 2005). This concept is supported by the fact that only a few antigen-specific cytotoxic T cells (2/100 islet-infiltrating $CD8^+$ lymphocytes) are required to initiate diabetes to a defined autoantigen expressed in β cells in a transgenic diabetes model (Christen et al., 2004).

In the immunogenetic arena, animal models have provided useful insights. For diseases that are determined by one or a few genes, and where no major epigenetic factors are involved, we have learnt much from immunodeficient animals as well as humans. However, in some cases the respective human immunodeficiency will yield a different phenotype than the genetic knockout mouse, i.e., ICOS, IRAK (Medzhitov et al., 1998; Dong et al., 2001; Grimbacher et al., 2003; Puel et al., 2004), illustrating differences between men and mice that are likely linked to epigenetic and environmental factors. For example, ICOS deficiency in humans has immunologic consequences that are found in some patients with common variable immunodeficiency. In contrast, the ICOS-deficient mouse has a rather uniform phenotype involving immune deviation of T-cell responses and their cytokine production, as well as effects on B-cell function. Overall, comparison between mouse models and the humans deficient in respective genes greatly contributes to our understanding of autoimmunity (AIRE, fox-P3, CD25) (Sakaguchi et al., 1982; Anderson, 2002; Ramsdell and Ziegler, 2003), even if complete phenotypic homology between knockout mice and the respective human genetic deficiency is lacking. Unfortunately, we can be certain that one single genetic defect or polymorphism will not be the cause for most human autoimmune disorders. We have learnt recently that the far more common scenario is a substantial degree of polygenic complexity involving many protective and predisposing genes that act in concert, leading to disease manifestation in a fraction of their bearers. For example, in addition to major histocompatibility complex (MHC) class II molecules that are found in significant association with several autoimmune diseases in mouse models and humans i.e. multiple sclerosis (MS), type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), other genetic factors with rather low LOD-scores (McDevitt, 1998; Todd and Wicker, 2001; Weber et al., 2003; Bielekova et al., 2004) contribute to the expression of autoimmune disease. Frequently, these associations are weak enough to allow for the possibility that environmental factors in addition to genetic ones determine penetrance of disease (Bielekova et al., 2004). In addition, frequently the genes or regions that are connected more weakly with a given disease functionally point towards immunologic mediators that are already known and whose effect on pathogenesis has been well studied, i.e. the interleukin 2 (IL-2) locus and polymorphism in cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) genes that are associated with T1D (Lyons and Wicker, 1999; Hill et al., 2000; Ueda et al., 2003; Tabeta et al., 2004). Therefore, immunogenetics in animal models is at an important crossroads: seeking direct relevance to the human disorder becomes very important, and the assumption that there is a necessity to identify genetic defects in animal models in order to obtain the best pathways to cure human disease may not be correct. Indeed, in today's medicine, there is little evidence that the optimal treatment for a given disease is always the elimination of its cause. Immunogenetics in animal models might still help the adoption of novel predictive strategies and subclassifications for autoimmune diseases, which might in the future allow for more individualized treatments. However, genetic studies carried out in humans become of paramount importance. Future promising strategies involve large-scale haplotype mapping initiatives in humans (van Heel et al., 2004). In addition, reverse genetic approaches carried out in animals can define novel genetic causes for diseases, since this approach is a phenotype- rather than a genotype-driven screening approach (Tabeta et al., 2004).

Animal models have helped us understand the complexity of biologic processes and be cautious in predicting the limitations of therapeutic interventions in humans. For example, in T1D, there are many different pathways to kill β cells. If one of them is inactivated (i.e., perforin or interferon-γ knockout), others will take over. The consequence is that it will be very difficult to engineer a "death-defying" β cell that could be used for islet transplantations, since too many death pathways have to be eliminated in order to achieve resistance to immune destruction (Thomas and Kay, 2001).

Animal Models—Advantages and Disadvantages

As illustrated in Box 26.1, there are several areas of investigation, where we have learned much from animal models. The first is investigation of immune responses at different locations. This is particularly important for under-

Box 26.1
What can animal models teach us that we cannot learn otherwise?

- *In vivo* immune kinetics at **sites that are difficult to access** in humans.
- **Proof of concept** using techniques that cannot be used in humans, i.e., adoptive transfers, genetic knockouts, conditional knockouts, etc.
- Large-scale assessment of **dose range, toxicity,** and immunization sites in drug and vaccine development.
- *In vivo* events that would never have been anticipated.

standing autoimmune diseases that frequently affect certain sites, cell types, or organs. Second, it is difficult to establish proof of concept in humans. Lastly, of course, the use of animal experimentation is at this point frequently indispensable to defining dose ranges and regimens for immunotherapies.

How far reaching should the conclusions that we draw from observations in a given animal model be? The prevailing attitude is still that once a discovery has been made in one of the animal models thought to faithfully represent the human disease (i.e., the NOD mouse for diabetes, EAE model for MS or NZB mouse for SLE) there is no urgent need to look at this issue in other model systems. If the same result is found, it is considered "confirmatory", if a different result is discovered, the "secondary" model is frequently labeled as not-as-good or even flawed, for which then various reasons are cited. This, we believe, can be treacherous and may hamper translation of research performed in animal models, because our pathogenetic insight into human diseases is often still rather limited due to ethical constraints. For example, only about 10% of patients with T1D exhibit the same clinical features as the NOD diabetic mouse, which is characterized by a polyglandular autoimmune syndrome affecting thyroid, salivary glands, and testes (Atkinson and Leiter, 1999; Roep et al. Nature Reviews Immunology 2004). However, there are also striking similarities between NOD and human diabetes, for example, the occurrence of autoantibodies that precedes the development of clinical disease in NOD mice and humans (Pietropaolo and Eisenbarth, 2001). Another caveat in directly applying scenarios from animal models to humans is the MRL-lymphoproliferation (lpr) mouse. The lpr mutation in the MRL mouse results in autoimmunity that is perfectly correctable by transfer of the Fas gene. Yet, defects in Fas expression and Fas-mediated signaling leads to autoimmune lymphoproliferative syndrome (ALPS) in humans (Silvestris et al., 1996; see also Chapter 70). Similarly, the generalized lymphoproliferative disease (gld) mutation (defective FasL expression) in mice leads to systemic autoimmunity, yet only very rare patients with SLE have a mutation in the FasL gene (Lin et al., 2004). It follows that we should elevate the importance of validation of crucial findings in other model settings.

ANIMAL MODELS FOR ORGAN-SPECIFIC AUTOIMMUNE DISEASES

Animal Models of Spontaneous Organ-Specific Autoimmunity

The widely utilized NOD mouse expresses multiple autoimmune features in various organs—sialitis, T1D, orchitis, thyroiditis (Atkinson and Leiter, 1999)—and is prone to develop other autoimmune disorders including autoimmune hepatitis in NOD congenics (Ridgway et al., 1999), EAE (Winer et al., 2001; 2003), neuritis in NOD 86 (B7.2) deficient strains (Salomon et al., 2001). It was discovered in Japan and exhibits spontaneous diabetes penetrance of 20–100% depending on the environment (see Chapter 36). In addition, females develop diabetes about four times as frequently as males. In contrast to most patients with diabetes, the NOD mouse develops multiglandular and other organ-specific autoimmunity. The immunologic causes for diabetes development are uncertain, but the genetics of the NOD has been studied extensively and is strikingly similar to the genetics of human T1D (Lyons and Wicker, 1999), particularly in MHC class II molecules linked to disease expression (McDevitt, 2002). In addition, susceptibility loci that contain, among others, the genes for IL-2 and CTLA-4 have been linked to disease or protection. In the case for CTLA-4, polymorphisms have been described in the NOD mouse that might play a similar role in the human disease. However, in both humans and NOD mice, environmental factors strongly influence disease expression. Monozygotic twins exhibit discordance in T1D penetrance, and NOD mice do not develop diabetes unless they are kept under specific pathogen-free (SPF) conditions. Current investigations indicate that viral infections can have both enhancing and protective effects in T1D animal models (Christen et al., 2004) and a large-scale investigation to define potential associations with infectious events or their absence is under way in humans (Graves et al., 2003).

Immunologically, it is still unclear to what degree NOD mice and humans with T1D are similar. The appearance of autoantibodies predicts the risk to develop islet autoimmunity and, in many cases, T1D on an individual basis; the antigens targeted, however, differ that for NOD mice autoantibodies almost exclusively recognize insulin, whereas anti-GAD and anti-IA2 are present in humans (Eisenbarth, 2003). T lymphocytes with a Th1-like profile correlate with destructive insulitis in the NOD and, as reported more recently using a panel of pro-insulin peptides, also in humans (Arif et al., 2004). However, the question of

which features characterize an "optimal" autoaggressive T cell is not resolved. Transfer studies in the NOD model have shown that Th1 as well as Th2 cells can cause disease in immunodeficient NOD/SCID or NOD/RAG hosts, when sufficient cell numbers are transferred (Pakala et al., 1997). There may be a determining factor that will always correlate with autoaggression, or the destructive immune response may be adaptable and able to use multiple pathways to destroy β cells, which seems the more likely answer (Kay et al., 2003). The situation is not that different for the responses of regulatory T cells (Tregs). Regulatory T cells are thought to control most immune responses and can be antigen-induced (adaptive) or spontaneous (likely autoantigen specific and mostly characterized by constitutive CD25 expression). In NOD mice, such cells can protect from diabetes, but until now only when cotransferred into immunodeficient recipients together with diabetogenic T cells. This is not the case in other animal models and their antigen-specific induction and transfer in immune competent hosts has been described (see below). In human T1D, lymphocytes with increased IL-10 production in response to autoantigens have been found in healthy but not prediabetic or diabetic individuals, indicating the possibility of autoreactive regulatory or beneficial responses (Peakman et al., 2001). Indeed, such physiological autoreactivity might be essential to maintain self-tolerance in healthy individuals. Overall, the predisposition to autoimmunity in the NOD mouse could also be linked to defective thymic selection and/or presentation of autoantigens in the thymus (Sprent and Surh, 2003; Stratmann et al., 2003). Here, mechanistically, the predisposing MHC class II allele may also play a role by possibly insufficiently presenting self-peptides, resulting in suboptimal elimination of a variety of autoreactive T-cell specificities. Indeed, autoantigens and their variants that are targeted by the aggressive organ-specific response might be characterized by insufficient expression in the thymus, which has been observed in several animal models as well as humans (Pietropaolo et al., 2002).

Genetic Engineered Animal Models for Organ-Specific Autoimmunity

Genetic engineered defects involving the systemic deficiency or overexpression of a distinct molecule will rarely result in a disease that is limited merely to one or a few target organs. Systemic knockouts that "take the brakes off" the immune system such as foxp3 (defective Tregs), AIRE (defective thymic negative selection), Cbl-b (defective signaling), lpr (defective lymphocyte elimination), TGF-β (defective regulation) usually result in disease affecting multiple organs and are, therefore, discussed under the respective section in systemic autoimmune diseases (Anderson et al., 2002; Gorelik and Flavell, 2002; Walker et al., 2003; Liu, 2004). There are, however, a few exceptions, one being the CD86-(B7.2)-deficient NOD strain (Salomon et al., 2001). The autoimmunity that is usually directed towards the pancreatic islets in the NOD was redirected to attack peripheral nerves, resulting in neuritis. The reason may be altered local regulation of costimulatory molecules as well as defective thymic selection, resulting in higher levels of naïve T cells that can recognize neuronal antigens.

In summary, although systemic immune defects will more likely lead to more severe autoimmunity affecting multiple organs, there are exceptions to the rule. In humans, a combination of genetic factors leads to an overall predisposition state, yet not to actual clinical disease. Then, manifestation of organ-specific autoimmunity may occur after a local inflammatory insult, either a virus infection (Von Herrath et al., 2003) or other events that could lead to expansion of autoaggressive T cells (Von Herrath, 1996).

Animal Models for Induced Organ-Specific Autoimmune Diseases

Among many antigen-specific models for diabetes and other organ-specific autoimmune disorders are those based on transgenic technology to express a defined autoantigen under the control of a tissue-specific promoter—e.g., the rat insulin promoter (RIP) to direct expression to β cells (von Herrath et al., 1994); the keratin promoter to direct expression to the skin (McGargill et al., 2002); myelin basic protein (MBP), neural-specific enolase (NSE), or glial-fibril acidic protein (GFAP) promoters to achieve expression in the brain, liver, or eye (de la Torre et al., 1993; Rall et al., 1995; Evans et al., 1996). For diabetes, examples include the mouse models RIP-Tag (large T-antigen from SV40 virus), RIP-Ova (ovalbumin), RIP-HA (influenza hemagluttinin) and RIP-LCMV (lymphocytic choriomeningitis virus-derived proteins) (Ohashi et al., 1991; von Herrath et al., 1994; Forster et al., 1995; Kurts et al., 1996; Murtaza et al., 2001). Such antigen-specific models have the advantage that the initiation of the autoaggressive response can be defined precisely, and at least the initial autoaggressive "driver" T cell is known. Notably, immunization with the protein antigen does not lead to diabetes in any of the RIP-antigen models. In some models, infection with a virus expressing the self-protein (transgene) or immunization in conjunction with Toll-like receptor (TLR) stimuli leads to T1D reliably (RIP-LCMV mice), whereas in other situations, tolerance cannot even be broken by viral infection (RIP-HA). In these cases, adoptive transfer of activated T cells is necessary to elicit disease (T1D), which works well in RIP-HA and Rat insulin promoter transgenic mice that express membrane-bound Ova under control of the rat insulin promoter (RIP-mOva). Overall, these antigen-induced diabetes models have been very useful to address a variety of defined questions.

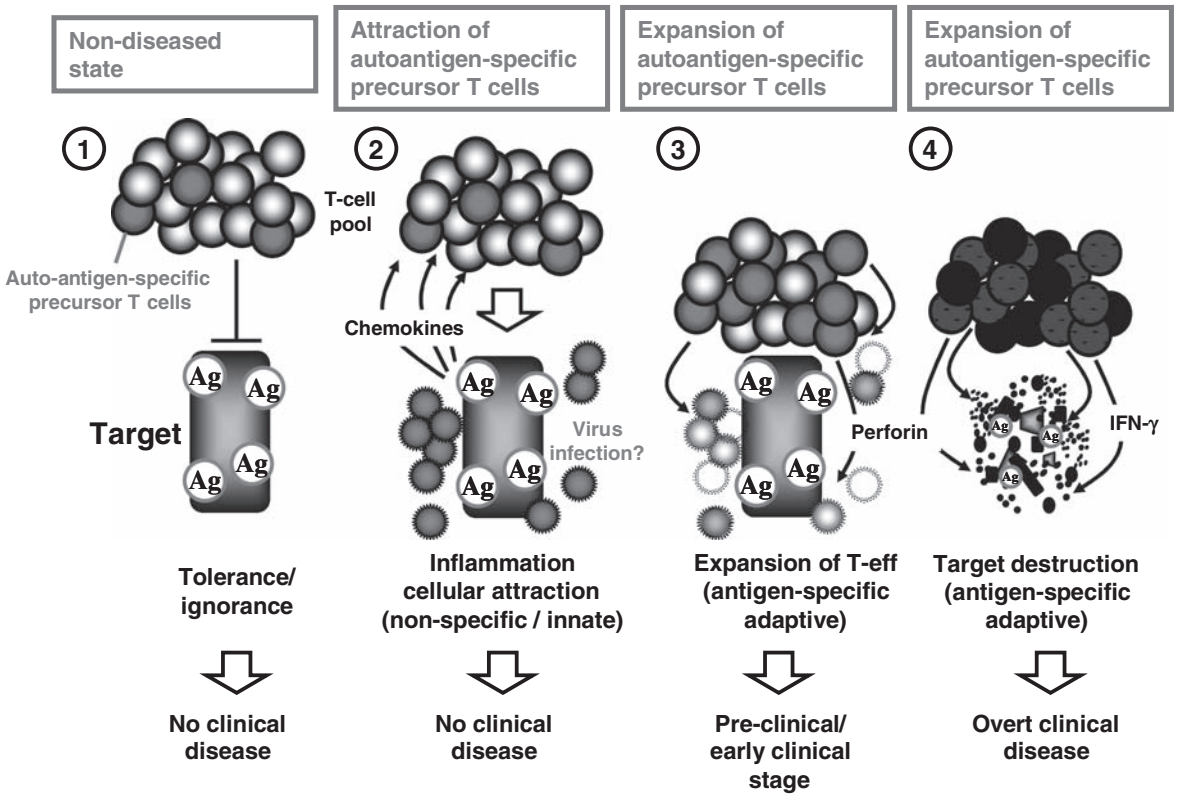


FIGURE 26.1 Pathogenesis of organ specific autoimmunity. For organ-specific autoimmunity one can assume that the initiating events might be antigen non-specific or specific. (1) A combination of genetic and environmental factors might lead to the breaking of self-tolerance and activation of autoreactive T cells. (2) Further inflammation might attract more such cells, for example, if a virus persists or autoantigens are being presented in a chronic manner. Certainly, activation of T-cell receptors and antigen-presenting cells will serve to propagate disease. (3) The ultimate outcome is determined by the magnitude and class of the autoreactive response. Many aggressive T cells, such as cytotoxic T cells and Th1 lymphocytes, will enhance progression, whereas the presence of (autoreactive) regulatory T cells (Tregs) will dampen inflammation. (4) Penetrance of clinical disease is directly correlated with the amount of target cell (organ) destruction and is determined by this balance of Tregs to aggressive T cells. Ag, antigen; IFNγ, interferon γ; Teff, effector T cell. See color plate section.

1. *Breaking tolerance to an autoantigen* is a "numbers game" and $CD8^+$ cytotoxic T lymphocytes (CTLs) can eliminate target cells such as keratinocytes or islet β cells, when activated and able to enter the organ in sufficient numbers (Figure 26.1). Interesting differences emerge here for different target organs that express the same antigen: the pancreas and skin more readily show clinical signs of autoimmunity, but the brain appears to be capable of suppressing activated autoaggressive T cells, even after they cross the blood–brain barrier, as evidenced by mild disease in MBP-LCMV-Ag transgenic mice despite intracerebral infiltration (Evans et al., 1996). $CD4^+$ help and local expression of the costimulatory CD80 (B7.1) can both reduce the requirement of $CD8^+$ cells to elicit disease, as can immunomodulatory approaches that either dampen regulatory components or enhance the lifespan (i.e., decrease apoptosis) of autoaggressive $CD8^+$ lymphocytes (von Herrath et al., 1994; 1995; Christen et al., 2001b; 2004). These rules appear generally applicable to various models. Thus, induction of organ-specific autoimmunity can be expected to follow quantitative rules and reduction of autoaggressive T cells beyond a certain threshold level will abrogate disease development.
2. *Inflammation within any specific target organ* (e.g., the pancreas, brain, eye or skin) induced by means of a local viral infection (von Herrath and Holz, 1997; Horwitz et al., 2002) can activate macrophages and "set the stage" for further development of disease. Thus, breaking of tolerance might require the association with localized specific or non-specific inflammatory processes that could have, in addition to viral infections, a variety of other causes. In some models it was demonstrated that viral recombinants that express autoantigenic mimics can aggravate disease in an antigen-specific direct way (Olson et al., 2001). In other situations, there may be activation of antigen-presenting cells that then increasingly present autoantigens (Horwitz et al., 1998). However,

viral infections can under certain conditions even protect from autoimmunity. Infection of prediabetic RIP-LCMV and NOD mice with Coxsackie virus or LCMV prevents disease in most scenarios. Hence, the autoimmune response can be redirected through an inflammatory insult, which is at least in part due to recruiting aggressive lymphocytes away from the islets (Christen et al., 2004).

3. *Regulatory cells* can prevent autoimmune disease. Using antigen-specific models one can clearly delineate the concept of bystander suppression, namely that a Treg with one antigenic specificity can downmodulate aggressive T cells with other antigenic specificities (Figure 26.2). This concept will, in our opinion, be instrumental in antigen-specific immunotherapy (Homann et al., 1999). The route of self-antigen administration to induce bystander suppressor autoreactive Tregs appears important. Oral or nasal routes induce regulatory cells more readily in various models. In this context, it is important to distinguish between direct elimination of aggressive T cells specific to the antigen used for immunization, and the induction of autoreactive regulators that can then act as bystander suppressors of a variety of antigen specific responses. Strategies that aim at eliminating or tolerizing autoaggressive T cells with specificity for one single antigenic epitope include high-dose tolerance (Vandenbark et al., 2000), the injection of 1-ethyl-3-(3-dimethylaminoproypl)-carbodiimide (ECDI)-coupled splenocytes (currently being investigated in clinical trials), and the coupling of toxic molecules to MHC tetramers or dimers. These strategies will be effective, if autoimmunity in a given individual is caused only by one or a few autoaggressive driver clones (Sercarz, 2000), or antigenic spreading has not progressed far (early in preclinical autoimmunity). In contrast, there will be a need for bystander suppression when T-cell populations with multiple autoaggressive specificities act in concert, and it might be necessary to dampen many to see an effect. At this point, it is not clear yet whether autoimmune processes are driven by a few or many specificities.
4. *Death of the target cells* (see also point 3). A close look at many of the antigen-specific autoimmune models suggests that the destruction of the attacked cell type or organ can occur via a variety of pathways that are not

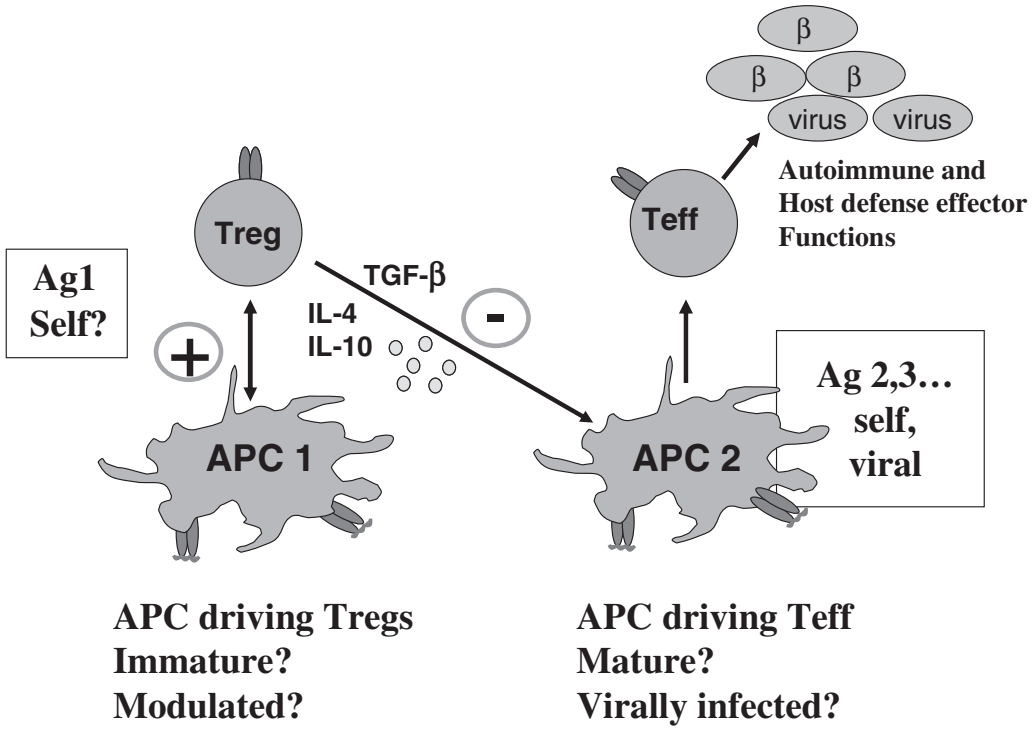


FIGURE 26.2 Regulation in autoimmunity: bystander suppression and the "drivers" of regulatory cells (Treg) and effector cells (Teff). Bystander suppression proposes that an autoreactive Treg with one specificity (Ag 1) can downmodulate autoaggressive Teff with different antigenic specificities (Ag 2, 3 . . . etc). Perhaps immature dendritic cells (DCs) favor Treg activation, whereas fully matured DCs favor Teff activation (see Chapter 4). In addition, Tregs might be set at different numeric levels *in vivo* varying in different disease stages and adaptive regulatory (Treg) function should be viewed in the context of the pathogenesis of the disease. Thus, each organ-specific autoimmune disease might have an optimal antigen-induced Treg, which is defined by the precise profile of effector cytokine or chemokine molecules secreted. Ag, antigen; APC, antigen-presenting cell; IL, interleukin; TGF-β, transforming growth factor-β.

mutually exclusive. In addition, the autoaggressive response exhibits plasticity in the sense that blocking of one specific effector arm, e.g., killing of CTL by Fas or perforin or inflammatory cytokines, will result in the emergence of alternative pathways.

How Do Animal Models Compare to Human Organ-Specific Autoimmune Disorders?

Every model will reflect certain aspects of the human disease and for this reason will be suited for some but not all of the scientific questions. Two examples of this are T1D and MS.

Type 1 Diabetes

One has to acknowledge that there is no conclusive proof that the cause for T1D in humans is (always) autoimmune, because autoantibodies found in almost all patients seem not to be necessary for disease development as evidenced by earlier plasmapheresis experiments, and the disease can occur in the absence of B lymphocytes (Martin et al., 2001). In addition, there is no association between maternal autoantibodies to islet antigens and diabetes incidence in offspring, unlike in the NOD model (Greeley et al., 2002; Ziegler et al., 2002). Therefore, perhaps the initiating events in human T1D and also major parts of the pathogenesis are not autoimmune in nature. Certain MHC class II alleles predispose to T1D, but genetic predisposition cannot alone explain development of disease (although it might fully explain autoimmunity), and other environmental factors have to be present as well (Gale, 2002) including viral infections and other, possibly epigenetic, factors. So, the NOD model resembles human T1D in many but not all aspects. In addition, it probably exhibits insufficient stringency to vigorously test immune-based interventions: some 190 interventions can prevent T1D in the NOD mouse when given early enough, but many have been unsuccessful in humans. Consequently, we should experimentally increase the stringency of the animal model test, and evaluate potential candidate interventions, both in NOD mice with recent-onset diabetes and in other diabetes models (see Chapter 75).

Multiple Sclerosis

In striking similarity to the NOD model for T1D, more than 90% of research on MS utilizes the EAE model. Just as virally-induced models are claimed to not reflect human disease faithfully, since we are not certain that a virus causes MS, we can be certain that immunization with myelin extract or peptides in conjunction with pertussis toxin and Freund's adjuvant does not cause human MS. Thus, we need a more open attitude towards alternative models that might highlight different and potentially important aspects of the human disease.

ANIMAL MODELS FOR SYSTEMIC AUTOIMMUNE DISEASES

Animal models have greatly facilitated the study of systemic autoimmune diseases, notably SLE and RA, and helped to develop rational new treatments (Figure 26.3).

Spontaneous Models of Systemic Autoimmunity

Murine models that develop spontaneously SLE have generated significant information on the role of hormones in the expression of autoimmunity (Fernandes and Talal, 1986; see Chapter 25); the contribution of aberrant immune regulation (Theofilopoulos and Dixon, 1985); the role of cytokines, chemokines, and adhesion molecules (Handwerger et al., 1994) in the expression of the disease; and, lastly, they have been used to identify loci that contribute to the genetic pool required for the development of disease (Drake et al., 1995; Choubey and Kotzin, 2002; Theofilopoulos and Kono, 2002; see Chapters 20 and 21). Gene complementation studies have shed light on the epistatic interactions of genes (Wakeland et al., 1997; Morel and Wakeland, 1998). Candidate genes have been identified including those of the CD2 family, the interferon (IFN)-inducible genes (Rozzo et al., 2001) and the complement receptor gene Cr2 (Boackle et al., 2001). These results have shown that introduction of an IFN-α/β receptor null gene into the NZB mouse results in decreased production of anti-erythrocytic antibodies (Santiago-Raber et al., 2003). Since IFN-α has been found to be increased in patients with SLE and to promote dendritic cell maturation (Blanco et al., 2001), a case can be made for the construction of biologics to limit the action of type 1 IFNs in systemic autoimmunity. Moreover, identification of contributing genes and loci in animal models has guided the search for orthologs in humans with systemic autoimmune diseases (Tsao et al., 1997; 2002; Gaffney et al., 1998; 2000; Harley et al., 1998; Moser et al., 1999).

The NZB (H-2^d) mouse develops anemia due to anti-erythrocytic antibodies. When crossed to the NZW (H-2^z), systemic autoimmunity ensues with anti-DNA antibodies and severe glomerulonephritis. The decreased average lifespan of these mice is 8 months for females and 13 months for males (Vyse et al., 1998; Ibnou-Zekri et al., 1999).

The MRL mouse, which has two doses of the lpr mutation and develops accelerated autoimmune disease, has allowed identification of genes whose products are central to expression of systemic disease. For example, while deletion of the cyclin-dependent kinase inhibitor p21 does not lead to autoimmunity (Lawson et al., 2002), transfer of the p21 null gene into the MRL$^{lpr/lpr}$ mouse results in reduced autoimmune disease by allowing T-cell death and

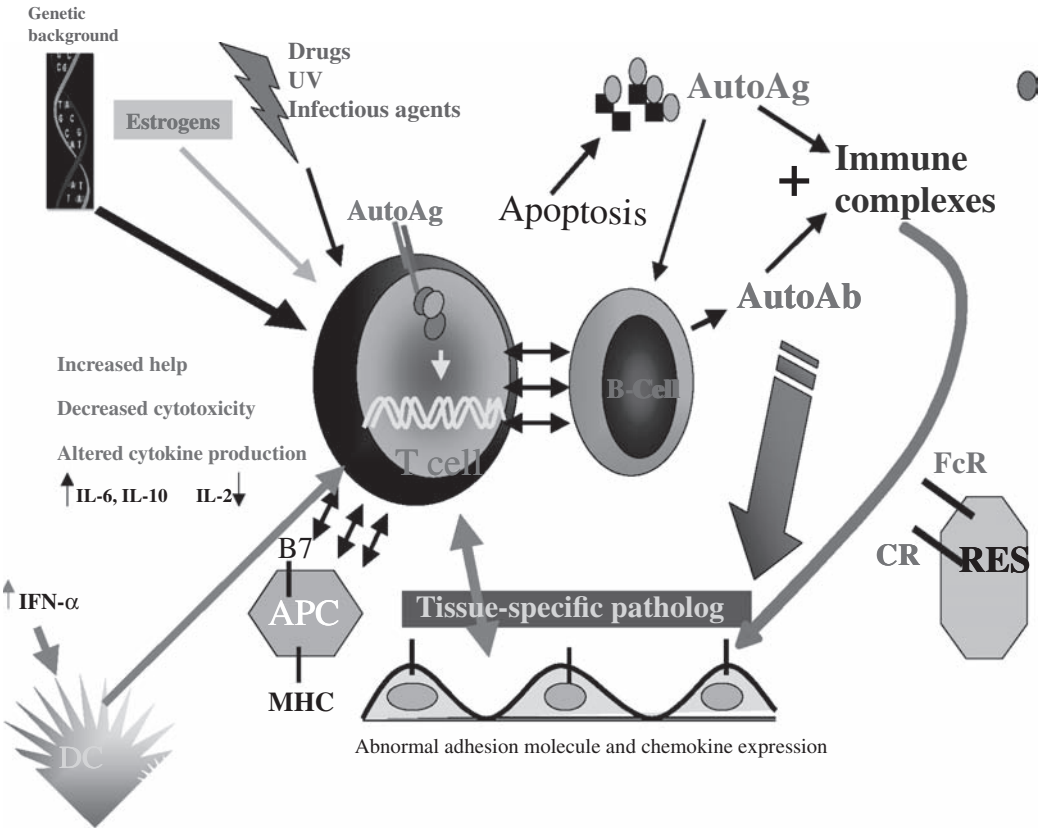


FIGURE 26.3 Pathogenesis of systemic autoimmunity compiled from studies on mice and humans with systemic lupus erythematosus (SLE). Similar to organ-specific autoimmunity, systemic autoimmunity is a function of a deleterious combination of genetic and environmental factors that lead in concert to the loss of self-tolerance. For example, certain genes such as CD40 ligand may be overtranscribed, whereas others, e.g. IL-2, may be undertranscribed. T cells may display numerous aberrations in the form of defective cytotoxic responses, defective immunoregulatory function, and increased ability to provide help to B cells to produce autoantibodies (AutoAbs). In systemic autoimmunity, autoantibodies are frequently directed against nuclear antigens, with which they form immune complexes. Defective clearance of such immune complexes is the result of defective expression and function of Fc and complement receptors, and increased levels of nuclear material may propagate the autoimmune response. Lastly, humans and mice with SLE have defects in the expression and function of costimulatory molecules and interferon-α (IFN-α) appears to be over-expressed, which stimulates dendritic cells and enhances autoantigen presentation. APC, antigen-presenting cell; AutoAg, autoantigen; DC, dendritic cell; IL, interleukin; MHC, major histocompatibility complex; UV, ultraviolet radiation. See color plate section.

decreasing the accumulation of G0/G1 arrested cells (Lawson et al., 2004).

Male only BXSB mice develop severe glomerulonephritis and autoantibodies. The genetic locus responsible for the expression of the disease is located in the Y chromosome and is known as Yaa (Y-chromosome accelerated autoimmunity). The disease is heavily dependent on the presence of the H-2^b allele, because its replacement with the H-2^d allele results in prolonged survival. Of interest, the BXSB × NZW F1 mouse develops thrombocytopenia and coronary artery disease accompanied by the presence of antiphospholipid antibodies (Kono and Theofilopoulos, 2002).

Palmerston North (PN) mice develop spontaneously autoimmunity manifested by the production of multiple autoantibodies, vasculitis and glomerulonephritis. PN × NZB F1 mice develop more severe disease (Luzina et al., 1999, Handwerger et al., 1999).

Mice homozygous for the ank mutation. ank/ank mice develop early-onset joint ankylosis in the spine and peripheral joints resembling spondyloarthropathies (Krug et al., 1989).

K/BxN T-cell receptor mice, a spontaneous mouse model of autoimmune arthritis, generated fortuitously by crossing a TCR transgenic line with the NOD strain (Monach et al., 2004), all spontaneously develop an autoimmune disease with most (although not all) of the clinical, histologic, and immunologic features of RA in humans. The murine disease is critically dependent on both T and B cells and is joint specific, but it is initiated and perpetuated by T- and B-cell autoreactivity to a ubiquitously expressed antigen, glucose

phosphate isomerase (GPI). Transfer of serum (or purified anti-GPI immunoglobulins) from arthritic K/BxN mice into healthy animals regularly provokes arthritis within a few days, even when recipients are devoid of lymphocytes. Complement components, Fc receptors, and mast cells are important for the expression of the disease. The relevance of the K/BxN model to human RA is supported by one report showing that serum from almost two-thirds of patients with RA contain anti-GPI antibodies, which are absent from serum of normal individuals or of patients with Lyme arthritis or Sjögren syndrome (Matsumoto et al., 2003). However, not all investigators agree. The K/BxN model has been particularly useful in illustrating the role of immune-inflammatory components in the development of arthritis and notably the characterization of the role of mast cells (Lee et al., 2002); yet, this model offers little evidence that autoantibodies are involved in the pathogenesis of RA.

There is a new murine model of *spontaneous, T-cell mediated arthritis*, SKG, due to a mutation of the gene encoding an Src homology 2 (SH2) domain of ζ-associated protein of 70 kDa (ZAP-70), a key signal transduction molecule in T cells. The disturbance in the thymic T-cell selection that results from absence of ZAP-70 results in the generation of arthritogenic T cells. Besides synovitis, SKG mice develop extra-articular lesions, including pneumonitis and vasculitis. Serologically, they develop high levels of rheumatoid factor (RF) and autoantibodies specific for type II collagen (Sakaguchi et al., 2003). A molecule termed synoviolin/Hrd1 was found to play a key role in the development of synovitis. This represents an E3 ubiquitin ligase, which, by promoting the growth of synoviocytes, facilitates the development of arthritis. Mice lacking synoviolin are resistant to arthritis (Amano et al., 2003).

Mice that develop spontaneous autoimmunity have helped our understanding of hormonal and immunoregulatory influences in autoimmunity, but obvious restraints limit direct transfer of this information to human disease. As for T1D in the NOD mouse model, some hundreds of measures are curative for SLE in these mice; yet, we are still left with just three (aspirin, prednisone and plaquenil) that are approved by the Food and Drug Administration (FDA) for the treatment of human SLE! Treatment of humans with hormones, cytotoxic drugs, and rational biologics (including anti-CD154 (CD40) ligand, tolerizing small DNA tetramers, and others) has had limited success or elicited serious side effects. However, some monoclonal antibody treatments such as tumor necrosis factor (TNF) blockade and anti-CD20 are promising (See Chapter 76). Perhaps, each of the models may only reflect a proportion of SLE patients, which may account for the failure to develop effective therapeutics so far. Further, human disease develops in individuals with a permissive genetic background in conjunction with environmental and stressful factors, possibly acting over a long period. Clinical researchers can study the disease only in patients in whom significant pathogenetic processes have occurred and tissue injury has begun. We may have to accept that it will be more difficult to alter pathogenic pathways that have already been set into motion and focus our attention on understanding and limiting the tissue injury-mediating processes.

Genetically Manipulated Models of Systemic Autoimmunity

The advent of genetic manipulation has led to development of numerous single-gene modified strains that develop autoantibodies and other features of SLE. These mice have provided great insights into the mechanisms that govern tolerance and autoimmunity and suggested novel rational treatments.

Defects in Signaling Molecules

Human SLE T cells are known to express less TCR-ζ chain (Liossis et al., 1998) and to use alternative signaling though the FcR-γ chain (Enyedy et al., 2001). Mice that lack TCR-ζ chain develop autoimmune manifestations and display disturbed positive and negative thymic selection (Yamazaki et al., 1997), and the phenotype can be rescued successfully by introducing the FcR-γ chain (Shores and Love, 1997). The pathophysiology for the murine and human phenotypes may well differ and, although in mice lack of the ζ chain may lead to autoimmunity by altering early thymic events that limit the export of autoimmune T cells, in humans the rewiring of the TCR with the newly upregulated FcR-γ chain will lead to increased TCR-mediated signaling processes (Tsokos et al., 2003).

The *B7-CD28/CTLA-4 costimulatory pathway* is pivotal for T-cell activation. Signaling through this pathway is complex due to the presence of at least two B7 family members, CD80 (B7-1) and CD86 (B7-2), and two counter-receptors, CD28 and CTLA-4. CTLA-4-deficient mice rapidly develop lymphoproliferative disease with multi-organ lymphocytic infiltration and tissue destruction, with particularly severe myocarditis and pancreatitis, and die by 3–4 weeks of age (Tivol et al., 1995). CTLA4-Ig limits effectively murine lupus (Finck et al., 1994) and the use of CTLA-4-Ig biologics has helped patients with RA (Kremer et al., 2003) and psoriatic arthritis (Abrams et al., 2000).

Program death-1 (*PD1*) is a member of the CD28 family of receptors and its intracytoplasmic domain defines an immunoreceptor tyrosine-based inhibition motif (ITIM), and when engaged it delivers a negative signal. Strains of mice lacking the PD1 gene develop either cardiomyopathy (Okazaki et al., 2003) or lupus glomerulonephritis (Nishimura et al., 1999). PD1 polymorphisms have been identified among SLE patients (Prokunina et al., 2004).

Src homology 2-containing phosphatase 1 (*SHP1)* is one of the best-characterized protein tyrosine phosphatases (PTPase). The homozygous loss in mice leads to the "moth-eaten" phenotype characterized by spotty hair loss and abnormalities in the immune system that lead to systemic autoimmunity and skin inflammation (Kozlowski et al., 1993; Tsui et al., 1993; Bignon and Siminovitch, 1994). T lymphocytes from these mice are hyper-responsive to TCR stimulation (Pani et al., 1996). Since neutrophils from "moth-eaten" mice demonstrate increased oxidant production, surface expression of CD18, and adhesion to protein-coated plastic, the autoimmune phenotype in these mice may not directly reflect lymphocyte aberrations (Kruger et al., 2000).

Other lines of research suggest T cells from *lupus prone mice (MRL/lpr) that are transgenic for a specific receptor* have decreased antigen-initiated T-cell stimulation thresholds (Vratsanos et al., 2001; Bouzahzah et al., 2003). A similar concept of an "over-excitable" antigen-initiated proximal lymphocyte signaling phenotype has been proposed for human SLE (Tsokos et al., 2003). There is no information on the levels and function of phosphatases in spontaneous models of autoimmunity or in human SLE and this appears a promising area of study.

Mice with *N-acetylglucosaminyltransferase deficiency* show decreased glycosylation of T-cell membrane proteins, which prevents galectin binding and thereby disrupts the galectin-glycoprotein lattice leading to increased clustering of TCR (Demetriou et al., 2001). Increased TCR clustering in these autoimmune mice provides a phenotype comparable with human SLE, involving lowered T-cell activation thresholds and increased TCR signaling. In human SLE T cells, increased association of the TCR-ζ chain with lipid-rafts, as well as membrane clustering, are claimed to lead to decreased tolerance and abnormal signaling (Nambiar et al., 2002). Thus, defects in the expression and function of glycosylation processes may predispose to autoimmunity and, along the same lines, glycosylation of the transcription factor Elf-1, which enables the 80-kDa form to transform to the DNA-binding 98-kDa form, is defective in human SLE T cells (Juang et al., 2002).

A state of B lymphocyte hyperactivity resembling SLE is seen in *mice lacking the src-family kinase Lyn* (Chan et al., 1997). Lyn is not required to initiate B-cell antigen receptor (BCR) signaling, but is an essential inhibitory component. $Lyn^{-/-}$ B cells have a delayed but increased calcium flux and exaggerated negative selection responses in the presence of antigen, and spontaneous hyperactivity in the absence of antigen. B cells in human SLE have a similar phenotype by displaying increased calcium responses following BCR stimulation and they appear to have decreased levels of Lyn kinase protein and mRNA (Liossis et al., 2001). On the other hand, Lyn deficiency causes increased tolerance of Ig^{HEL} B cells to soluble hen-egg lysozyme (HEL) (Cornall et al., 1998). Sustained activation of Lyn *in vivo* using a targeted gain-of-function mutation ($Lyn^{up/up}$ mice) led to the development of autoantibodies and lethal autoimmune glomerulonephritis. Interestingly, B cells show a heightened calcium flux in response to BCR stimulation (Hibbs et al., 2002). These data in humans and mice suggest that mechanisms that lead to sustained BCR signaling may override control mechanisms and lead to autoimmunity.

CD22 is a B lineage-specific protein member of the sialoadhesin family with a still unknown ligand. The intracytoplasmic domain defines three immunoreceptor tyrosine-based inhibitory motifs (ITIM), which provide anchors for inhibitory downstream molecules. The BCR-stimulated calcium fluxes are increased in B cells from $CD22^{-/-}$ mice, and one line of such mice develops autoimmune features (O'Keefe et al., 1999).

Toll-like receptors recognize bacterial components and they share MyD88 to process signaling following the engagement of proper ligands. The AM14 transgenic mouse expresses a heavy chain that is a component of a rheumatoid-factor heavy chain that recognizes IgG2a and corresponding immune complexes (Wang and Shlomchik, 1997). The use of MyD88-deficient mice has enabled the molecular characterization of the role of infectious agents in the expression of autoimmunity, since B cells from $AM14/MyD88^{-/-}$ animals fail to respond to IgG2a immune complexes containing chromatin (Leadbetter et al., 2002). Use of the already available TLR (1 through 9) knockout mice in this and other systems should help to delineate the mechanisms whereby infectious agents influence the expression of autoimmunity at the molecular level. It is known that patients with SLE experience a flare after an infection.

Increased levels of DNA/anti-DNA immune complexes can initiate tissue inflammation and also may provide positive feedback to B cells through cognate recognition of nuclear antigens by the BCR, and costimulation through TLR9. Mice defective in DNAse1 develop systemic autoimmunity (Napirei et al., 2000). In addition, there are reports of two patients with SLE with defects at DNAse1 locus (Yasutomo et al., 2001). These findings reinforce the concept that complement proteins clear nuclear material from the circulation, and suggest that it may be useful to focus on novel treatments to lower circulating DNA levels in SLE.

Antigen presented in the context of immune complexes engages not only the BCR but also the FcγRIIb, which results in the phosphorylation of the ITIM defined by its intracytoplasmic domain. Recruitment of the phosphatases SHIP (Src homology 2 domain-containing inositol-5-phosphatase) and SHP1 suppresses signaling. Introduction of the FcγRIIb null phenotype into the C57BL/6 background caused production of autoantibodies and glomerulonephritis (Bolland and Ravetch, 2000). Yet the same null phenotype on the BALB/c background did not result in autoimmunity.

Although SHIP is considered responsible for FcγRIIb-mediated suppression, the SHIP null phenotype does not result in autoimmunity (Helgason et al., 2000). In humans, polymorphisms of the Fc receptors have been associated with systemic autoimmunity and particularly SLE and Wegener's granulomatosis (Kimberly et al., 1995; Gibson et al., 1999). The therapeutic effect of many biologics that contain IgG Fc components could be determined by the FcR polymorphism expressed by the treated subject.

Complement Protein Knockout Mice

The B6/lpr mouse develops minor autoimmune features. Introduction of the *CR1/CR2 deficiency* into this strain permits development of intense autoimmune features (Boackle and Holers, 2003) indicating that complement receptors are important in the elimination of B cells that display reactivity with self antigens. This explanation assumes that self antigens initiate a strong B-cell signal, which leads to B-cell death and the absence of the CR2-mediated enhancement of the signal permits their survival (Tsokos et al., 1990; Dempsey et al., 1996). When the complement receptor deficiency was introduced into the HEL double transgenic mouse model for cell tolerance, the paucity of production of anti-HEL antibodies was not reversed; yet, the numbers of circulating B cells increased, and they responded normally to stimulation (Fischer et al., 1998; Prodeus et al., 1998) indicating that there is some effect on B-cell biology, but not enough to break tolerance. These two sets of experiments suggest a relative role for CR2 in the maintenance of tolerance for self antigens—that is, the absence of CR2 can cause autoimmune disease only when there is genetic predisposition to autoimmunity. Also, evidence for the involvement of Cr2 in systemic autoimmunity comes from the NZM2410 mouse. A major murine SLE susceptibility locus, Sle1, which corresponds to three loci independently affecting loss of tolerance to chromatin, has been identified in this congenic mouse. The congenic interval corresponding to Sle1c contains Cr2, which encodes CR1/CR2 (CD35/CD21). NZM2410/NZW Cr2 exhibits a single nucleotide polymorphism that introduces a novel glycosylation site, resulting in higher molecular weight proteins. This polymorphism, located in the C3d binding domain, reduces ligand binding and receptor-mediated cell signaling. Molecular modeling based on the recently solved CR2 structure in complex with C3d has revealed that this glycosylation interferes with receptor dimerization (Boackle et al., 2001).

In contrast to these experiments, when $Cr2^{-/-}$ mice were immunized with myosin, they failed to develop the expected myocarditis (Kaya et al., 2001). Regardless of the claimed role of CR2 on the surface of T cells as part of an activated T-cell phenotype, the role of complement in this inducible model of autoimmune disease must be different from that in the spontaneous models of autoimmunity. This implies distinct functions for complement as an effector of tissue pathology and regulator of positive and negative selection.

C1q deficiency alone is sufficient to cause autoimmunity in humans, whereas in mice a genetic lack of C1q does not suffice, since there was no evidence of autoimmunity in C1q-deficient C57BL/6 and C57BL/$6^{lpr/lpr}$ mice. However, in C1q-deficient MRL/$Mp^{+/+}$ mice, there was an acceleration of both the onset and the magnitude of antinuclear antibodies (ANA) and of glomerulonephritis, particularly in females, which developed severe crescentic glomerulonephritis. Thus, the expression of autoimmunity in C1q-deficient mice is strongly influenced by other background genes (Botto and Walport, 2002; Moldenhauer et al., 1987).

Since *disruption of the C1q, C4 and CR1/CR2* leads to reduced selection against autoreactive B cells and to impaired humoral responses, C1 and C4 could act through CR1/CR2 to enhance humoral immunity and suppress autoimmunity, but each complement component appears to act independently (Chen et al., 2000). High titers of spontaneous ANA and "SLE-like" autoimmunity developed in all $C4^{-/-}$ females and most male animals but not in $Cr2^{-/-}$ animals. The fact that the clearance of circulating immune complexes was impaired in pre-autoimmune $C4^{-/-}$, but not $Cr2^{-/-}$ mice favors the role of nuclear antigen-ANA immune complexes in the development of autoimmune disease.

Membranoproliferative glomerulonephritis (MPGN) occurs in *factor H-deficient humans* and pigs. Pickering et al. (2002) showed that mice deficient in factor H ($Cfh^{-/-}$ mice) develop MPGN spontaneously and are predisposed to renal injury caused by immune complexes. The contribution of complement activation was confirmed, because, after a second mutation in the gene encoding complement factor B was introduced which prevents C3 turnover *in vivo*, the phenotype of $Cfh^{-/-}$ was not expressed any more. Thus, uncontrolled C3 activation *in vivo* is essential for the development of MPGN due to deficiency of factor H. The mechanism can include a direct effect of C5b-9 on mesangial cell proliferations (Niculescu et al., 1997) or through the production of C5a. Thus, in patients with systemic autoimmunity, tissue injury is not caused not only by circulating immune complexes but also through the action of complement-activation products.

Glomerular deposition of C3 is quite typical for glomerulonephritis in both humans and mice. However, some humans with C3 deficiency have been found to develop glomerulonephritis, whereas C3- and C4-deficient mice were protected from the development of anti-glomerular basement membrane (GBM)-mediated nephritis (Sheerin et al., 1997). In B6/lpr mice that produce low titer anti-DNA antibodies without developing glomerular disease, C4 deficiency enhances autoantibody production and proliferative glomerulitis occurs. C3 deficiency, however, neither

influenced antibody production nor led to renal disease (Prodeus et al., 1998). In MRL/lpr mice, C3 deficiency had minimal to no effect on skin disease, spleen size, B-cell or T-cell numbers, B-cell activation, pathologic renal scores, or production of autoantibodies. C3 deficiency did, however, result in significantly greater albuminuria and glomerular IgG deposition compared with wild-type C3-producing littermates (Sekine et al., 2001). Therefore, C3 may actually not be needed for the development of glomerulonephritis, and its role could be protective: it may serve either in the facilitation of clearance of immune complexes, or indirectly control autoreactive cells during their expansion.

The experiments discussed above point to a dichotomous effect of complement in immunopathology: one effect is on the negative and positive selection of the immune cell repertoire and the other is on effector processes.

Tumor Necrosis Factor/Tumor Necrosis Factor Receptor Family

The TNR/TNFR system acts on the homeostasis of the immune system in different ways. Tumor necrosis factor is produced in response to many stimuli, and after binding to TNFR1 (p55) or TNFR2 (p75) it may result in cell death or cell survival, respectively. The role of TNF in the expression of human and murine autoimmunity is complex and anti-TNF treatment of some autoimmune disease, with the exception of RA, was limited by side effects. HLA-DR3$^+$ patients with SLE, and lpr/lpr and BXSB mice, produce high levels of TNF whereas HLADR2$^+$ SLE patients and NZB $\times$ NZWF1 mice produce less TNF. Transgenic mice expressing wild-type p75TNFR develop a severe inflammatory syndrome (Douni and Kollias, 1998; Kollias and Kontoyiannis, 2002; Kollias et al., 2002). Accordingly, the lpr phenotype is accelerated in p55$^{-/-}$lpr/lpr mice (Zhou et al., 1996), and TNF$^{-/-}$gld/gld mice have less autoimmune disease (Korner et al., 2000). These excerpts from the vast literature indicate the complexity of the effects of cytokines coupled with the major role of the genetic background in the expression of the clinical phenotype. It also points to the complexities and unpredictability of the use of some biologics in the modulation of human disease.

Tumor Necrosis Factor Overexpression and Autoimmunity

The successful clinical introduction of treatment with anti-TNF confirmed the biologic relevance of TNF function in chronic inflammatory diseases, particularly RA and Crohn's disease (see Chapters 32 and 52). The introduction of a modified human TNF-globin hybrid transgene in mice was the first demonstration in animal models that TNF has arthritogenic properties. These mice (tg 197) spontaneously develop (with 100% penetrance and a predictable time of onset) a chronic, erosive, inflammatory polyarthritis with histologic lesions resembling human RA (Kontoyiannis et al., 1999). AU-rich elements (AREs) are important for TNF mRNA destabilization and translational repression in hematopoietic and stromal cells. Development of two specific pathologies in mutant mice—chronic inflammatory arthritis and colitis akin to Crohn's disease—suggests that a defective function of AREs may participate in the pathogenesis of analogous human pathologies. These mice have proven quite informative in dissecting the pleiotropic effects of TNF on the immune response and on the expression of various forms of autoimmune pathology.

Interleukin-2 and its Receptors

Mice that are deficient in IL-2 and IL-2Rα have disrupted immunologic homeostasis that eventually leads to fatal autoimmune manifestations (Nelson, 2002). Specifically, these mice develop autoimmune hemolytic anemia and colitis. IL-2R$\beta^{-/-}$ mice likewise develop anemia, splenomegaly, and lymphadenopathy, but not colitis. In humans, IL-2 deficiency is clinically manifested as severe combined immunodeficiency, whereas a patient lacking IL-2Rα is immunocompromised, and several organs are infiltrated with inflammatory cells (Sharfe et al., 1997). The autoimmune manifestations depend on the presence of both T and B cells and environmental antigens since, if the mice are kept under pathogen-free conditions, they do not develop autoimmunity. Activation-induced cell death (AICD) is central for the elimination of activated autoreactive cells, and this depends on IL-2 signaling. Defective AICD obviously plays a role in the development of autoimmunity. Humans with SLE have defective AICD that appears to be multifactorial: defective TNF-α (Kovacs et al., 1996) and IL-2 production (Tsokos et al., 1996) or increased cyclooxygenase-2 (COX-2) expression (Xu et al., 2004). COX-2$^{-/-}$ mice do not have significantly increased AICD. It could be helpful to know whether correction of IL-2 production alters the severity of autoimmune disease in the MRL-lpr/lpr mouse that produces limited amounts of IL-2 (Theofilopoulos and Dixon, 1985), as this may represent a form of treatment for patients with SLE. It is of interest that when the IL-2$^{-/-}$ locus is crossed into the MRL-lpr/lpr mouse, both autoimmune syndromes are corrected (Xiao et al., 2003), an observation calling for consideration of gene subtraction rather than gene supplementation in the treatment of autoimmune disease.

Ubiquitination-Protein Ligases

Ubiquitination-protein ligases (Casitas B-lineage lymphoma [cbl]-family of proteins) are important in the signal-

ing transduction process and as determinants of energy and activation (Liu, 2004). Cbl-b is considered a negative regulator of T-cell activation, and Cbl-b$^{-/-}$ T cells proliferate and produce more IL-2 following TCR stimulation (Rudd and Schneider, 2000). Cbl-b-deficient mice display massive mononuclear cell infiltration of many organs and peripheral T cells express activation markers (Bachmaier et al., 2000). One intriguing disease model is Cbl-b deficiency in T1D (Yoko et al. Nature Genetics 2000), but a human counterpart for Cbl-b deficiency has not yet been described.

Apoptosis-Related Genes

The lpr mutation represents a CD95(Fas), CD152(FasL) gene mutation and its presence limits apoptosis of T cells. The lpr mutation is recessive and greatly accelerates disease in autoimmune-prone strains such as MRL and BXSB. When present in C57BL/6 mice, it results in the production of autoanti-bodies and limited glomerulonephritis (Kono and Theofilopoulos, 1999). Heterozygous mutations in CD95, CD95 ligand or caspase-10 in humans underlie most cases of autoimmune lymphoproliferative syndrome (ALPS), a human disorder that is characterized by defective lymphocyte apoptosis, lymphadenopathy, splenomegaly, and autoimmunity (Chun and Lenardo, 2001; see Chapter 70). In contrast, mutations in caspase-8 lead to immunodeficiency (Chun et al., 2002).

In a similar fashion the gld mutation accelerates autoimmunity in predisposed mouse strains and leads to milder disease in non-autoimmune prone strains. A patient with SLE has been described with a mutation in the CD178 (FasL) gene (Wu et al., 1996). On the other hand, the expression of CD178 (FasL) is increased in T cells from SLE patients (Tsokos et al., 1996; Kovacs et al., 1997) and this may reflect resistance of autoimmune T cells to killing.

The association of CD95/CD178 (Fas/FasL) defects with "lupus-like disease" susceptibility strongly suggests that mutations of other genes whose products are involved in the apopto-sis signaling cascade should similarly contribute to such expressions. Indeed, overexpression of the apoptosis inhibitor Bcl-2 leads to mild autoimmunity (Strasser et al., 1990; 1991), and patients with SLE have been reported to have higher levels of Bcl-2 protein (Aringer et al., 1994). Similarly, overexpression of Bcl-2 in C57BL/6-lpr mice leads to accelerated autoimmune disease (Reap et al., 1995) as does the overexpression of another apoptosis inhibitor, the cytoplasmic serine/threonine kinase Pim-1 (Moroy et al., 1993).

Mice lacking the membrane tyrosine kinase c-mer have impaired clearance of infused apoptotic cells, and develop progressive lupus-like autoimmunity, with antibodies to chromatin, DNA, and IgG. Since they have minimal polyclonal B-cell activation, the development of autoimmunity in these mice favors the concept that autoantigens present in apoptotic debris can enhance systemic autoimmunity (Scott et al., 2001; see Chapter 15).

Induced Models of Systemic Autoimmunity

Information has been acquired over many years from study of models based on induction of autoimmunity, including immunoregulatory events that lead to the expression of clinical disease and proximal events associated with triggering of autoimmune disease.

The *isoprenoid alkane pristane* induces autoantibodies characteristic of SLE, including anti-Sm, anti-dsDNA, and anti-ribosomal P in BALB/c and SJL/J mice (Richards et al., 1999) and CD1d deficiency exacerbates lupus nephritis induced by pristane (Yang et al., 2003). For unexplained reasons, the lpr and gld mutations protect mice from the production of antibodies routinely induced by pristane (Satoh et al., 2000).

Graft-versus-host induced models of systemic autoimmunity involve the injection of parent cells into F1 offspring and clarified early events in the induction of autoimmunity. Donor CD4$^+$ cells are stimulated by recipient F1 MHC class II cells, which presumably present a chromatin-associated nuclear antigen and produce initially IL-2 and later on IL-4 and IL-10. Anti-DNA antibodies are produced, while the generation of CD8$^+$ CTL cells is silenced (Via and Shearer, 1988).

Intraperitoneal injection of cell walls from varying types of bacteria results in a biphasic inflammatory arthritis. Initially, there is an acute inflammatory arthritis over 1–2 weeks followed by a month-long chronic arthritis (Cromartie et al., 1977). This model of arthritis has led to the characterization of cell, cytokine, and chemokine factors that are responsible for the synovial inflammation and the destruction of diarthrodial joints and, in parallel, to establishing the efficacy of various anticytokine treatments in arthritis.

Injection of *allogeneic collagen type II (CII)* or certain peptides of this protein in complete or incomplete Freund's adjuvant into susceptible strains of rats or mice results in collagen-induced arthritis (CIA) resembling RA. The role of MHC in the expression of CIA is indicated, only in H-2^q mice after injection of chicken CII, whereas H-2^r mice are susceptible to pig CII (Stuart et al., 1983; Watson et al., 1987). CD4$^+$ cells and various cytokines including IL-1 and TNF-α, as well as antibody to CII, have been shown to participate in the expression of CIA.

Immunization of BALB/c mice with *partially deglycosylated human aggrecan* induces chronic progressive polyarthritis and spondylitis (Glant et al., 2003). This proteoglycan (aggrecan)-induced arthritis (PGIA) resembles RA (as judged by clinical assessments, laboratory tests,

radiography, and histopathology of the peripheral joints). Occurrence of PGIA depends on development of cross-reactive T- and B-cell responses between the immunizing human and self (mouse) cartilage aggrecan.

How Do Animal Models of Systemic Autoimmunity Resemble Human Disease?

Animal models of systemic human autoimmune disease have served us well for understanding autoimmunity. Given the facts that human systemic autoimmune disease is highly heterogeneous and its origins and pathogenetic processes are multiple and highly complicated, each model will likely mimic pathogenesis of disease just in a small proportion of patients. Therefore, some therapies that are highly successful in animal models have only limited utility in humans. However, many false hypotheses have been dispelled by proper manipulation of animal models. For example, although B cells are needed for the development of glomerulonephritis, antibody production is not mandatory (Chan et al., 1999). Wakeland and colleagues (Wakeland et al., 1997; Mohan et al., 1999) have solidified the concept that several genetic loci have to act in concert for expression of autoimmunity and even more are needed for expression of disease. This explains the clinical experience that the presence of autoantibodies alone does not necessarily herald an upcoming disease. In addition, mutation of a gene often leads to disparate diseases in humans and mice, e.g., the Fas and FasL mutations that promote autoimmunity in mice and ALPS in humans (discussed above). In other situations, mice and humans can develop very similar autoimmune disease, e.g., when the DNAse1 gene is mutated (discussed above). Therefore, experiments should be designed to address specific questions that arise from the close study of human disease, but for which no answer is available from *in vivo* or *ex vivo* experiments in humans.

CONCLUSIONS

Our message is that a given animal model is only as good as the question it is used to answer, and particular models are well-suited for some but not all questions. Consequently, a careful choice is needed in advance. Inbred mouse strains that are used to limit variability of *in vivo* studies will essentially tell the story of just one or a few human patients—for translating findings in animal models to human therapy, this is a particularly important realization—and a much higher degree of variation can be expected in human populations. In addition, it follows that each model will only reflect certain aspects of a given autoimmune disease accurately and, therefore, immunotherapeutic approaches should be validated in more than one model if possible. Nevertheless, the numerous animal models that are available to us are indispensable tools to study *in vivo* pathogenesis and treatments, and are a crucial step when moving from the bench to the bedside.

References

Abrams, J.R., Kelley, S.L., Hayes, E., Kikuchi, T., Brown, M.J., Kang, S., Lebwohl, M.G., Guzzo, C.A., Jegasothy, B.V., Linsley, P.S., and Krueger, J.G. (2000). Blockade of T lymphocyte costimulation with cytotoxic T lymphocyte-associated antigen 4-immunoglobulin (CTLA4Ig) reverses the cellular pathology of psoriatic plaques, including the activation of keratinocytes, dendritic cells, and endothelial cells. *J. Exp. Med. 192,* 681–694.

Amano, T., Yamasaki, S., Yagishita, N., Tsuchimochi, K., Shin, H., Kawahara, K., Aratani, S., Fujita, H., Zhang, L., Ikeda, R., Fujii, R., Miura, N., Komiya, S., Nishioka, K., Maruyama, I., Fukamizu, A., and Nakajima, T. (2003). Synoviolin/Hrd1, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy. *Genes Dev. 17,* 2436–2449.

Anderson, M.S. (2002). Autoimmune endocrine disease. *Curr. Opin. Immunol. 14,* 760–764.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science 298,* 1395–1401.

Arif, S., Tree, T.I., Astill, T.P., Tremble, J.M., Bishop, A.J., Dayan, C.M., Roep, B.O., and Peakman, M. (2004). Related Articles. Links Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J. Clin. Invest. 113*, 415–463.

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.

Atkinson, M.A. and Leiter, E.H. (1999). The NOD mouse model of type 1 diabetes: as good as it gets? *Nat. Med. 5,* 601–604.

Bach, J.F., Koutouzov, S., and van Endert, P.M. (1998). Are there unique autoantigens triggering autoimmune diseases? *Immunol. Rev. 164,* 139–155.

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,* 211–216.

Bielekova, B., Sung, M.H., Kadom, N., Simon, R., McFarland, H., and Martin, R. (2004). Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. *J. Immunol. 172,* 3893–3904.

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,* 168–179.

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.

Boackle, S.A. and Holers, V.M. (2003). Role of complement in the development of autoimmunity. *Curr. Dir. Autoimmun. 6,* 154–168.

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,* 775–785.

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.

Botto, M. and Walport, M.J. (2002). C1q, autoimmunity and apoptosis. *Immunobiology, 205,* 395–406.

Bouzahzah, F., Jung, S., and Craft, J. (2003). CD4+ T cells from lupus-prone mice avoid antigen-specific tolerance induction *in vivo*. *J. Immunol. 170,* 741–748.

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,* 1639–1648.

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.

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.

Choubey, D. and Kotzin, B.L. (2002). Interferon-inducible p202 in the susceptibility to systemic lupus. *Front Biosci. 7,* e252–262.

Christen, U., Benke, D., Wolfe, T., Rodrigo, E., Rhode, A., Hughes, A.C., Oldstone, M.B., and von Herrath, M.G. (2004). Cure of prediabetic mice by viral infections involves lymphocyte recruitment along an IP-10 gradient. *J. Clin. Invest. 113,* 74–84.

Christen, U., Wolfe, T., Mohrle, U., Hughes, A.C., Rodrigo, E., Green, A., Flavell, R., and von Herrath, M. (2001a). A dual role for TNF-alpha in type 1 diabetes: Islet-specific expression abrogates the ongoing autoimmune process when induced late but not early during pathogenesis. *J. Immunol. 166,* 7023–7032.

Chun, H.J. and Lenardo, M.J. (2001). Autoimmune lymphoproliferative syndrome: types I, II and beyond. *Adv. Exp. Med. Biol. 490,* 49–57.

Chun, H.J., Zheng, L., Ahmad, M., Wang, J., Speirs, C.K., Siegel, R.M., Dale, J.K., Puck, J., Davis, J., Hall, C.G., Skoda-Smith, S., Atkinson, T.P., Straus, S.E., and Lenardo, M.J. (2002). Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature 419,* 395–399.

Cope, A., Ettinger, R., and McDivitt, H. (1997). The role of TNF alpha and related cytokines in the development and function of the autoreactive T-cell repertoire. *Res. Immunol., 148,* 307–312.

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,* 497–508.

Cromartie, W.J., Craddock, J.G., Schwab, J.H., Anderle, S.K., and Yang, C.H. (1977). Arthritis in rats after systemic injection of streptococcal cells or cell walls. *J. Exp. Med. 146,* 1585–1602.

Croxford, J.L., Olson, J.K., and Miller, S.D. (2002). Epitope spreading and molecular mimicry as triggers of autoimmunity in the Theiler's virus-induced demyelinating disease model of multiple sclerosis. *Autoimmun. Rev. 1,* 251–260.

de la Torre, J.C., Rall, G., Oldstone, C., Sanna, P.P., Borrow, P., and Oldstone, M.B. (1993). Replication of lymphocytic choriomeningitis virus is restricted in terminally differentiated neurons. *J. Virol. 67,* 7350–7359.

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.

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.

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 97–101*.

Douni, E., and Kollias, G. (1998). A critical role of the p75 tumor necrosis factor receptor (p75TNF-R) in organ inflammation independent of TNF, lymphotoxin alpha, or the p55TNF-R. *J. Exp. Med. 188,* 1343–1352.

Drake, C.G., Rozzo, S.J., Vyse, T.J., Palmer, E., and Kotzin, B.L. (1995). Genetic contributions to lupus-like disease in (NZB × NZW)F1 mice. *Immunol. Rev. 144,* 51–74.

Eisenbarth, G.S. (2003). Insulin autoimmunity: immunogenetics/immunopathogenesis of type 1A diabetes. *Ann. N. Y. Acad. Sci. 1005,* 109–118.

Eisenbarth, G.S., Moriyama, H., Robles, D.T., Liu, E., Yu, L., Babu, S., Redondo, M., Gottlieb, P., Wegmann, D., and Rewers, M. (2002). Insulin autoimmunity: prediction/precipitation/prevention type 1A diabetes. *Autoimmun. Rev. 1,* 139–145.

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.

Evans, C.F., Horwitz, M.S., Hobbs, M.V., and Oldstone, M.B.A. (1996). Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. *J. Exp. Med. 184,* 2371–2384.

Fernandes, G. and Talal, N. (1986). SLE: hormones and diet. *Clin. Exp. Rheumatol. 4,* 183–185.

Finck, B.K., Linsley, P.S., and Wofsy, D. (1994). Treatment of murine lupus with CTLA4Ig. *Science 265,* 1225–1227.

Fischer, M.B., Goerg, S., Shen, L., Prodeus, A.P., Goodnow, C.C., Kelsoe, G., and Carroll, M.C. (1998). Dependence of germinal center B cells on expression of CD21/CD35 for survival. *Science 280,* 582–585.

Forster, I., Hirose, R., Arbeit, J.M., Clausen, B.E., and Hanahan, D. (1995). Limited capacity for tolerization of CD4+ T cells specific for a pancreatic beta cell neo-antigen. *Immunity 2,* 573–585.

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. U.S.A. 95,* 14875–14879.

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.

Gale, E.A. (2002). A missing link in the hygiene hypothesis? *Diabetologia 45,* 588–594.

Gibson, A.W., Wu, J., Edberg, J.D., and Kimberly, R.P. (1999). Fcg receptor polymorphisms; insights into pathogenesis. In G.M. Kammer, and G.C. Tsokos (eds), *Insights into Pathogenesis*. Totowa: Humana Press.

Glant, T.T., Finnegan, A., and Mikecz, K. (2003). Proteoglycan-induced arthritis: immune regulation, cellular mechanisms, and genetics. *Crit. Rev. Immunol. 23,* 199–250.

Gorelik, L. and Flavell, R.A. (2002). Transforming growth factor-beta in T-cell biology. *Nat. Rev. Immunol. 2,* 46–53.

Graves, P.M., Rotbart, H.A., Nix, W.A., Pallansch, M.A., Erlich, H.A., Norris, J.M., Hoffman, M., Eisenbarth, G.S., and Rewers, M. (2003). Prospective study of enteroviral infections and development of beta-cell autoimmunity. Diabetes autoimmunity study in the young (DAISY). *Diabetes Res. Clin. Pract. 59,* 51–61.

Greeley, S.A.W., Katsumata, M., Yu, L., Eisenbarth, G.S., Moore, D.J., Goodarzi, H., Barker, C. F., Naji, A., and Noorchashm, H. (2002). Elimination of maternally-transmitted autoantibodies prevents diabetes in nonobese diabetic mice. *Nat. Med. 8*, 399–402.

Green, E.A. and Flavell, R.A. (2000). The temporal importance of TNF alpha expression in the development of diabetes. *Immunity 12,* 459–469.

Grimbacher, B., Hutloff, A., Schlesier, M., Glocker, E., Warnatz, K., Drager, R., Eibel, H., Fischer, B., Schaffer, A.A., Mages, H.W., Kroczek, R.A., and Peter, H.H. (2003). Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nat. Immunol. 4,* 261–268.

Hafler, D.A., Kent, S.C., Chen, Y., Bregoli, L., Clemmings, S.M., Kenyon, N.S., Ricordi, C., Hering, B.J. (2005). Expanded T Cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature*, *435*, 224–8.

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.

Handwerger, B.S., Storrer, C.E., Wasson, C.S., Movafagh, F., and Reichlin, M. (1999). Further characterization of the autoantibody response of Palmerston North mice. *J. Clin. Immunol. 19,* 45–57.

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.

Helgason, C.D., Kalberer, C.P., Damen, J.E., Chappel, S.M., Pineault, N., Krystal, G., and Humphries, R.K. (2000). A dual role for Src homology 2 domain-containing inositol-5-phosphatase (SHIP) in immunity: aberrant development and enhanced function of b lymphocytes in ship –/– mice. *J. Exp. Med. 191,* 781–794.

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,* 1593–1604.

Hill, N.J., Lyons, P.A., Armitage, N., Todd, J.A., Wicker, L.S., and Peterson, L.B. (2000). NOD Idd5 locus controls insulitis and diabetes and overlaps the orthologous CTLA4/IDDM12 and NRAMP1 loci in humans. *Diabetes 49,* 1744–1747.

Homann, D., Holz, A., Bot, A., Coon, B., Wolfe, T., Petersen, J., Dyrberg, T.P., Grusby, M.J., and von Herrath, M.G. (1999). Autoreactive CD4+ T cells protect from autoimmune diabetes via bystander suppression using the IL-4/Stat6 pathway. *Immunity 11,* 463–472.

Horwitz, M.S., Bradley, L.M., Harbertson, J., Krahl, T., Lee, J., and Sarvetnick, N. (1998). Diabetes induced by Coxsackie virus: Initiation by bystander damage and not molecular mimicry. *Nat. Med. 4,* 781–785.

Horwitz, M.S., Ilic, A., Fine, C., Rodriguez, E., and Sarvetnick, N. (2002). Presented antigen from damaged pancreatic beta cells activates autoreactive T cells in virus-mediated autoimmune diabetes. *J. Clin. Invest. 109,* 79–87.

Hummel, M., Williams, A.J., Norcross, A., Standl, E., Bonifacio, E., Ziegler, A.G., and Bingley, P.J. (2001). Proinsulin-specific autoantibodies are relatively infrequent in young offspring with pre-type 1 diabetes. *Diabetes Care. 10*, 1843–1844.

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,* 275–280; discussion 281.

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 TCR zeta-chain. *J. Immunol. 168,* 2865–2871.

Katz-Levy, Y., Neville, K.L., Girvin, A.M., Vanderlugt, C.L., Pope, J.G., Tan, L.J., and Miller, S.D. (1999). Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. *J. Clin. Invest. 104,* 599–610.

Kay, T.W., Darwiche, R., Irawaty, W., Chong, M.M., Pennington, H.L., and Thomas, H.E. (2003). The role of cytokines as effectors of tissue destruction in autoimmunity. *Adv. Exp. Med. Biol. 520,* 73–86.

Kaya, Z., Afanasyeva, M., Wang, Y., Dohmen, K.M., Schlichting, J., Tretter, T., Fairweather, D., Holers, V.M., and Rose, N.R. (2001). Contribution of the innate immune system to autoimmune myocarditis: a role for complement. *Nat. Immunol. 2,* 739–745.

Kimberly, R.P., Salmon, J.E., and Edberg, J.C. (1995). Receptors for immunoglobulin G. Molecular diversity and implications for disease. *Arthritis Rheum. 38,* 306–314.

Kollias, G. and Kontoyiannis, D. (2002). Role of TNF/TNFR in autoimmunity: specific TNF receptor blockade may be advantageous to anti-TNF treatments. *Cytokine Growth Factor Rev. 13,* 315–321.

Kollias, G., Kontoyiannis, D., Douni, E., and Kassiotis, G. (2002). The role of TNF/TNFR in organ-specific and systemic autoimmunity: implications for the design of optimized 'anti-TNF' therapies. *Curr. Dir. Autoimmun. 5,* 30–50.

Kono, D.H. and Theofilopoulos, A.N. (1999). Genetic susceptibility to spontaneous lupus in mice. *Curr. Dir. Autoimmun. 1,* 72–98.

Kono, D.H. and Theofilopoulos, A.N. (2002). Genes and genetics of murine lupus. In A.N. Theofilopoulos, and C.A. Bona (eds), *The Molecular Pathology of Autoimmune Diseases.* New York: Taylor and Francis.

Kontoyiannis, D., Pasparakis, M., Pizarro, T.T., Cominelli, F., and Kollias, G. (1999). Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity 10,* 387–398.

Korner, H., Cretney, E., Wilhelm, P., Kelly, J.M., Rollinghoff, M., Sedgwick, J.D., and Smyth, M.J. (2000). Tumor necrosis factor sustains the generalized lymphoproliferative disorder (gld) phenotype. *J. Exp. Med. 191,* 89–96.

Kovacs, B., Liossis, S.N., 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 25,* 213–221.

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.

Kozlowski, M., Mlinaric-Rascan, I., Feng, G.S., Shen, R., Pawson, T., and Siminovitch, K.A. (1993). Expression and catalytic activity of the tyrosine phosphatase PTP1C is severely impaired in motheaten and viable motheaten mice. *J. Exp. Med. 178,* 2157–2163.

Kremer, J.M., Westhovens, R., Leon, M., Di Giorgio, E., Alten, R., Steinfeld, S., Russell, A., Dougados, M., Emery, P., Nuamah, I.F., Williams, G.R., Becker, J.C., Hagerty, D.T., and Moreland, L.W. (2003). Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N. Engl. J. Med. 349,* 1907–1915.

Krug, H.E., Mahowald, M.L., and Clark, C. (1989). Progressive ankylosis (ank/ank) in mice: an animal model of spondyloarthropathy. III. Proliferative spleen cell response to T cell mitogens. *Clin. Exp. Immunol. 78,* 97–101.

Kruger, J., Butler, J.R., Cherapanov, V., Dong, Q., Ginzberg, H., Govindarajan, A., Grinstein, S., Siminovitch, K.A., and Downey, G.P. (2000). Deficiency of Src homology 2-containing phosphatase 1 results in abnormalities in murine neutrophil function: studies in motheaten mice. *J. Immunol. 165,* 5847–5859.

Kurts, C., Heath, W.R., Carbone, F.R., Allison, J., Miller, J.F., and Kosaka, H. (1996). Constitutive class I-restricted exogenous presentation of self antigens *in vivo*. *J. Exp. Med. 184,* 923–930.

Lawson, B.R., Baccala, R., Song, J., Croft, M., Kono, D.H., and Theofilopoulos, A.N. (2004). Deficiency of the cyclin kinase inhibitor p21(WAF-1/CIP-1) promotes apoptosis of activated/memory t cells and inhibits spontaneous systemic autoimmunity. *J. Exp. Med. 199,* 547–557.

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,* 5928–5932.

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature 416,* 603–607.

Lee, D.M., Friend, D.S., Gurish, M.F., Benoist, C., Mathis, D., and Brenner, M.B. (2002). Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science 297,* 1689–1692.

Lin, S.C., Yen, J.H., Tsai, J.J., Tsai, W.C., Ou, T.T., Liu, H.W., and Chen, C.J. (2004). Association of a programmed death 1 gene polymorphism with the development of rheumatoid arthritis, but not systemic lupus erythematosus. *Arthritis Rheum. 50,* 770–775.

Liossis, S.N., Ding, X.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.

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. Investig. Med. 49,* 157–165.

Liu, Y.C. (2004). Ubiquitin ligases and the immune response. *Ann. Rev. Immunol. 22,* 81–127.

Lundin, K.E., Scott, H., Hansen, T., Paulsen, G., Halstensen, T.S., Fausa, O., Thorsby, E., and Sollid, L.M. (1993). Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J. Exp. Med. 178,* 187–196.

Luzina, I.G., Knitzer, R.H., Atamas, S.P., Gause, W.C., Papadimitriou, J.C., Sztein, M.B., Storrer, C.E., and Handwerger, B.S. (1999). Vasculitis in the Palmerston North mouse model of lupus: phenotype and cytokine production profile of infiltrating cells. *Arthritis Rheum. 42,* 561–568.

Lyons, P.A. and Wicker, L.S. (1999). Localising quantitative trait loci in the NOD mouse model of type 1 diabetes. *Curr. Dir. Autoimmun. 1,* 208–225.

Mantegazza, R., Cristaldini, P., Bernasconi, P., Baggi, F., Pedotti, R., Piccini, I., Mascoli, N., Mantia, L.L., Antozzi, C., Simoncini, O., Cornelio, F., and Milanese, C. (2004). Anti-MOG autoantibodies in Italian multiple sclerosis patients: specificity, sensitivity and clinical association. *Int. Immunol. 16,* 559–565.

Martin, S., Wolf-Eichbaum, D., Duinkerken, G., Scherbaum, W.A., Kolb, H., Noordzij, J.G., and Roep, B.O. (2001). Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. *N. Engl. J. Med. 345,* 1036–1040.

Matsumoto, I., Lee, D.M., Goldbach-Mansky, R., Sumida, T., Hitchon, C.A., Schur, P.H., Anderson, R.J., Coblyn, J.S., Weinblatt, M.E., Brenner, M., Duclos, B., Pasquali, J.L., El-Gabalawy, H., Mathis, D., and Benoist, C. (2003). Low prevalence of antibodies to glucose-6-phosphate isomerase in patients with rheumatoid arthritis and a spectrum of other chronic autoimmune disorders. *Arthritis Rheum. 48,* 944–954.

McDevitt, H. (2002). The discovery of linkage between the MHC and genetic control of the immune response. *Immunol. Rev. 185,* 78–85.

McDevitt, H.O. (1998). The role of MHC class II molecules in susceptibility and resistance to autoimmunity. *Curr. Opin. Immunol. 10,* 677–681.

McGargill, M.A., Mayerova, D., Stefanski, H.E., Koehn, B., Parke, E.A., Jameson, S.C., Panoskaltsis-Mortari, A., and Hogquist, K.A. (2002). A spontaneous CD8 T cell-dependent autoimmune disease to an antigen expressed under the human keratin 14 promoter. *J. Immunol. 169,* 2141–2147.

Medzhitov, R., Preston-Hurlburt, P., Kopp, E., Stadlen, A., Chen, C., Ghosh, S., and Janeway, C. A., Jr. (1998). MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol. Cell 2,* 253–258.

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,* 1685–1695.

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.

Monach, P.A., Benoist, C., and Mathis, D. (2004). The role of antibodies in mouse models of rheumatoid arthritis, and relevance to human disease. *Adv. Immunol. 82,* 217–248.

Morel, L. and Wakeland, E.K. (1998). Susceptibility to lupus nephritis in the NZB/W model system. *Curr. Opin. Immunol. 10,* 718–725.

Moriyama, H., Abiru, N., Paronen, J., Sikora, K., Liu, E., Miao, D., Devendra, D., Beilke, J., Gianani, R., Gill, R.G., and Eisenbarth, G.S. (2003). Evidence for a primary islet autoantigen (preproinsulin 1) for insulitis and diabetes in the nonobese diabetic mouse. *Proc. Natl. Acad. Sci. U.S.A. 100,* 10376–10381.

Moroy, T., Grzeschiczek, A., Petzold, S., and Hartmann, K.U. (1993). Expression of a Pim-1 transgene accelerates lymphoproliferation and inhibits apoptosis in lpr/lpr mice. *Proc. Natl. Acad. Sci. U.S.A. 90,* 10734–10738.

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.

Murtaza, A., Nugent, C.T., Tailor, P., Asensio, V.C., Biggs, J.A., Campbell, I.L., and Sherman, L.A. (2001). Altered functional and biochemical response by CD8+ T cells that remain after tolerance. *Int. Immunol. 13,* 1085–1093.

Nambiar, M.P., Enyedy, E.J., Fisher, C.U., Krishnan, S., Warke, V.G., Gilliland, W.R., Oglesby, R.J., and Tsokos, G.C. (2002). Abnormal expression of various molecular forms and distribution of T cell receptor zeta chain in patients with systemic lupus erythematosus. *Arthritis Rheum. 46,* 163–174.

Napirei, M., Karsunky, H., Zevnik, B., Stephan, H., Mannherz, H.G., and Moroy, T. (2000). Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat. Genet. 25,* 177–181.

Nelson, B.H. (2002). Interleukin-2 signaling and the maintenance of self-tolerance. *Curr. Dir. Autoimmun. 5,* 92–112.

Niculescu, F., Rus, H., van Biesen, T., and Shin, M.L. (1997). Activation of Ras and mitogen-activated protein kinase pathway by terminal complement complexes is G protein dependent. *J. Immunol. 158,* 4405–4412.

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.

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.

Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., Malissen, B., Zinkernagel, R.M., and Hengartner, H. (1991). Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell 65,* 305–317.

Okazaki, T., Tanaka, Y., Nishio, R., Mitsuiye, T., Mizoguchi, A., Wang, J., Ishida, M., Hiai, H., Matsumori, A., Minato, N., and Honjo, T. (2003). Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med. 9,* 1477–1483.

Olson, J.K., Croxford, J.L., Calenoff, M.A., Dal Canto, M.C., and Miller, S.D. (2001). A virus-induced molecular mimicry model of multiple sclerosis. *J. Clin. Invest. 108,* 311–318.

Pakala, S.V., Kurrer, M.O., and Katz, J.D. (1997). T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice. *J. Exp. Med. 186,* 299–306.

Pani, G., Fischer, K.D., Mlinaric-Rascan, I., and Siminovitch, K.A. (1996). Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J. Exp. Med. 184,* 839–852.

Peakman, M., Tree, T.I., Endl, J., van Endert, P., Atkinson, M.A., and Roep, B.O. (2001). Characterization of preparations of GAD65, proinsulin, and the islet tyrosine phosphatase IA-2 for use in detection of autoreactive T-cells in type 1 diabetes: report of phase II of the Second International Immunology of Diabetes Society Workshop for Standardization of T-cell assays in type 1 diabetes. *Diabetes 50,* 1749–1754.

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. *Nat. Genet. 31,* 424–428.

Pietropaolo, M. and Eisenbarth, G.S. (2001). Autoantibodies in human diabetes. *Curr. Dir. Autoimmun. 4,* 252–282.

Pietropaolo, M., Giannoukakis, N., and Trucco, M. (2002). Cellular environment and freedom of gene expression. *Nat. Immunol. 3,* 335; author reply 336.

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,* 721–731.

Prokunina, L., Gunnarsson, I., Sturfelt, G., Truedsson, L., Seligman, V.A., Olson, J.L., Seldin, M.F., Criswell, L.A., and Alarcon-Riquelme, M.E. (2004). The systemic lupus erythematosus-associated PDCD1 polymorphism PD1.3A in lupus nephritis. *Arthritis Rheum. 50,* 327–328.

Puel, A., Picard, C., Ku, C.L., Smahi, A., and Casanova, J.L. (2004). Inherited disorders of NF-kappaB-mediated immunity in man. *Curr. Opin. Immunol. 16,* 34–41.

Rall, G.F., Mucke, L., and Oldstone, M. B. (1995). Consequences of cytotoxic T lymphocyte interaction with major histocompatibility complex class I-expressing neurons *in vivo*. *J. Exp. Med. 182,* 1201–1212.

Ramsdell, F. and Ziegler, S.F. (2003). Transcription factors in autoimmunity. *Curr. Opin. Immunol. 15,* 718–724.

Reap, E.A., Leslie, D., Abrahams, M., Eisenberg, R.A., and Cohen, P.L. (1995). Apoptosis abnormalities of splenic lymphocytes in autoimmune lpr and gld mice. *J. Immunol. 154,* 936–943.

Richards, H.B., Satoh, M., Shaheen, V.M., Yoshida, H., and Reeves, W.H. (1999). Induction of B cell autoimmunity by pristane. *Curr. Top. Microbiol. Immunol. 246,* 387–392; discussion 393.

Ridgway, W.M., Fasso, M., and Fathman, C.G. (1999). A new look at MHC and autoimmune disease. *Science 284,* 749, 751.

Robinson, W.H., Utz, P.J., and Steinman, L. (2003). Genomic and proteomic analysis of multiple sclerosis. Opinion. *Curr. Opin. Immunol. 15,* 660–667.

Roep, B.O., Atkinson, M., and von Herrath, M.G. (2004). Satisfaction (not) guarenteed: re-evaluating the use of animal models of type 1 diabetes. *Nat. Rev. Immunol. 4*, 989–997.

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,* 435–443.

Rudd, C.E. and Schneider, H. (2000). Lymphocyte signaling: Cbl sets the threshold for autoimmunity. *Curr. Biol. 10,* R344–347.

Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., and Sakaguchi, S. (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature 426,* 454–460.

Sakaguchi, S., Takahashi, T., and Nishizuka, Y. (1982). Study on cellular events in postthymectomy autoimmune oophoritis in mice. I. Requirement of Lyt-1 effector cells for oocytes damage after adoptive transfer. *J. Exp. Med. 156,* 1565–1576.

Salomon, B., Rhee, L., Bour-Jordan, H., Hsin, H., Montag, A., Soliven, B., Arcella, J., Girvin, A. M., Padilla, J., Miller, S.D., and Bluestone, J.A. (2001). Development of spontaneous autoimmune peripheral polyneuropathy in B7-2-deficient NOD mice. *J. Exp. Med. 194,* 677–684.

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,* 777–788.

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.

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,* 207–211.

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,* 6444–6451.

Sercarz, E.E. (2000). Driver clones and determinant spreading. *J. Autoimmun. 14,* 275–277.

Sharfe, N., Dadi, H.K., Shahar, M., and Roifman, C.M. (1997). Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. *Proc. Natl. Acad. Sci. U.S.A. 94,* 3168–3171.

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,* 403–409.

Shores, E.W. and Love, P.E. (1997). TCR zeta chain in T cell development and selection. *Curr. Opin. Immunol. 9,* 380–389.

Silvestris, F., Frassanito, M.A., Cafforio, P., Potenza, D., Di Loreto, M., Tucci, M., Grizzuti, M.A., Nico, B., and Dammacco, F. (1996). Antiphosphatidylserine antibodies in human immunodeficiency virus-1 patients with evidence of T-cell apoptosis and mediate antibody-dependent cellular cytotoxicity. *Blood 87,* 5185–5195.

Sprent, J. and Surh, C.D. (2003). Knowing one's self: central tolerance revisited. *Nat. Immunol. 4,* 303–304.

Strasser, A., Harris, A.W., and Cory, S. (1991). bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. *Cell 67,* 889–899.

Strasser, A., Harris, A.W., Vaux, D.L., Webb, E., Bath, M.L., Adams, J.M., and Cory, S. (1990). Abnormalities of the immune system induced by dysregulated bcl-2 expression in transgenic mice. *Curr. Top. Microbiol. Immunol. 166,* 175–181.

Stratmann, T., Martin-Orozco, N., Mallet-Designe, V., Poirot, L., McGavern, D., Losyev, G., Dobbs, C.M., Oldstone, M.B., Yoshida, K., Kikutani, H., Mathis, D., Benoist, C., Haskins, K., and Teyton, L. (2003). Susceptible MHC alleles, not background genes, select an autoimmune T cell reactivity. *J. Clin. Invest. 112,* 902–914.

Stuart, J.M., Tomoda, K., Yoo, T.J., Townes, A.S., and Kang, A.H. (1983). Serum transfer of collagen-induced arthritis. II. Identification and localization of autoantibody to type II collagen in donor and recipient rats. *Arthritis Rheum. 26,* 1237–1244.

Tabeta, K., Georgel, P., Janssen, E., Du, X., Hoebe, K., Crozat, K., Mudd, S., Shamel, L., Sovath, S., Goode, J., Alexopoulou, L., Flavell, R.A., and Beutler, B. (2004). Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. *Proc. Natl. Acad. Sci. U.S.A. 101,* 3516–3521.

Theofilopoulos, A.N. and Dixon, F.J. (1985). Murine models of systemic lupus erythematosus. *Adv. Immunol. 37,* 269–390.

Theofilopoulos, A.N. and Kono, D.H. (2002). A genetic analysis of lupus. *Allergy 57* Suppl 72, 67–74.

Thomas, H.E. and Kay, T. W. (2001). How beta cells die in type 1 diabetes. *Curr. Dir. Autoimmun. 4,* 144–170.

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.

Todd, J.A. and Wicker, L.S. (2001). Genetic protection from the inflammatory disease type 1 diabetes in humans and animal models. *Immunity 15,* 387–395.

Tsao, B.P., Cantor, R.M., Grossman, J.M., Kim, S.K., Strong, N., Lau, C.S., Chen, C.J., Shen, N., Ginzler, E.M., Goldstein, R., Kalunian, K.C., Arnett, F.C., Wallace, D.J., and Hahn, B.H. (2002). Linkage and interaction of loci on 1q23 and 16q12 may contribute to susceptibility to systemic lupus erythematosus. *Arthritis Rheum. 46,* 2928–2936.

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.

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. *Arthritis Rheum. 39,* 600–609.

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.

Tsokos, G.C., Nambiar, M.P., Tenbrock, K., and Juang, Y.T. (2003). Rewiring the T-cell: signaling defects and novel prospects for the treatment of SLE. *Trends Immunol. 24,* 259–263.

Tsui, H.W., Siminovitch, K.A., de Souza, L., and Tsui, F.W. (1993). Motheaten and viable motheaten mice have mutations in the haematopoietic cell phosphatase gene. *Nat. Genet. 4,* 124–129.

Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., Rainbow, D.B., Hunter, K.M., Smith, A.N., Di Genova, G., Herr, M. H., Dahlman, I., Payne, F., Smyth, D., Lowe, C., Twells, R.C., Howlett, S., Healy, B., Nutland, S., Rance, H.E., Everett, V., Smink, L. J., Lam, A.C., Cordell, H.J., Walker, N.M., Bordin, C., Hulme, J., Motzo, C., Cucca, F., Hess, J.F., Metzker, M.L., Rogers, J., Gregory, S., Allahabadia, A., Nithiyananthan, R., Tuomilehto-Wolf, E., Tuomilehto, J., Bingley, P., Gillespie, K.M., Undlien, D.E., Ronningen, K.S., Guja, C., Ionescu-Tirgoviste, C., Savage, D.A., Maxwell, A.P., Carson, D.J., Patterson, C.C., Franklyn, J.A., Clayton, D.G., Peterson, L.B., Wicker, L.S., Todd, J.A., and Gough, S.C. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature 423,* 506–511.

van Heel, D.A., Fisher, S.A., Kirby, A., Daly, M.J., Rioux, J.D., and Lewis, C.M. (2004). Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. *Hum. Mol. Genet. 13,* 763–770.

Vandenbark, A.A., Barnes, D., Finn, T., Bourdette, D.N., Whitham, R., Robey, I., Kaleeba, J., Bebo, B.F., Jr., Miller, S.D., Offner, H., and Chou, Y.K. (2000). Differential susceptibility of human T(h)1 versus T(h) 2 cells to induction of anergy and apoptosis by ECDI/antigen-coupled antigen-presenting cells. *Int. Immunol. 12,* 57–66.

Veldman, C.M., Gebhard, K.L., Uter, W., Wassmuth, R., Grotzinger, J., Schultz, E., and Hertl, M. (2004). T cell recognition of desmoglein 3 peptides in patients with pemphigus vulgaris and healthy individuals. *J. Immunol. 172,* 3883–3892.

Via, C.S. and Shearer, G.M. (1988). T-cell interactions in autoimmunity: insights from a murine model of graft-versus-host disease. *Immunol. Today 9,* 207–213.

Von Herrath, M. (1996). Virus-induced autoimmune disease. *Curr. Opin. Immunol. 8,* 878–885.

Von Herrath, M., Fujinami, R.S., and Whitton, J.L. (2003). Microorganisms and autoimmunity: making the barren field fertile . . . ? *Nat. Rev. Microbiol. 1*, 151–157.

von Herrath, M.G., Dockter, J., and Oldstone, M.B. (1994). How virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. *Immunity 1,* 231–242.

von Herrath, M.G., Guerder, S., Lewicki, H., Flavell, R.A., and Oldstone, M.B. (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.

von Herrath, M.G. and Holz, A. (1997). Pathological changes in the islet milieu precede infiltration of islets and destruction of beta-cells by autoreactive lymphocytes in a transgenic model of virus-induced IDDM. *J. Autoimmun. 10,* 231–238.

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.

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,* 2757–2766.

Wakeland, E.K., Morel, L., Mohan, C., and Yui, M. (1997). Genetic dissection of lupus nephritis in murine models of SLE. *J. Clin. Immunol. 17,* 272–281.

Walker, M.R., Kasprowicz, D.J., Gersuk, V.H., Benard, A., Van Landeghen, M., Buckner, J.H., and Ziegler, S.F. (2003). Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J. Clin. Invest. 112,* 1437–1443.

Wang, H. and Shlomchik, M.J. (1997). High affinity rheumatoid factor transgenic B cells are eliminated in normal mice. *J. Immunol. 159,* 1125–1134.

Watson, W.C., Brown, P.S., Pitcock, J.A., and Townes, A.S. (1987). Passive transfer studies with type II collagen antibody in B10.D2/old and new line and C57Bl/6 normal and beige (Chediak-Higashi) strains: evidence of important roles for C5 and multiple inflammatory cell types in the development of erosive arthritis. *Arthritis Rheum. 30,* 460–465.

Weber, A., Infante-Duarte, C., Sawcer, S., Setakis, E., Bellmann-Strobl, J., Hensiek, A., Rueckert, S., Schoenemann, C., Benediktsson, K., Compston, A., and Zipp, F. (2003). A genome-wide German screen for linkage disequilibrium in multiple sclerosis. *J. Neuroimmunol. 143,* 79–83.

Winer, S., Astsaturov, I., Cheung, R.K., Gunaratnam, L., Kubiak, V., Cortez, M.A., Moscarello, M., O'Connor, P.W., McKerlie, C., Becker, D.J., and Dosch, H.M. (2001). Type I diabetes and multiple sclerosis patients target islet plus central nervous system autoantigens; nonimmunized nonobese diabetic mice can develop autoimmune encephalitis. *J. Immunol. 166,* 2831–2841.

Winer, S., Tsui, H., Lau, A., Song, A., Li, X., Cheung, R.K., Sampson, A., Afifiyan, F., Elford, A., Jackowski, G., Becker, D.J., Santamaria, P., Ohashi, P., and Dosch, H.M. (2003). Autoimmune islet destruction in spontaneous type 1 diabetes is not beta-cell exclusive. *Nat. Med. 9,* 198–205.

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.

Xiao, S., Sung, S.S., Fu, S.M., and Ju, S.T. (2003). Combining Fas mutation with interleukin-2 deficiency prevents Colitis and Lupus: impli-

cating interleukin-2 for auto-reactive T cell expansion and Fas ligand for colon epithelial cell death. *J. Biol. Chem. 278,* 52730–52738.

Xu, L., Zhang, L., Yi, Y., Kang, H.K., and Datta, S.K. (2004). Human lupus T cells resist inactivation and escape death by upregulating COX-2. *Nat. Med. 10,* 411–415.

Yamazaki, T., Arase, H., Ono, S., Ohno, H., Watanabe, H., and Saito, T. (1997). A shift from negative to positive selection of autoreactive T cells by the reduced level of TCR signal in TCR-transgenic CD3 zeta-deficient mice. *J. Immunol. 158,* 1634–1640.

Yang, J.Q., Singh, A.K., Wilson, M.T., Satoh, M., Stanic, A.K., Park, J.J., Hong, S., Gadola, S. D., Mizutani, A., Kakumanu, S.R., Reeves, W.H., Cerundolo, V., Joyce, S., Van Kaer, L., and Singh, R.R. (2003). Immunoregulatory role of CD1d in the hydrocarbon oil-induced model of lupus nephritis. *J. Immunol. 171,* 2142–2153.

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. *Nat. Genet. 28,* 313–314.

Yokoi, N., Komeda, K., Wang, H.Y., Yano, H., Kitada, K., Saitoh, Y., Seino, Y., Yasuda, K., Serikawa, T., and Seino, S. (2002). Cblb is a major susceptibility gene for rast type 1 diabetes mellitus. *Nat. Genet. 31*, 391–394. Epub 2002 Jul 15.

Zhou, T., Edwards, C.K., 3rd, 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.

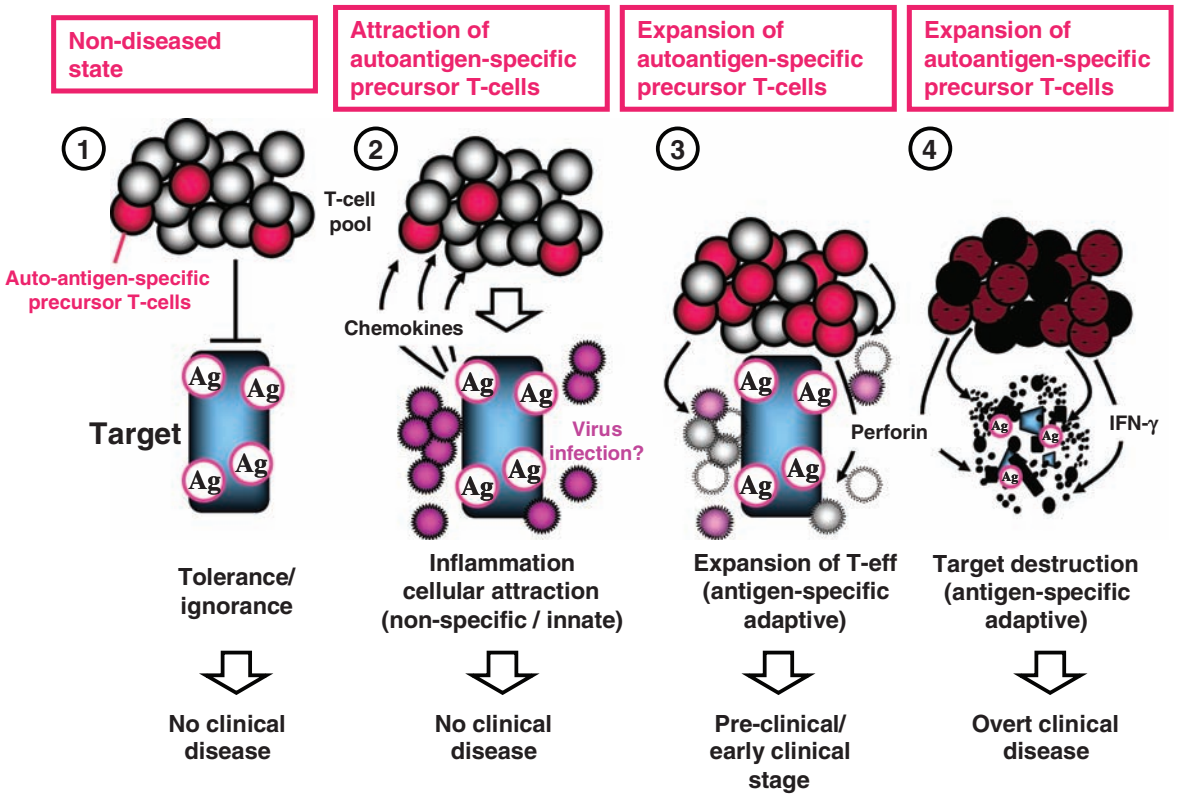


FIGURE 26.1 Pathogenesis of organ specific autoimmunity. For organ-specific autoimmunity one can assume that the initiating events might be antigen non-specific or specific. (1) A combination of genetic and environmental factors might lead to the breaking of self-tolerance and activation of autoreactive T cells. (2) Further inflammation might attract more such cells, for example, if a virus persists or autoantigens are being presented in a chronic manner. Certainly, activation of T-cell receptors and antigen-presenting cells will serve to propagate disease. (3) The ultimate outcome is determined by the magnitude and class of the autoreactive response. Many aggressive T cells, such as cytotoxic T cells and Th1 lymphocytes, will enhance progression, whereas the presence of (autoreactive) regulatory T cells (Tregs) will dampen inflammation. (4) Penetrance of clinical disease is directly correlated with the amount of target cell (organ) destruction and is determined by this balance of Tregs to aggressive T cells. Ag, antigen; IFNγ, interferon γ; Teff, effector T-cell.

CHAPTER

27

Systemic Lupus Erythematosus and Related Diseases: Clinical Features

MICHELLE PETRI
Johns Hopkins University School of Medicine
Baltimore, Maryland, USA

SYSTEMIC LUPUS ERYTHEMATOSUS

Epidemiology

Systemic lupus erythematosus (SLE) is the classic multisystem autoimmune disease. It occurs more frequently in women, with a 9 : 1 ratio, and is more common in African-Americans, Asian-Americans, and Hispanic-Americans than in whites. Its incidence may have tripled since the 1960s and 70s (Uramoto et al., 1999). The incidence is approximately 4 in 100,000 in most countries, with an especially high incidence of 8.7 in 100,000 in Brazil (Vilar and Sato, 2002).

There is a genetic predisposition to SLE, but only 2% of children of affected women develop SLE. The genetic predisposition may involve over 100 different genes. Recent work has found that an "interferon signature" characterizes many genes that are activated in SLE (Baechler et al., 2003). Identified predisposing genes include HLA DR and DQ alleles that are associated with the production of specific autoantibodies (Arnett et al., 1991; Olsen et al., 1993). Both complement deficiency states and certain Fc-γ receptor alleles predispose to SLE, and affect clearance of immune complexes (see Chapter 16).

Multiple environmental factors contribute to the pathogenesis of SLE. Ultraviolet radiation is the best understood, leading to the exposure of Ro on the surface of keratinocytes. Other environmental triggers of SLE include certain drugs. For idiopathic lupus, echinacea and sulfonamide antibiotics can trigger SLE or SLE flares. For drug-induced lupus, isoniazid, hydralazine, procainamide, minocycline, and anti-tumor necrosis factor (TNF) biologics have been identified. Infections including Epstein–Barr virus (James et al., 2001) and parvovirus are associated with SLE. A recently identified toxic exposure that increases risk of SLE is silica (Parks et al., 2002). Smoking is a risk factor for SLE (Costenbader et al., 2004) and, in addition, in patients with established SLE, smoking increases the risk of discoid lupus.

The hormonal milieu is important in the gender ratio and timing of SLE. In children, the gender ratio is equal. In women, SLE usually develops after puberty. Women with SLE tend to be "super women," in that they preferentially employ hydroxylation pathways that lead to the production of more feminizing metabolites of estradiol (Lahita et al., 2004) (see Chapter 25). In women with SLE, levels of the adrenal hormone dehydroepiandrosterone (DHEA) are usually low, and drop even lower if the patient is treated with prednisone.

Immunopathogenesis

The hallmark of SLE is the production of autoantibodies by B cells. However, there is also ample evidence for

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

abnormal T-cell function (Tsokos, 2001) and abnormal reticuloendothelial system function. One of the most important discoveries in SLE was the finding that the proteins to which SLE patients make autoantibodies are exposed in nuclear blebs during apoptosis (Casciola-Rosen et al., 1994). This has led to the "waste disposal" theory of SLE, namely that ineffective clearance of apoptotic bodies leads to the initial break in self-tolerance (Manderson et al., 2004) (see Chapter 15).

Clinical Presentation

The most common organ system presentations are cutaneous, musculoskeletal, and renal. Cutaneous involvement includes photosensitivity, malar rash, discoid rash, nasal and/or oral ulcers, and cutaneous vasculitis. Cutaneous lupus often first presents after ultraviolet light exposure, with a maculopapular rash in sun-exposed areas. Musculoskeletal features consist of polyarthralgias or polyarthritis, and, more rarely, myositis. Lupus arthritis affects the small joints of the hand (posterior interphalangeal [PIP] and metacarpophalangeal [MCP] joints) and wrists. It may be initially confused with rheumatoid arthritis. Unlike rheumatoid arthritis, however, it is rarely erosive. Instead, over time, it may lead to reducible deformities, secondary to tendon and ligament laxity, called Jaccoud arthropathy. Renal lupus presents as proteinuria, hematuria, and/or red blood cell casts.

Almost any organ can be involved. Hematologic abnormalities include leukopenia, lymphopenia, thrombocytopenia, and hemolytic anemia. Serositis manifests as pleurisy or pleural effusion, pericarditis with or without effusion, and rarely as abdominal pain or ascites. Neurologic lupus runs the gamut from cognitive impairment (common) to rare features such as seizures, psychosis, transverse myelitis, cranial or peripheral neuropathy, mononeuritis multiplex, encephalopathy, and stroke.

Almost 50% of SLE patients make antiphospholipid antibodies (lupus anticoagulant, anticardiolipin, or anti-β2 glycoprotein 1), which lead to hypercoagulability. The antiphospholipid syndrome is defined as arterial or venous thrombosis or vasculopathy or pregnancy morbidity (early losses, late fetal loss, or severe pre-eclampsia or placental insufficiency) (Wilson et al., 1999) (see Chapter 30). Lupus nephritis is described in Chapter 59.

Diagnosis

The American College of Rheumatology has devised classification criteria that require the presence of four of the following eleven to "classify" SLE: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder (psychosis or seizures), hematologic disorder (hemolytic anemia, leucopenia, lymphopenia or thrombocytopenia), immunologic disorder (anti-DNA, anti-Sm, or antiphospholipid antibodies) and positive antinuclear antibodies (ANA) (Tan et al., 1982).

However, the clinical diagnosis of SLE can be made without four criteria. Alopecia and Raynaud's phenomenon occur frequently in SLE. Several other autoantibodies may be helpful, although not specific for SLE, including antiribonuclear protein (anti-RNP), anti-Ro, or anti-La. Low complement (C3, C4, or CH50) is very common in SLE. Several neurologic manifestations, including encephalopathy, mononeuritis multiplex, and transverse myelitis may lead to the consideration of SLE.

Autoantibodies

Of all the autoantibodies associated with SLE, ANA is one of the least helpful in diagnosis. A negative ANA makes the diagnosis of SLE unlikely. A positive ANA, especially at lower titers, can occur in 20% of normal young women, in localized autoimmune disorders, in other multisystem autoimmune diseases, in vasculitis, in infections, in ageing, with many drugs, and in malignancy.

Anti-dsDNA is specific for SLE, especially if the assay used is immunofluoresence on Crithidia. Low titers of anti-DNA in enzyme-linked immunosorbant assay (ELISA) may not be specific for SLE. Patients with SLE and anti-dsDNA are at greater risk of renal disease. Anti-Sm is also specific for SLE, but is less frequent than anti-dsDNA. Patients with SLE who are positive for anti-Sm are also at greater risk for renal disease.

Anti-Ro and anti-La are classically considered to be Sjögren syndrome antibodies. In SLE, secondary Sjögren syndrome can occur with or without these autoantibodies. Patients with anti-Ro are more likely to be photosensitive. Anti-Ro and anti-La increase the risk of congenital heart block during pregnancy (see Chapter 71).

Anti-RNP can also occur in rheumatoid arthritis and in mixed connective tissue disease. Patients with SLE who are positive for anti-RNP are more likely to have Raynaud's phenomenon or myositis.

Differential Diagnosis

Early symptoms, including low-grade fever, fatigue, malaise, mild hair loss, and arthralgias, are not specific for SLE. Often there is a delay of several years before a diagnosis of SLE is made. Other multisystem autoimmune diseases that share features with SLE include Sjögren syndrome, rheumatoid arthritis, and systemic sclerosis. Vasculitis, especially hypocomplementemic urticarial vasculitis, overlaps with SLE. Other vasculitides, including Wegener's granulomatosis, polyarteritis nodosa, vasculitis secondary to hepatitis B or hepatitis C, and lymphomatoid granulomatosis need to be considered. Sarcoidosis can share

some features with SLE, including fever, lymphadenopathy, and polyarthritis. Several viral infections, including cytolomegalovirus, parvovirus, human imunodeficiency virus (HIV), and hepatitis B can cause polyarthritis, and, sometimes, autoantibodies. Malignancy can lead to polyarthritis, vasculitis, and autoantibodies including ANA.

Disease Course

The classic pattern of SLE is a relapsing-remitting, or "flare" pattern, but some patients have chronic activity. Periods of long quiescence can occur, but are rare (Barr et al., 1999). Lack of flares (Swaak et al., 1989) and lack of organ damage (Rahman et al., 2001) are associated with normal survival. However, more than 50% of SLE patients have one or more organs that are chronically damaged. Some organs are damaged by the disease itself, such as renal insufficiency (or renal failure), but a large component of organ damage in SLE is also due to chronic treatment with corticosteroids. Corticosteroid-induced damage includes osteoporotic fractures, osteonecrosis, cataracts, and diabetes mellitus.

Osteopenia or osteoporosis occurs in the majority of SLE patients on chronic corticosteroids. Bone loss from corticosteroids occurs early. If corticosteroids are stopped, some bone loss may be reversible. However, there is also evidence that SLE itself is a risk factor for osteoporosis, likely due to inflammatory cytokines such as interleukin 6 (IL-6). Other risk factors for osteoporosis include post-menopausal status, being white, smoking, and sedentary lifestyle. All women with SLE should be on calcium and vitamin D supplementation. Those with osteoporosis should start bisphosphonate therapy, unless there is a contraindication such as renal insufficiency/failure or plans for a future pregnancy.

Osteonecrosis (or avascular necrosis of bone) is a complication of high-dose corticosteroid use, usually greater than 30 mg prednisone (or equivalent) daily. The pathogenesis is poorly understood, but it is thought that corticosteroids lead to fat replacement in the marrow, increased intraosseous pressure, and necrosis of bone. Hypercoagulability may further increase the risk (Mont et al., 1997a). If detected early, core decompression of the affected joint may allow growth of new bone (Mont et al., 1997b). Hips are most frequently involved, followed by knees and shoulders. Joint involvement is almost always bilateral. Most patients eventually require total joint replacement. Prevention is avoidance of high-dose corticosteroids. There is interest in calcium-channel blockers, which may reduce the intraosseous pressure (Barbosa et al., 1995).

Both cataracts and glaucoma are increased in SLE patients treated with corticosteroids. Both high-dose daily prednisone and chronic low-dose (<7.5 mg/day) prednisone increase the risk of posterior subcapsular cataracts. Hydroxychloroquine is rarely associated with retinopathy, which is nearly always reversible (Rynes and Bernstein, 1993). The dose of hydroxychloroquine should be reduced in renal insufficiency.

Diabetes mellitus is increased in SLE patients on corticosteroids, especially in those with a positive family history or who have gained weight. Weight gain is one of the most common complications of chronic corticosteroid treatment of SLE.

The major cause of late death in SLE is actually cardiovascular disease (Urowitz et al., 1976). The rate of myocardial infarction in young women with SLE is 50 times higher than Framingham offspring controls (Manzi et al., 1997). The increased risk of cardiovascular disease remains even after adjustment for traditional cardiovascular risk factors (Esdaile et al., 2001).

Surprisingly, the SLE patients at risk for angina pectoris and myocardial infarction are not those who clinically have the most severe disease (Zonana-Nacach et al., 1995). The patients at greatest risk are those chronically on corticosteroids, usually low-dose. Similarly, SLE patients with atherosclerosis detected by noninvasive means are those on less treatment and with less autoantibodies (Roman et al., 2003). Thus, the "lupus factor" associated with atherosclerosis cannot be identified using clinical indices of disease activity or serologic markers such as anti-dsDNA or low complement.

Traditional cardiovascular risk factors predictive of later myocardial infarction in SLE include age, male sex, hypertension, diabetes mellitus, renal insufficiency, homocysteine, and the lupus anticoagulant. Homocysteine is a risk factor for later stroke (or any arterial thrombotic event) (Petri et al., 1996). Reduction in and control of traditional cardiovascular risk factors is an essential part of the management of SLE.

Infection is a major cause of both morbidity and mortality in SLE. The disease itself is associated with infection; the risk increases with the use of corticosteroids and immunosuppressive drugs (Petri, 1998). Patients with SLE are especially susceptible to certain bacterial infections (pneumococcus, meningococcus, salmonella), and viral (cytomegalovirus, herpes zoster, warts) infections.

Malignancy is increased with the use of cytotoxic drugs such as cyclophosphamide. Recent studies suggest SLE itself is associated with malignancy, including lymphoma (Sweeney et al., 1995) (see Chapter 69). Cervical dysplasia and cervical cancer are increased in SLE (Blumenfeld et al., 1994).

Treatment

Treatment is determined by organ involvement. Photosensitive rashes are treated by sun avoidance, sun block, and by hydroxychloroquine, an antimalarial drug with an immunomodulating effect. Arthritis is managed with

nonsteroidal anti-inflammatory drugs (NSAIDs) and with hydroxychloroquine; severe cases may require immunosuppressive drugs, including methotrexate or leflunomide. Anti-TNF biologic agents, approved for rheumatoid arthritis, are generally avoided in SLE arthritis, because they have been associated with rare cases of drug-induced lupus and with the development of anticardiolipin and anti-dsDNA antibodies. Mild serositis may be controlled with NSAIDs, whereas severe cases may require initial high-dose corticosteroid therapy (often given as a 3-day methylprednisolone "pulse") followed by taper of oral prednisone. A severe pericardial effusion that leads to pericardial tamponade may require a pericardial window. Mild hematologic lupus often requires no treatment, but severe hemolytic anemia or thrombocytopenia is treated with corticosteroids and/or intravenous immunoglobulin. Recalcitrant hematologic lupus may require splenectomy. Granulocyte colony-stimulating factor (G-CSF), which is used to treat neutropenia (including neutropenia from cyclophosphamide) is associated with SLE flares (Euler et al., 1997).

Severe internal organ involvement will require immunosuppressive regimens. Lupus nephritis occurs in mild (mesangial, focal) and severe (diffuse proliferative) forms, as well as in the form of membranous nephritis. Mycophenolate mofetil is appropriate initial therapy, along with corticosteroids (Chan et al., 2000; Contreras et al., 2004). Rapidly progressive nephritis may require intravenous cyclophosphamide, usually given monthly for six months as "induction therapy" (Boumpas et al., 1992), followed by a maintenance regimen of mycophenolate mofetil, azathioprine, or quarterly cyclophosphamide. Angiotensin converting enzyme (ACE) inhibitor therapy is given to most nephritis patients to retard renal sclerosis, and may reduce proteinuria in membranous nephritis.

Encephalopathy mononeuritis multiplex and transverse myelitis are treated by high-dose corticosteroids, often with the addition of intravenous "pulse" cyclophosphamide. Seizures due to active lupus are treated with both corticosteroids and anti-epileptics. Psychosis can be caused by both SLE and by high-dose corticosteroids. Cognitive impairment occurs in 80% of SLE patients within 10 years of diagnosis. Early detection is difficult, given lack of access to cognitive function testing. In some studies, antiphospholipid positivity appears to be associated with progression (Hanly et al., 1999; Menon et al., 1999).

New approaches are being developed for life-threatening SLE, recalcitrant to current regimens. Both high-dose cyclophosphamide alone (Petri et al., 2003) and high-dose cyclophosphamide followed by stem-cell rescue (Burt et al., 1998) have shown long-term benefit, but, over time, relapses are frequent. New approaches that target B cells include rituximab (Leandro et al., 2002) and monoclonals directed against the B lymphocyte stimulator protein (Stohl et al., 2003) (see Chapter 76).

CHRONIC CUTANEOUS LUPUS

Lupus also occurs in a form limited to the skin, usually as discoid lupus. Over time, only 5% of patients presenting as chronic cutaneous lupus evolve to the systemic form. Initial clues to the subgroup, which may progress to SLE, include discoid lesions above and below the neck, or the presence of polyarthralgias. Other rare forms of chronic cutaneous lupus include hypertrophic (verrucous) lupus, lupus panniculitis, mucosal lupus, tumid lupus, chilblains lupus, and discoid lupus/lichen planus overlap. Subacute cutaneous lupus is an exception to the rule, in that as many as 50% may have SLE.

Chronic cutaneous lupus is treated with topical corticosteroids, corticosteroid injections, antimalarial drugs including hydroxychloroquine and quinacrine, thalidomide, and mycophenolate mofetil.

MIXED CONNECTIVE TISSUE DISEASE

Mixed connective tissue disease (MCTD) is defined in patients with high-titer RNP and features of SLE, scleroderma, and myositis, including arthritis, Raynaud phenomenon, esophageal dysmotility, decreased carbon monoxide diffusion opacity in the lung (DLCO) and myositis (Sharp et al., 1976). However, this utility of the diagnosis over time has been questioned, because of a high rate of progression to SLE or systemic sclerosis (Isenburg and Black, 1999).

UNDIFFERENTIATED CONNECTIVE TISSUE DISEASES

About 5% of women have symptoms or signs of autoimmune disease without meeting classification criteria for SLE, rheumatoid arthritis, systemic sclerosis, myositis, or vasculitis. Onset is typically in women in their thirties. Arthralgias/arthritis, cutaneous, and Raynaud phenomenon are the most frequent symptoms and signs (Danieli et al, 1999). Ten to thirty-five percent of patients with undifferentiated multisystem autoimmune diseases progress to a classifiable autoimmune disease over five years or longer of follow-up (Danieli et al, 1999; Williams et al., 1999; Mosca et al., 2002; Bodolay et al., 2003). Progression to a defined illness usually occurs within the first 2 years after onset.

References

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.

Baechler, E.C., Batliwalla, F.M., Karypis, G., Gaffney, P.M., Ortmann, W.A., Espe, K.J., Shark, K.B., Grande, W.J., Hughes, K.M., Kapur, V., Gregerson, P.K., and Behrens, T.W. (2003). Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. U.S.A. 100*(5), 2610–2615.

Barbosa, L.M., Gauthier, V.J., and Davis, C.L. (1995). Bone pain that responds to calcium channel blockers. A retrospective and prospective study of transplant recipients. *Transplantation 59*(4), 541–544.

Barr, S., Zonana-Nacach, A., Magder, L., and Petri, M. (1999). Patterns of disease activity in systemic lupus erythematosus. *Arthritis Rheum. 42,* 2682–2688.

Blumenfeld, Z., Lorber, M., Yoffe, N., and Scharf, Y. (1994). Systemic lupus erythematosus: predisposition for uterine cervical dysplasia. *Lupus 3,* 59–61.

Bodolay, E., Csiki, Z., Szekanecz, Z., Ben, T., Kiss, E., Zeher, M., Szucs, G., Danko, K., and Szegedi, G. (2003). Five-year follow-up of 665 Hungarian patients with undifferentiated connective tissue disease (UCTD). *Clin. Exp. Rheumatol. 21*(3), 313–320.

Boumpas, D.T., Austin, III 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.

Burt, R.K., Traynor, A.E., Pope, R., Schroeder, J., Cohen, B., Karlin, K.H., et al. (1998). Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. *Blood 92,* 3505–3514.

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.

Chan, T.M., Li, F.K., Tang, C.S., Wong, R.W., Fang, G.X., Ji, Y.L., Lau, C.S., Wong, A.K., Tong, M.K., Chan, K.W., and Lai, K.N. (2000). Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. *N. Engl. J. Med. 343*(16), 1156–1162.

Contreras, G., Pardo, V., Leclercq, B., Lenz, O., Tozman, E., O'Nan, P., and Roth, D. (2004). Sequential therapies for proliferative lupus nephritis. *N. Engl. J. Med. 350*(10), 971–980.

Costenbader, K.H., Kim, D.J., Peerzada, J., Lockman, S., Nobles-Knight, D., Petri, M., and Karlson, E.W. (2004). Cigarette smoking and the risk of systemic lupus erythematosus: a meta-analysis. *Arthritis Rheum. 50*(3), 849–857.

Danieli, M.G., Fraticelli, P., Franceschini, F., Cattaneo, R., Farsi, A., Passaleva, A., Pietrogrande, M., Invernizzi, F., Vanoli, M., Scorza, R., Sabbadini, M.G., Gerli, R., Corvetta, A., Farina, G., Salsano, F., Priori, R., Valesini, G., and Danieli G. (1999). Five-year follow-up of 165 Italian patients with undifferentiated connective tissue diseases. *Clin. Exp. Rheumatol. 17*(5), 585–591.

Esdaile, J.M., Abrahamowicz, M., Grodzicky, T., Li, Y., Panaritis, C., du Berger, R., Cjte, R., Grover, S.A., Fortin, P.R., Clarke, A.E., and Senecal, J.L. (2001). Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum. 44*(10), 2331–2337.

Euler, H.H., Harten, P., Zeuner, R.A., and Schwab, U.M. (1997). Recombinant human granulocyte colony stimulating factor in patients with systemic lupus erythematosus associated neutropenia and refractory infections. *J. Rheumatol. 24,* 2153–2157.

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*(4), 728–734.

Isenberg, D. and Black, C. (1999). Naming names! Comment on the article by Smolen and Steiner. *Arthritis Rheum. 42*(1), 191–196.

James, J.A., Neas, B.R., Moser, K.L., Hall, T., Bruner, G.R., Sestak, A.L., and Harley, J.B. (2001). Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum. 44*(5), 1122–1126.

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.

Leandro, M.J., Edwards, J.C., Cambridge, G., Ehrenstein, M.R., and Isenberg, D.A. (2002). An open study of B lymphocyte depletion in systemic lupus erythematosus. *Arthritis Rheum. 46*(10), 2673–2677.

Manderson, A.P., Botto, M., and Walport, M.J. (2004). The role of complement in the development of systemic lupus erythematosus. *Ann. Rev. Immunol. 22,* 431–456.

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 infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am. J. Epidemiol. 145*(5), 408–415.

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*(4), 735–741.

Mont, M.A., Glueck, C.J., Pacheco, I., Wang, P., Hungerford, D.S., and Petri, M. (1997a). Risk factors for osteonecrosis in systemic lupus erythematosus. *J. Rheumatol. 24,* 654–662.

Mont, M.A., Fairbank, A.C., Petri, M., and Hungerford, D.S. (1997b). Core decompression for osteonecrosis of the femoral head in systemic lupus erythematosus. *Clin. Orthop.* 91–97.

Mosca, M., Neri, R., Bencivelli, W., Tavoni, A., and Bombardieri, S. (2002). Undifferentiated connective tissue disease: analysis of 83 patients with a minimum followup of 5 years. *J. Rheumatol. 29*(11), 2345–2349.

Olsen, M., Arnett, C., and Reveille, J. (1993). Contrasting molecular patterns of MHC Class II alleles associated with the anti-Sm and anti-RNP precipitin autoantibodies in systemic lupus erythematosus. *Arthritis Rheum. 36,* 94–104.

Parks, C.G., Cooper, G.S., Nylander-French, L.A., Sanderson, W.T., Dement, J.M., Cohen, P.L., Dooley, M.A., Treadwell, E.G., St Clair, E.W., Gilkeson, G.S., Hoppin, J.A., and Savitz, D.A. (2002). Occupational exposure to crystalline silica and risk of systemic lupus erythematosus: a population-based, case-control study in the southeastern United States. *Arthritis Rheum. 46*(7), 1840–1850.

Petri, M. (1998). Infection in systemic lupus erythematosus. *Rheum. Dis. Clin. N. Am. 24,* 423–456.

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.

Petri, M., Jones, R.J., and Brodsky, R.A. (2003). High dose immunoblative cyclophosphamide without stem cell transplantation in systemic lupus erythematosus. *Arthritis Rheum. 48,* 166–173.

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.

Roman, M.J., Shanker, B.A., Davis, A., Lockshin, M.D., Sammaritano, L., Simantov, R., Crow, M.K., Schwartz, J.E., Paget, S.A., Devereux, R.B., and Salmon, J.E. (2003). Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N. Engl. J. Med. 349*(25), 2399–2406.

Rynes, R.I. and Bernstein, H.N. (1993). Ophthalmologic safety profile of antimalarial drugs. *Lupus 2* (Suppl 1), S17–S19.

Sharp, G.C., Irvin, W.S., May, C.M., Holman, H.R., McDuffie, F.C., Hess, E.V., and Schmid, F.R. (1976). Association of antibodies to ribonucleoprotein and Sm antigens with mixed connective-tissue disease,

systematic lupus erythematosus and other rheumatic diseases. *N. Engl. J. Med. 295*(21), 1149–1154.

Stohl, W., Metyas, S., Tan, S.M., Cheema, G.S., Oamar, B., Xu, D., Roschke, V., Wu, Y., Baker, K.P., and Hilbert, D.M. (2003). B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum. 48*(12), 3475–3486.

Swaak, A.J.G., Nossent, J.C., Bronsveld, W., van Rooyen, A., Nieuwenhuys, E.J., Theuns, L., and Smeenk, R.J.T. (1989). Systemic lupus erythematosus. II. Observations on the occurrence of exacerbations in the disease course: Dutch experience with 110 patients studied prospectively. *Ann. Rheum. Dis. 48,* 455–460.

Sweeney, D.M., Manzi, S., Janosky, J., Selvaggi, K.J., Ferri, W., Medsger, T.A., Jr., and Ramsey-Goldman, R. (1995). Risk of malignancy in women with systemic lupus erythematosus. *J. Rheumatol. 22*(8), 1478–1482.

Tan, E.M., Cohen, A.S., Fries, J.F., Masi, A.T., McShane, D.J., Rothfeld, 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.

Tsokos, G.C. (2001). Systemic lupus erythematosus. A disease with a complex pathogenesis. *Lancet 358* (Suppl), S65.

Uramoto, K.M., Michet, C.J.J., Thumboo, J., Sunku, J., O'Fallon, W.M., and Gabriel, S.E. (1999). Trends in the incidence and mortality of systemic lupus erythematosus, 1950–1992. *Arthritis Rheum. 42,* 46–50.

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.

Vilar, M.J. and Sato, E.I. (2002). Estimating the incidence of systemic lupus erythematosus in a tropical region (Natal, Brazil). *Lupus 11*(8), 528–532.

Williams, H.J., Alarcon, G.S., Joks, R., Steen, V.D., Bulpitt, K., Clegg, D.O., Ziminski, C.M., Luggen, M.E., St Clair, E.W., Wilkens, R.F., Yarboro, C., Morgan, J.G., Egger, M.J., and Ward, J.R. (1999). Early undifferentiated connective tissue disease (CTD). VI. An inception cohort after 10 years: disease remissions and changes in diagnoses in well established and undifferentiated CTD. *J. Rheumatol. 26*(4), 816–825.

Wilson, W.A., Gharavi, A.E., Koike, T., Lockshin, M.D., Branch, D.W., Peitte, M.D., Brey, R., Dersken, 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: report of an international workshop. *Arthritis Rheum. 42,* 1309–1311.

Zonana-Nacach, A., Salas, M., Sánchez, M., Camargo-Coronel, A., Bravo-Gatica, C., and Mintz, G. (1995). Measurement of clinical activity of systemic lupus erythematosus and laboratory abnormalities: a 12-month prospective study. *J. Rheumatol. 22,* 45–49.

CHAPTER

28

Systemic Lupus Erythematosus: Immunologic Features

INSOO KANG* AND JOE CRAFT*†

**Section of Rheumatology, Department of Medicine, and †Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06520-8031*

Systemic lupus erythematosus (SLE) is unique among autoimmune illnesses in its breadth of clinical manifestations, in the variability of disease progression, and in the nature of its underlying immunologic abnormalities (Vratsanos et al., 2001a). The hallmark of lupus is the presence of serum antibodies directed against nuclear antigens (antinuclear antibodies or ANA) (Friou, 1957; Holman and Kunkel, 1957; Deicher et al., 1959) that indicate the breakdown of tolerance to self-antigens. Such an event is secondary to complex genetic and environmental interactions that lead to the activation of autoantibody-producing B cells. We have previously reviewed these interactions (Vratsanos et al., 2001a), and they are recounted and updated below. In this review, we have used the terms SLE and lupus interchangeably, identifying the same systemic disease.

AUTOANTIBODIES

Autoantibodies against chromatin and ribonucleoproteins, in addition to being serologic hallmarks for SLE (Tan, 1989) appear to have a pathogenic role in lupus (Shlomchik et al., 2001). The deposition of immune complexes of these autoantibodies with their respective autoantigens in target organs, such as the kidney, leads to activation of complement and Fc receptor binding with subsequent tissue injury (Lefkowith and Gilkeson, 1996; Clynes et al., 1998). Presumably, similar mechanisms account for tissue inflammation in other organs, such as the skin and joints.

Evidence of the pathogenicity of certain autoantibodies comes from studies of human polyclonal anti-dsDNA and murine monoclonal anti-dsDNA or anti-nucleosome (anti-chromatin) IgG antibodies. The passive transfer of these antibodies into non-autoimmune mice induces glomerular pathology that is similar to that seen in human lupus (Raz et al., 1989; Vlahakos et al., 1992). Mice that transgenically express immunoglobulin genes coding for anti-dsDNA antibodies also develop immune complex-mediated proliferative glomerulonephritis (Roark et al., 1995a; 1995b). In addition, genetic disruption of the ability of B cells to produce (auto)antibodies results in markedly diminished glomerular pathology in lupus-prone mice (Shlomchik et al., 1994; Chan et al., 1999). In humans, increases in serum titer often precede, or are coincident with, worsening of renal disease (Koffler et al., 1967; Hecht et al., 1976). Autoantibodies other than those directed against chromatin may also incite immune complex renal disease, and end-organ injury may also result from non-antibody dependent mechanisms (Chan and Shlomchik, 1998; Chan et al., 1999; Shlomchik et al., 2001).

The precise site of interactions between autoantibody and autoantigen is unclear. Three hypotheses have been proposed to explain the process by which immune complexes containing anti-chromatin antibodies are formed (reviewed in Gilkeson, 1999). The first proposes that the interaction between these autoantibodies and antigen occurs exclusively in the serum, with subsequent deposition in tissues. This explanation now appears unlikely since multiple attempts to detect immune complexes containing DNA in the serum of lupus patients have failed (Izui et al., 1977; Sano and

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Morimoto, 1981). Further, the passive transfer of preformed complexes containing anti-DNA antibodies in mice has not resulted in glomerular inflammation (Emlen and Mannik, 1982). A second postulates that anti-chromatin or anti-DNA antibodies may cross-react with antigens, such as laminin or actin, in the glomerulus to form immune complexes (Jacob et al., 1985; Faaber et al., 1986; Madaio et al., 1987; Raz et al., 1993; Mostoslavsky et al., 2001; Deocharan et al., 2002; Mason et al., 2004; Zhao et al., 2005), and/or that a subset of autoantibodies directly binds glomerular structures (Liang et al., 2004). An alternative possibility is that circulating antigen is trapped *in situ* within the kidney and subsequently binds anti-dsDNA antibodies. Evidence for the latter hypothesis comes from the observation that circulating nucleosomes, normally generated during the process of apoptosis (Bell et al., 1990; Emlen et al., 1994; Tax et al., 1995), can bind to proteins within the glomerular basement membrane, particularly type IV collagen (Izui et al., 1976; Schmiedeke et al., 1989; Bernstein et al., 1995; Di Valerio et al., 1995). Regardless of the precise mechanism(s) involved, immune complexes that are located within the kidney can induce inflammation and tissue destruction by activating phagocytic cells bearing Fc and complement receptors (Clynes et al., 1998).

Properties of autoantibodies influence pathogenicity. These properties include the net charge of the antigen-binding region, isotype, and avidity of the antibody for DNA. Cationic antibodies are more likely to be pathogenic (Lefkowith et al., 1996; Liang et al., 2004), presumably due to increased avidity for negatively charged antigens such as DNA, and preferentially bind in the kidney (Dang and Harbeck, 1984). Isotypes that bind complement well (IgG2a) are more pathogenic in murine models than other isotypes (IgG1). Some studies have found no striking difference in the variable (V) gene segment usage of heavy or light immunoglobulin chains found between lupus-prone and non-autoimmune mice, or in studies of human lupus patients (Gilkeson, 1999), suggesting that germline gene segment rearrangement does not affect the pathogenicity of anti-dsDNA antibodies; however, ANA isolated from lupus-prone mice do have increased utilization of certain gene families, compared with non-ANA binding antibodies (Liang et al., 2004).

Anti-dsDNA antibodies may also induce tissue injury in the brain. Recent studies have demonstrated that a subset of anti-dsDNA antibodies in the mouse can cross-react with *N*-methyl-D-aspartate (NMDA) receptors in the brain, with the functional capacity to induce excitotoxic neuronal cell death, providing a putative mechanism for induction of pathologic responses in central nervous system (CNS) lupus (DeGiorgio et al., 2001). More recent work by these investigators has shed light on this issue, with the demonstration that lipopolysaccharide (LPS), a ligand for Toll-like receptor (TLR) 4, can accentuate autoantibody-mediated brain injury in a peptide-induced lupus model (Kowal et al., 2004). Given the capacity of LPS to induce a breach in the blood–brain barrier (BBB) (Xaio et al., 2001), this work suggests an initiating mechanism whereby autoantibodies produced in the periphery in SLE can enter the brain.

ABNORMALITIES OF B-CELL ACTIVATION

Experimental evidence from murine and human lupus has consistently suggested that there is an intrinsic abnormality in lupus B cells that leads to dysregulated production of autoantibodies. Work done over a decade ago supported this conclusion with the demonstration that pre-B cells isolated from the fetal liver of lupus-prone NZB × NZW F1 mice (NZB/W), transferred into scid mice, led to IgM and IgG hyperglobulinemia, production of antinuclear antibodies, and, in some mice, lupus-like deposition of IgG in the kidney with significant proteinuria (Reininger et al., 1992). The transfer of pre-B cells from non-autoimmune mice did not lead to these characteristic signs of autoimmunity. B cells from lupus-prone mice are also more sensitive to stimulation from T cells (Jongstra-Bilen et al., 1997). These data are consistent with studies in human lupus that demonstrate that B cells are hyperproliferative and produce more antibodies spontaneously, including autoantibodies (Blaese et al., 1980).

The mechanism(s) responsible for this hyper-effector phenotype of lupus B cells remains largely unknown. Such alterations can be secondary to changes in B-cell subsets, cytokine production, expression of effector molecules, and/or cell signaling pathways. An expansion of the early plasma cells ($CD38^+$ $CD19^+$ $CD10^-$ $CD20^-$ $CD21^+$ $CD24^-$ $CD39^+$ $CD5^-$ VLA-4^+ VLA-5^- MPC-1^- and surface membrane IgM^-) has been noticed in peripheral blood of lupus patients (Harada et al., 1996). Others have confirmed these results by showing an increased frequency and absolute number of plasmablasts and plasma cells ($CD19^+$ $CD27^{high}$ B cells) in lupus patients (Odendahl et al., 2000; Arce et al., 2001). Correlation between circulating $CD27^{high}$ plasma cells and disease activity in patients with SLE is also found (Jacobi et al., 2003).

Abnormal cytokine production and expression of effector molecules on the cell surface have been investigated in both human and murine lupus. B cells and monocytes from lupus patients produce more interleukin 10 (IL-10) and are more sensitive to IL-10 stimulation (Llorente et al., 1993; 1994; 1995; Csiszar et al., 2000). Further, B cells from lupus patients with active disease abnormally express CD40 ligand, compared with those isolated from patients in remission or from healthy controls (Desai-Mehta et al., 1995). Expression of CD40L could be induced on B cells from lupus patients in remission but not on control B cells,

supporting the idea of an intrinsic difference in lupus B-cell phenotype. Indeed, a short course of anti-CD40L antibody has improved serologic activity and hematuria in lupus patients with nephritis (Boumpas et al., 2003). Such treatment has reduced the frequency of IgG and IgG anti-DNA antibody-producing B cells (Huang et al., 2002) and eliminated CD38 (bright) Ig-secreting cells that are found in lupus patients, in particular those with active disease (Grammer et al., 2003). Taken together, these findings demonstrate that, in lupus, opportunities for T/B interaction exist that do not exist in unaffected individuals. It seems likely that aberrant production of cytokines (such as IL-10) and expression of surface effector molecules such as CD154 (CD40L) sets up a self-perpetuating cycle of lymphocyte activation that ultimately enhances disease progression.

Evidence suggests that signal transduction in B cells is abnormal in lupus. Abnormalities in both positive and negative regulation of B-cell receptor (BCR) signaling have been described. For example, in human lupus, B cells differ in their biochemical imprint after BCR stimulation, having greater protein tyrosine phosphorylation and free calcium release than control B cells after BCR stimulation (Liossis et al., 1996). The reasons for these differences are unclear, although genetic studies from mice indicate that deficiencies in molecules that down-regulate B-cell activation lead to a lupus phenotype. For example, CD22 is a B-cell specific transmembrane glycoprotein, which is phosphorylated after BCR signaling and assists in the recruitment of SHP-1 phosphatase into the BCR signaling complex. It, therefore, serves as an important downregulator of B-cell activation (O'Keefe et al., 1996). B cells from mice deficient in CD22 (through targeted gene knockout) are also hyper-responsive after BCR stimulation and develop high titers of IgG anti-dsDNA antibodies (O'Keefe et al., 1996; 1999). Similarly, genetic disruption of other negative signaling molecules in mouse B cells, for example the inhibitory receptor FcγRIIB, also lead to a lupus-like phenotype, depending upon the background murine strain (Bolland and Ravetch, 2000; Fukuyama et al., 2005). The role of CD22, inhibitory Fc receptors, and other B-cell receptor-associated proteins, such as CD21, in regulating B-cell activity in human lupus is unknown and is an important area for investigation.

The role of B cells as antigen-presenting cells (APCs) also appears important to the development of lupus. Lupus-prone MRL/Fas^{lpr} mice genetically deficient in B cells had a significantly reduced number of activated and memory T cells with a shift towards a naïve phenotype, when compared with B-cell intact animals (Chan and Shlomchik, 1998). As a consequence, T-cell infiltration of the skin and kidney was markedly reduced.

In summary, lupus B cells appear to be intrinsically abnormal in terms of BCR signaling, with enhanced production of, and sensitivity to, cytokines tropic to B cells as well as aberrant expression of effector molecules. These abnormalities result in dysregulation of antibody production and may also lead to inappropriate activation of T cells and abnormal T/B collaboration.

The conclusions that autoantibodies against chromatin and other nucleoproteins are central to the pathogenesis of lupus, and that B-cell abnormalities exist in disease, assume even greater significance in view of emerging evidence from several laboratories that TLR stimulation by endogenous ligands such as chromatin may play an important role in the pathogenesis of SLE. Toll-like receptors are pattern recognition receptors that recognize various pathogen-associated molecular structures including LPS, dsRNA, ssRNA and hypomethylated cytosine and guanosine sequence (CpG) DNA (Iwasaki and Medzhitov, 2004). Signals from TLR9 upon engagement by chromatin, presumably released from apoptotic cells, may potentiate autoimmune responses in lupus (Leadbetter et al., 2002). TLR9, as well as TLR3, recognize ligands not restricted to pathogens, unmethylated DNA present in mammalian chromatin (Viglianti et al., 2003), and dsRNA, respectively (for review, see Pasare and Medzhitov, 2003). In the former example, DNA upon direct engagement of anti-DNA-specific surface Ig, even in the absence of immune complexes, can initiate TLR9 signaling and sequential autoreactive B-cell activation (Viglianti et al., 2003); i.e., chromatin engaged by surface anti-DNA Ig may be delivered to intracellular TLR9 after endocytosis (Ahmad-Nejad et al., 2002). Such a scenario may be likely in SLE with its apparent abnormalities in the signaling threshold for B cells (Liossis et al., 1996), enabling them to bypass the normal anergic signals induced via surface Ig in contact with continually present self-antigen (Rui et al., 2003), and allowing them to garner help from available self-reactive, chromatin-specific, $CD4^+$ T cells (Mohan et al., 1993) (Figure 28.1).

T CELLS ARE REQUIRED FOR AUTOANTIBODY GENESIS AND DISEASE

The production of antibodies against chromatin and ribonucleoproteins is an antigen-driven process that is dependent on T-cell help (see Figure 28.1). This conclusion is based upon two essential lines of evidence. First, genetic analyses of high affinity, pathogenic murine and human IgG anti-DNA autoantibodies has revealed clonal selection and somatic mutation (Shlomchik et al., 1987; 1990; Davidson et al., 1990; Marion et al., 1990; O'Keefe et al., 1990). Apparent antigen selection and somatic hypermutation of autoantibodies suggests that autoreactive B cells in lupus require autoantigen-specific cognate and contact-dependent αβ T-cell help (reviewed in Craft et al., 1999), given the requirement of αβ T cells for immunoglobulin somatic

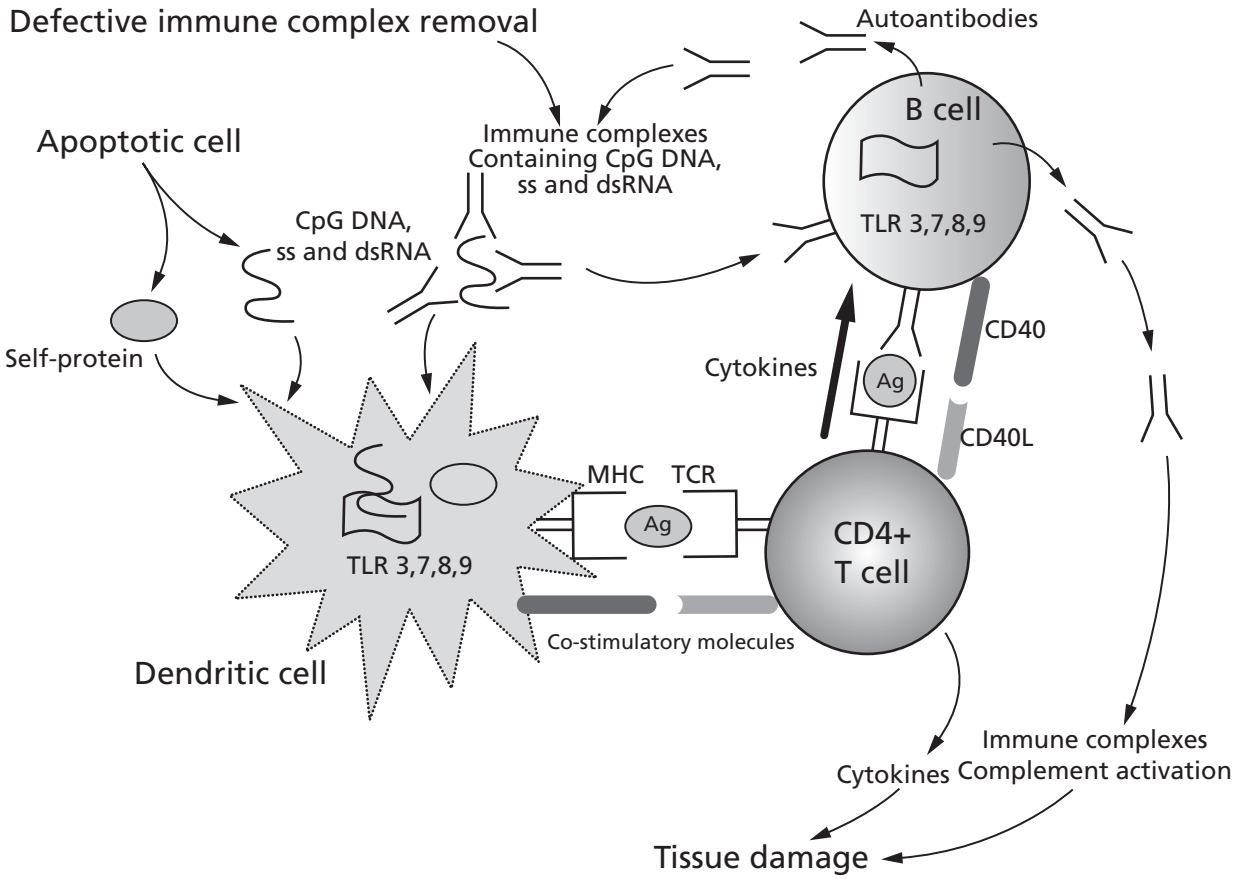


FIGURE 28.1 Immunologic pathogenesis of systemic lupus erythematosus (SLE). The figure depicts potential immunologic mechanisms involved in the development of SLE. These include defects in immune complex removal, apoptosis, and antigen presentations by antigen presenting cells (APCs) such as dendritic cells, as well as altered T- and B-cell interactions and their cytokine and autoantibody production. Nucleic acids (CpG DNA and ssRNA) from self and from microorganisms can also participate in stimulating APCs and B cells through Toll-like receptors (TLRs). Ag, antigen; ds, double stranded; MHC, major histocompatibility complex; ss, single stranded; TCR, T-cell receptor.

hypermutation and isotype switching in secondary lymphoid organs.

Second, early work demonstrating that neonatal thymectomy of lupus-prone mice led to abrogation of IgG anti-dsDNA synthesis and glomerulonephritis, and to improved mortality, indicated the T-cell dependence of autoantibody production (Steinberg et al., 1980). Similar results were obtained after treatment of these animals with antibodies that depleted $Thy1^+$ cells (Wofsy et al., 1985) or $CD4^+$ cells (Santoro et al., 1988). Genetic studies targeting $CD4^+$ T cells (Jevnikar et al., 1994; Koh et al., 1995) or αβ T-cell receptors (Peng et al., 1996b) in lupus-prone mice have provided further evidence for the T-cell dependence of disease in lupus. Furthermore, T cells have been isolated from (NZB × NZW)F1 and from (SWR × NZB)F1 (SNF1) lupus-prone mice that help anti-DNA production *in vitro* and accelerated disease when transferred to pre-nephritic mice *in vivo* (Ando et al., 1987; Mohan et al., 1993; Singh et al., 1995; Kaliyaperumal et al., 1996). Presumably similar pathways exist in SLE that account for the production of pathogenic autoantibodies by chromatin-peptide specific autoreactive T cells (Lu et al., 1999).

T cells that are reactive with lupus nuclear antigens including DNA-histones and small nuclear ribonucleoproteins have been isolated from peripheral blood of lupus patients (Rajagopalan et al., 1990; Crow et al., 1994; Desai-Mehta et al., 1995; Hoffman, 2004). These cells are typically $CD4^+$ and can provide help to B cells in producing anti-DNA and antihistone antibodies, supporting the idea that T cells that promote autoantibody generation are autoreactive. In fact, lupus-prone mice with transgenic T cells recognizing foreign antigens failed to promote the development of lymphadenopathy or pathogenic immune-complex disease, as assayed by cutaneous, renal, and salivary gland lesions (Peng et al., 1996a).

As in a normal immune response, αβ T-cell help in lupus is an MHC-restricted interaction between an αβ T-cell and B-cell recognizing epitopes derived from the same antigen.

This interaction is dependent upon binding of CD154 (CD40L) on the activated T cells to CD40 on the B-cell surface. Interruption of either CD154-CD40 or the CD28 CD80/86 (B7.1/B7.2) ligand pair interactions in lupus-prone mice by antibodies, fusion protein (CTLA-4Ig), or genetic knockout results in diminished autoantibody synthesis and end-organ disease (Finck et al., 1994; Mohan et al., 1995; Early et al., 1996; Ma et al., 1996; Daikh et al., 1997; Peng et al., 1997; Kalled et al., 1998). In addition, soluble CD154, cleaved from the cell membrane by matrix metalloproteinases, may serve as an additional pathway to activation of both cognate and non-cognate APCs (Kato et al., 1999; Vakkalanka et al., 1999). In fact, soluble CD154 levels are elevated in lupus patients, particularly in those with active disease (Kato et al., 1999; Vakkalanka et al., 1999).

ABNORMALITIES OF T CELLS

In human and murine lupus, αβ T cells are necessary for full penetrance of autoantibody production and disease. It appears that activation of such cells in lupus is a consequence of peripheral tolerance abrogation, as central tolerance appears intact to conventional peptide antigens (Singer and Abbas, 1994; Fatenejad et al., 1998), with the subsequent development of autoantigen-reactive T cells (Hoffman et al., 1993; Yan and Mamula, 2002). The events that lead to this are largely unknown. The potential mechanisms for the loss of T-cell tolerance to autoantigens in lupus include exposure to cryptic or self-antigens during apoptosis (Lanzavecchia, 1995; Doyle and Mamula, 2001), an abnormal activation threshold (Tsokos et al., 2003), excess costimulation (Desai-Mehta et al., 1996; Crow and Kirou, 2001), or inappropriate control by regulatory T cells (Shevach, 2000; Powrie and Maloy, 2003).

Regarding these possibilities, evidence suggests that lupus T cells may have intrinsic (genetic) defects that render them more susceptible to activation through their T-cell receptor (TCR)–CD3 complex after contact with self-peptides. This hypothesis stems from several observations. First, T cells from humans with SLE appear to have abnormalities in TCR signaling (Kammer et al., 1996; Vassilopoulos et al., 1995; Wong et al., 1999; Tsokos et al., 2003), anergy avoidance (Yi et al., 2000; Xu et al., 2004) and apoptosis (Elkon, 1994; Kovacs et al., 1996; Budagyan et al., 1998), as well as in expression of effector molecules, including CD40 ligand (CD154) (Desai-Mehta et al., 1996; Koshy et al., 1996; Kato et al., 1999; Vakkalanka et al., 1999). Second, lupus-prone mice have increased numbers of activated T cells (Rozzo et al., 1994; Sabzevari et al., 1997; Ishikawa et al., 1998) as well as enhanced responsiveness to TCR triggering *in vitro* and *in vivo* (Vratsanos et al., 2001b; Bouzahzah et al., 2003; Zielinski et al., 2005). Moreover, initial B-cell help and follicular migration in lupus appears to depend upon polyclonally activated T cells, rather than merely upon monoclonal or oligoclonal populations (Busser et al., 2003), adding credence to the notion that polyclonal activation is critical for disease initiation. Third, a genetic locus on chromosome 7 from lupus-prone New Zealand mice (NZM; New Zealand mixed) contributes to a heightened threshold of T-cell activation and a lower threshold for apoptotic death (Morel et al., 1997; Mohan et al., 1999) although these latter defects may be secondary to APC abnormalities (Sobel et al., 2002).

A series of defects in intracellular signaling have been identified in human lupus T cells (Kammer, 2005). Such T cells appear hyper-responsive after TCR engagement, with exaggerated intracellular calcium concentration and increased cytoplasmic protein tyrosine phosphorylation following anti-CD3 antibody stimulation (Liossis et al., 1998; Tsokos et al., 2003). In addition, lupus T cells have decreased TCR/CD3ζ expression, with upregulated FcRγ chain that replaces the TCR/CD3ζ chain (Enyedy et al., 2001). FcRγ forms a complex with Syk kinase, which is known to signal 100 times more effectively than ζ-ZAP-70 complex. This rewiring of T-cell signaling in lupus could be responsible for abnormally increased T-cell responses (Tsokos et al., 2003), and may be a consequence of abnormal lipid raft formation observed in T cells from patients (Jury et al., 2004; Krishnan et al., 2004). In addition to these proximal defects in TCR-mediated signal transduction, more distal signaling abnormalities also have been identified, with the ultimate activation of the Ca^{2+}-calmodulin kinase IV signaling cascade and downregulation of IL-2 gene transcription (Elliott et al., 2004; Juang et al., 2005) (reviewed in Kammer et al., 2002; Kammer, 2005). Given the role of IL-2 in tolerance maintenance, these signaling events could lead to aberrant T-cell function in SLE (Kammer, 2005).

Coincident with the above explanation are the several studies that have reported abnormalities in T-cell activation-induced cell death in lupus (Kovacs et al., 1996; Budagyan et al., 1998; Xu et al., 2004). For example, a recent study has demonstrated that activated T cells of lupus patients resist anergy and apoptosis by markedly upregulating and sustaining cyclooxygenase-2 (COX-2) expression (Xu et al., 2004). Inhibition of COX-2 induced apoptosis of the anergy-resistant lupus T cells by augmenting Fas signaling and markedly decreasing the survival molecule c-FLIP (cellular homolog of viral FLICE inhibitory protein). Of interest, the gene encoding COX-2 is located in a lupus-susceptibility region on chromosome 1, which supports the notion of genetic (intrinsic) T-cell defects in lupus.

T cells expressing $CD4^+CD25^+$ are regulatory T cells that suppress immune responses and maintain self-tolerance (Shevach, 2000). A decreased frequency of regulatory T cells has been reported in peripheral blood of lupus patients during active disease, when compared with healthy controls

(Liu et al., 2004). However, the result of this study is complicated by the fact that activated T cells also express CD25, the α chain of the IL-2 receptor. It will be useful to employ an additional marker such as forkhead box P3 (FOXP3) for better understanding of the role of regulatory T cells in lupus (Coffer and Burgering, 2004; Mills, 2004).

Abnormalities in T-cell-mediated cytotoxic activity also have been reported in lupus patients. T cells from lupus patients have impaired mitogen-induced cytotoxicity (Stohl, 1995; Warrington and Rutherford, 1990) and decreased cytotoxic response against allogeneic or xenogeneic targets (Charpentier et al., 1979; Tsokos et al., 1985). However, the effect of such abnormalities on controlling of pathogens was not known. Recently, Kang and colleagues demonstrated that lupus patients have increased Epstein–Barr virus (EBV) viral loads in peripheral blood cells that likely stems from inadequate $CD8^+$ T-cell responses against EBV (Kang et al., 2004). However, these patients can mount appropriate EBV-specific $CD4^+$ T-cell responses that account for lack of the development of EBV-associated lymphoproliferative diseases. The results of this study support the notion that lupus patients have abnormal $CD8^+$ T-cell function that can potentially affect the control of pathogens.

T-cell cytokines have been extensively studied in lupus patients. For example, spontaneous production of IL-10, a Th2 cytokine, from peripheral blood cells is significantly higher in lupus patients compared with controls (Llorente et al., 1993; 1994). This result is further validated by the findings of increased IL-10 mRNA expression in peripheral blood cells (Csiszar et al., 2000) and increased serum IL-10 levels in lupus patients (Houssiau et al., 1995). Such increased IL-10 production in lupus may have a pathogenic role since this cytokine is a potent stimulator of B-cell proliferation and differentiation. In fact, serum IL-10 levels correlate with disease activity and anti-dsDNA antibody titers (Houssiau et al., 1995; Park et al., 1998; Tyrrell-Price et al., 2001). Although T cells produce IL-10, the predominant source of IL-10 in lupus appears to be non-T cells such as B cells and monocytes (Llorente et al., 1993; 1994; Csiszar et al., 2000). In fact, a recent study has demonstrated that immune complexes in SLE sera stimulate IL-10 production from peripheral blood mononuclear cells (PBMC) possibly acting through FcγRII expressed by $CD14^+$ monocytes (Ronnelid et al., 2003).

INNATE IMMUNITY AND LUPUS

The potential role of interferon-α (IFN-α) in the pathogenesis of lupus was suggested in the late 1970s by a study reporting increased serum levels of IFN-α in lupus patients (Hooks et al., 1979). This notion has been recently revived by several groups that have demonstrated increased expression of interferon-induced genes in peripheral blood cells from lupus patients, using microarray analyses (Baechler et al., 2003; Bennett et al., 2003). The role of IFN-α in lupus is further supported by the clinical findings that some patients receiving IFN-α for the treatment of viral hepatitis and malignancy develop drug-induced lupus and other autoimmune diseases (Baechler et al., 2004; Crow and Kirou, 2004).

Interferon-α can affect innate and adaptive immune cell function. It has antiproliferative effects on T cells (Petricoin et al., 1997) and enhances immunoglobulin isotype switching by stimulating dendritic cells (Doyle and Mamula, 2001). Interferon-α induces maturation of dendritic cells and drives monocytes to become more effective in stimulating allogeneic T cells (Radvanyi et al., 1999; Blanco et al., 2001; Dalod et al., 2003). In addition, IFN-α can regulate the production of cytokines such as IL-10 and IL-12 by monocytes in response to bacterial stimulation (Hermann et al., 1998). The results of murine lupus studies also provide evidence for the role of IFN-α in the development of lupus. Administration of poly(inosine-cytosine), a synthetic dsRNA and strong IFN-α inducer, accelerated development of disease in (NZB × NZW) F1 mice (Reeves et al., 1981). Lupus-prone mice lacking receptors for type I interferons including IFN-α had reduced anti-dsDNA antibodies and disease activity (Santiago-Raber et al., 2003).

The mechanisms of induction of IFN-α in lupus patients are not clear yet. Obviously, viral infection is a strong inducer of IFN-α. However, there is no convincing data yet showing that lupus patients have chronic active viral infection that causes increased IFN-α production. Of interest, Rönnblom and Alm have demonstrated that immune complexes in lupus serum containing autoantibodies associated with apoptotic fragments, DNA, or RNA can stimulate plasmacytoid dendritic cells (PDCs) to produce IFN-α (Rönnblom and Alm, 2001a; 2001b). Such events can be mediated by TLRs (Baechler et al., 2004; Crow and Kirou, 2004). Plasmacytoid dendritic cells express TLR9, which recognize CpG DNA (Iwasaki and Medzhitov, 2004). Thus, immune complexes in lupus serum containing CpG DNA and anti-DNA antibodies can stimulate PDC to produce IFN-α (see Figure 28.1). This notion is supported, as discussed above, by two studies showing that murine B-cell hybridomas with specificity to rheumatoid factor or DNA activate B cells by engaging both immunoglobulin receptors and TLR9 (Leadbetter et al., 2002; Viglianti et al., 2003). A similar concept has been raised by recent studies demonstrating the role of TLR7 and TLR8 in cellular responses to ssRNA (Diebold et al., 2004; Heil et al., 2004) since antibodies against small nuclear RNA and protein complexes are often found in lupus patients (Baechler et al., 2004; Crow and Kirou, 2004).

SUMMARY AND FUTURE DIRECTIONS

The immunopathogenesis of SLE involves the broad spectrum of the immune system from innate to adaptive immunity. In the latter, both T and B cells are critical for the development of clinical disease, with autoantibodies against chromatin and ribonucleoproteins as serologic hallmarks (Shlomchik et al., 2001). B and T cells in SLE appear to be intrinsically abnormal in terms of antigen–receptor mediated activation, events likely related to genetic abnormalities (Shlomchik et al., 2001; Vratsanos et al., 2001a). Recently, sizeable data supporting a role for aberrant innate immune responses in SLE have accumulated, based on the studies demonstrating upregulation of IFN-α inducible genes in peripheral blood cells of patients, and activation of PDCs with IFN-α production in response to the immune complexes containing DNA and RNA via TLRs (reviewed in Crow and Kirou, 2004). However, there are still a number of missing links in these observations. We do not know the initial event(s) leading to the development of SLE and the characteristic pathogenic autoantibodies, and whether such an event is continuously required for the maintenance of autoantibody production and clinical disease. Although there is a potential link between innate and adaptive immunity as suggested by DC and IFN-α studies, it is unclear how IFN-α affects the function of adaptive immune cells like B- and T cells. Furthermore, fuller identification of the potential genetic alterations responsible for intrinsic B- and T-cell abnormalities and the immunologic consequences of such abnormalities are needed in human lupus. The results of these investigations will provide new diagnostic and therapeutic insights in SLE.

Acknowledgments

This work was supported by a NIH K08 award to IK and grants to JC from the NIH (AR 44076 and AR 40072), the Arthritis Foundation, the Lupus Foundation of America, the S.L.E. Foundation, Inc, Rheuminations, Inc, and a Kirkland Scholars Award.

References

Ahmad-Nejad, P., Hacker, H., Rutz, M., Bauer, S., Vabulas, R.M., and Wagner, H. (2002). Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur. J. Immunol. 32*, 1958–1968.

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. The *J. Immunol. 138*, 3185–3190.

Arce, E., Jackson, D.G., Gill, M.A., Bennett, L.B., Banchereau, J., and Pascual, V. (2001). Increased frequency of pre-germinal center B cells and plasma cell precursors in the blood of children with systemic lupus erythematosus. *J. Immunol. 167*, 2361–2369.

Baechler, E.C., Batliwalla, F.M., Karypis, G., Gaffney, P.M., Ortmann, W.A., Espe, K.J., Shark, K.B., Grande, W.J., Hughes, K.M., Kapur, V., Gregersen, P.K., and Behrens, T.W. (2003). Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. U.S.A. 100*, 2610–2615.

Baechler, E.C., Gregersen, P.K., and Behrens, T.W. (2004). The emerging role of interferon in human systemic lupus erythematosus. *Curr. Opin. Immunol. 16*, 801–807.

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*, 1487–1496.

Bennett, L., Palucka, A.K., Arce, E., Cantrell, V., Borvak, J., Banchereau, J., and Pascual, V. (2003). Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med. 197*, 711–723.

Bernstein, K.A., Valerio, R.D., 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.

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.

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.

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.

Boumpas, D.T., Furie, R., Manzi, S., Illei, G.G., Wallace, D.J., Balow, J.E., and Vaishnaw, A. (2003). A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum. 48*, 719–727.

Bouzahzah, F., Jung, S., and Craft, J. (2003). CD4+ T cells from lupus-prone mice avoid antigen-specific tolerance induction *in vivo*. *J. Immunol. 170*, 741–748.

Budagyan, V.M., Bulanova, E.G., Sharova, N.I., Nikonova, M.F., Stanislav, M.L., and Yarylin, A.A. (1998). The resistance of activated T-cells from SLE patients to apoptosis induced by human thymic stromal cells. *Immunol. Letts 60*, 1.

Busser, B.W., Adair, B.S., Erikson, J., and Laufer, T.M. (2003). Activation of diverse repertoires of autoreactive T cells enhances the loss of anti-dsDNA B cell tolerance. *J. Clin. Invest. 112*, 1361–1371.

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*, 51–59.

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*, 1639–1648.

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.

Clynes, R., Dumitru, C., and Ravetch, J.V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science 279*, 1052–1054.

Coffer, P.J., and Burgering, B.M. (2004). Forkhead-box transcription factors and their role in the immune system. *Nat. Rev. Immunol. 4*, 889–899.

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*, 245–257.

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.

Crow, M.K. and Kirou, K.A. (2001). Regulation of CD40 ligand expression in systemic lupus erythematosus. *Curr. Opin. Rheumatol. 13*, 361–369.

Crow, M.K. and Kirou, K.A. (2004). Interferon-alpha in systemic lupus erythematosus. *Curr. Opin. Rheumatol. 16*, 541–547.

Csiszar, A., Nagy, G., Gergely, P., Pozsonyi, T., and Pocsik, E. (2000). Increased interferon-gamma (IFN-gamma), IL-10 and decreased IL-4 mRNA expression in peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol. 122*, 464–470.

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.

Dalod, M., Hamilton, T., Salomon, R., Salazar-Mather, T.P., Henry, S.C., Hamilton, J.D., and Biron, C. A. (2003). Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon alpha/beta. *J. Exp. Med. 197*, 885–898.

Dang, H. and Harbeck, R.J. (1984). The *in vivo* and *in vitro* glomerular deposition of isolated anti-double- stranded-DNA antibodies in NZB/W mice. *Clin. Immunol. Immunopathol. 30*, 265–278.

Davidson, A., Mannheimer-Lory, A., Aranow, C., Peterson, R., Hannigan, N., and Diamond, B. (1990). Molecular characterization of a somatically mutated anti-DNA antibody bearing two SLE related idiotypes. *J. Clin. Invest. 85*, 1401–1409.

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. *Nat. Med. 7*, 1189–1193.

Deicher, H.R., Holman H.R., and Kunkel, H.G. (1959). The precipitin reaction between DNA and a serum factor in SLE. *J. Exp. Med. 109*, 97.

Deocharan, B., Qing, X., Lichauco, J., and Putterman, C. (2002). Alpha-actinin is a cross-reactive renal target for pathogenic anti-DNA antibodies. *J. Immunol. 168*, 3072–3078.

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.

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.

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.

Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., and Reis e Sousa, C. (2004). Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science 303*, 1529–1531.

Doyle, H.A., and Mamula, M.J. (2001). Post-translational protein modifications in antigen recognition and autoimmunity. *Trends Immunol. 22*, 443–449.

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.

Elkon, K.B. (1994). Apoptosis in SLE: too little or too much? *Clin. Exp. Rheumatol. 12*, 553.

Elliott, M.R., Shanks, R.A., Khan, I.U., Brooks, J.W., Burkett, P.J., Nelson, B.J., Kyttaris, V., Juang, Y.T., Tsokos, G.C., and Kammer, G.M. (2004). Down-regulation of IL-2 production in T lymphocytes by phosphorylated protein kinase A-RIIbeta. *J. Immunol. 172*, 7804–7812.

Emlen, W. and Mannik, M. (1982). Clearance of circulating DNA-anti-DNA immune complexes in mice. *J. Exp. Med. 155*, 1210–1215.

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.

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.

Faaber, P., Rijke, T.P., van de Putte, L.B., Capel, P.J., and Berden, J.H. (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.

Fatenejad, S., Peng, S.L., Disorbo, O., and Craft, J. (1998). Central T cell tolerance in lupus-prone mice: influence of autoimmune background and the lpr mutation. *J. Immunol. 161*, 6427–6432.

Finck, B.K., Linsley, P.S., and Wofsy, D. (1994). Treatment of murine lupus with CTLA4Ig. *Science 265*, 1225–1227.

Friou, G.J. (1957). Clinical application of lupus serum; nucleoprotein reaction using fluorescent antibody technique. *J. Clin. Invest. 36*, 890.

Fukuyama, H., Nimmerjahn, F., and Ravetch, J.V. (2005). The inhibitory Fcgamma receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. *Nat. Immunol. 6*, 99–106.

Gilkeson, G.S. (1999). Glomerular binding antibodies in systemic lupus erythematosus. In G.M. Kammer, and G.C. Tsokos (eds.), *Lupus: Molecular and Cellular Pathogenesis.* Totowa, N.J.: Humana Press.

Grammer, A.C., Slota, R., Fischer, R., Gur, H., Girschick, H., Yarboro, C., Illei, G.G., and Lipsky, P.E. (2003). Abnormal germinal center reactions in systemic lupus erythematosus demonstrated by blockade of CD154–CD40 interactions. *J. Clin. Invest. 112*, 1506–1520.

Harada, Y., Kawano, M.M., Huang, N., Mahmoud, M.S., Lisukov, I.A., Mihara, K., Tsujimoto, T., and Kuramoto, A. (1996). Identification of early plasma cells in peripheral blood and their clinical significance. *Br. J. Haematol. 92*, 184–191.

Hecht, B., Siegel, N., Adler, M., Kashgarian, M., and Hayslett, J.P. (1976). Prognostic indices in lupus nephritis. *Medicine (Baltimore) 55*, 163–181.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., and Bauer, S. (2004). Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science 303*, 1526–1529.

Hermann, P., Rubio, M., Nakajima, T., Delespesse, G., and Sarfati, M. (1998). IFN-alpha priming of human monocytes differentially regulates gram-positive and gram-negative bacteria-induced IL-10 release and selectively enhances IL-12p70, CD80, and MHC class I expression. *J. Immunol. 161*, 2011–2018.

Hoffman, R.W. (2004). T cells in the pathogenesis of systemic lupus erythematosus. *Clin. Immunol. 113*, 4–13.

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.

Holman, H.R., and Kunkel, H.G. (1957). Affinity between the lupus erythematosus serum factor and cell nuclei and nucleoprotein. *Science 126*, 162.

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.

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.

Huang, W., Sinha, J., Newman, J., Reddy, B., Budhai, L., Furie, R., Vaishnaw, A., and Davidson, A. (2002). The effect of anti-CD40 ligand antibody on B cells in human systemic lupus erythematosus. *Arthritis Rheum. 46*, 1554–1562.

Ishikawa, S., Akakura, S., Abe, M., Terashima, K., Chijiiwa, K., Nishimura, H., Hirose, S., and Shirai, T. (1998). A subset of CD4+ T cells expressing early activation antigen CD69 in murine lupus: possible abnormal regulatory role for cytokine imbalance. *J. Immunol. 161*, 1267–1273.

Iwasaki, A. and Medzhitov, R. (2004). Toll-like receptor control of the adaptive immune responses. *Nat. Immunol. 5*, 987–995.

Izui, S., Lambert, P.H., and Miescher, P.A. (1976). *In vitro* demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA–anti-DNA complexes in systemic lupus erythematosus. *J. Exp. Med. 144*, 428–443.

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 erythematosus. *Clin. Exp. Immunol. 30*, 384–392.

Jacob, L., Lety, M.A., Louvard, D., and Bach, J.F. (1985). Binding of a monoclonal anti-DNA autoantibody to identical protein(s) present at the surface of several human cell types involved in lupus pathogenesis. *J. Clin. Invest. 75*, 315–317.

Jacobi, A.M., Odendahl, M., Reiter, K., Bruns, A., Burmester, G.R., Radbruch, A., Valet, G., Lipsky, P.E., and Dorner, T. (2003). Correlation between circulating CD27high plasma cells and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum. 48*, 1332–1342.

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*, 1137–1143.

Jongstra-Bilen, J., Vukusic, B., Boras, K., and Wither, J.E. (1997). Resting B cells from autoimmune lupus-prone New Zealand Black and (New Zealand Black × New Zealand White)F1 mice are hyper-responsive to T cell-derived stimuli. *J. Immunol. 159*, 5810–5820.

Juang, Y.T., Wang, Y., Solomou, E.E., Li, Y., Mawrin, C., Tenbrock, K., Kyttaris, V., and Tsokos, G.C. (2005). Systemic lupus erythematosus serum IgG increases CREM binding to the IL-2 promoter and suppresses IL-2 production through CAMVIV. *J. Clin. Invest. 115*, 996–1005.

Jury, E.C., Kabouridis, P.S., Flores-Borja, F., Mageed, R.A.I., and Isenberg, D.A. (2004). Altered lipid raft-associated signaling and ganglioside expression in T lymphocytes from patients with systemic lupus erythematosus. *J. Clin. Invest. 113*, 1176–1187.

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.

Kalled, S.L., Cutler, A.H., Datta, S.K., and Thomas, D.W. (1998). Anti-CD40 ligand antibody treatment of SNF1 mice with established nephritis: preservation of kidney function. *J. Immunol. 160*, 2158–2165.

Kammer, G.M. (2005). Altered regulation of IL-2 production in systemic lupus erythematosus: an evolving paradigm. *J. Clin. Invest. 115*, 836–840.

Kammer, G.M., Khan, I.U., Kammer, J.A., Olorenshaw, I., and Mathis, D. (1996). Deficient type I protein kinase A isozyme activity in systemic lupus erythematosus T lymphocytes: II. Abnormal isozyme kinetics. *J. Immunol. 157*, 2690–2698.

Kammer, G.M., Perl, A., Richardson, B.C., and Tsokos, G.C. (2002). Abnormal T cell signal transduction in systemic lupus erythematosus. *Arthritis Rheum. 46*, 1139–1154.

Kang, I., Quan, T., Nolasco, H., Park, S.H., Hong, M.S., Howe, J.G., and Craft, J. (2004). Defective control of latent Epstein-Barr virus infection in systemic lupus erythematosus. *J. Immunol. 172*, 1287–1294.

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.

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.

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*, 2558–2562.

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.

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.

Kowal, C., DeGiorgio, L.A., Nakaoka, T., Hetherington, H., Huerta, P.T., Diamond, B., and Volpe, B.T. (2004). Cognition and immunity; antibody impairs memory. *Immunity 21*, 179–188.

Krishnan, S., Nambiar, M.P., Warke, V.G., Fisher, C.U., Mitchell, J., Delaney, N., and Tsokos, G.C. (2004). Alterations in lipid raft composition and dynamics contribute to abnormal T cell responses in systemic lupus erythematosus. *J. Immunol. 172*, 7821–7831.

Lanzavecchia, A. (1995). How can cryptic epitopes trigger autoimmunity? *J. Exp. Med. 181*, 1945–1948.

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature 416*, 603–607.

Lefkowith, J.B., Di Valerio, R., Norris, J., Glick, G.D., Alexander, A.L., Jackson, L. and Gilkeson, G.S. (1996). Murine glomerulotropic monoclonal antibodies are highly oligoclonal and exhibit distinctive molecular features. *J. Immunol. 157*, 1297–1305.

Lefkowith, J.B. and Gilkeson, G.S. (1996). Nephritogenic autoantibodies in lupus: current concepts and continuing controversies. *Arthritis Rheum. 39*, 894–903.

Liang, Z., Xie, C., Chen, C., Kreska, D., Hsu, K., Li, L., Zhou, X., and Mohan, C. (2004). Pathogenic profiles and molecular signatures of antinuclear autoantibodies rescued from NZM2410 lupus mice. *J. Exp. Med. 199*, 381–398.

Liossis, S.N., Ding, X.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.

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.

Liu, M.F., Wang, C.R., Fung, L.L., and Wu, C.R. (2004). Decreased CD4+CD25+ T cells in peripheral blood of patients with systemic lupus erythematosus. *Scand. J. Immunol. 59*, 198–202.

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.

Llorente, L., Richaud-Patin, Y., Wijdenes, J., Alcocer-Varela, J., Maillot, M.C., Durand-Gasselin, I., Fourrier, B.M., Galanaud, P., and Emilie, D. (1993). Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine Netw. 4*, 421–427.

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., 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.

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.

Ma, J., Xu, J., Peng, Q., Zhang, J., Grewal, I.S., Flavell, R.A., and Craft, J. (1996). Autoimmune *lpr/lpr* mice deficient in CD40 ligand: spontaneous immunoglobulin class switching with dichotomy of autoantibody responses. *J. Immunol. 157*, 417–426.

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.

Marion, T.N., Tillman, D.M., and Jou, N. (1990). Interclonal and intraclonal diversity among anti-DNA antibodies from an (NZB × NZW)F1 mouse. *J. Immunol. 145*, 2322–2332.

Mason, L.J., Ravirajan, C.T., Rahman, A., Putterman, C., and Isenberg, D.A. (2004). Is alpha-actinin a target for pathogenic anti-DNA antibodies in lupus nephritis? *Arthritis Rheum. 50*, 866–870.

Mills, K.H. (2004). Regulatory T cells: friend or foe in immunity to infection? *Nat. Rev. Immunol. 4*, 841–855.

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.

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*, 1685–1695.

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.

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.

Mostoslavsky, G., Fischel, R., Yachimovich, N., Yarkoi, Y., Rosenmann, E., Monestier, M., Baniyash, M., and Eilat, D. (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.

Odendahl, M., Jacobi, A., Hansen, A., Feist, E., Hiepe, F., Burmester, G.R., Lipsky, P.E., Radbruch, A., and Dorner, T. (2000). Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J. Immunol. 165*, 5970–5979.

O'Keefe, T.L., Bandyopadhyay, S., Datta, S. K., and Imanishi-Kari, T. (1990). V region sequences of an idiotypically connected family of pathogenic anti-DNA autoantibodies. *J. Immunol. 144*, 4275–4283.

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.

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.

Park, Y.B., Lee, S.K., Kim, D.S., Lee, J., Lee, C.H., and Song, C.H. (1998). Elevated interleukin–10 levels correlated with disease activity in systemic lupus erythematosus. *Clin. Exp. Rheumatol. 16*, 283–288.

Pasare, C. and Medzhitov, R. (2003). Toll-like receptors: balancing host resistance with immune tolerance. *Curr. Opin. Immunol. 15*, 677–682.

Peng, S.L., Fatenejad, S., and Craft, J. (1996a). Induction of non-pathologic, humoral autoimmunity in lupus-prone mice by a class II-restricted, transgenic ab T cell: separation of autoantigen-specific and -nonspecific help. *J. Immunol. 157*, 5225–5230.

Peng, S.L., Madaio, M.P., Hughes, D.P., Crispe, I.N., Owen, M.J., Wen, L., Hayday, A.C., and Craft, J. (1996b). Murine lupus in the absence of alpha beta T cells. *J. Immunol. 156*, 4041–4049.

Peng, S.L., McNiff, J.M., Madaio, M.P., Ma, J., Owen, M.J., Flavell, R.A., Hayday, A.C., and Craft, J. (1997). ab T cell regulation and CD40 ligand dependence in murine systemic autoimmunity. *J. Immunol. 158*, 2464–2470.

Petricoin, E. F., 3rd, Ito, S., Williams, B.L., Audet, S., Stancato, L.F., Gamero, A., Clouse, K., Grimley, P., Weiss, A., Beeler, J., Finbloom, D.S., Shores, E.W., Abraham, R., and Larner, A.C. (1997). Antiproliferative action of interferon-alpha requires components of T-cell-receptor signalling. *Nature 390*, 629–632.

Powrie, F. and Maloy, K.J. (2003). Immunology. Regulating the regulators. *Science 299*, 1030–1031.

Radvanyi, L.G., Banerjee, A., Weir, M., and Messner, H. (1999). Low levels of interferon-alpha induce CD86 (B7.2) expression and accelerates dendritic cell maturation from human peripheral blood mononuclear cells. *Scand. J. Immunol. 50*, 499–509.

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. U.S.A. 87*, 7020–7024.

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.

Raz, E., Brezis, M., Rosenmann, E., and Eilat, D. (1989). Anti-DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. *J. Immunol. 142*, 3076–3082.

Reeves, J.P., Taurog, J.D., and Steinberg, A.D. (1981). Polyclonal B-cell activation of autoantibodies (CBA/N × NZB)F1 mice by polyinosinic polycytidylic acid. *Clin. Immunol. Immunopathol. 19*, 170–180.

Reininger, L., Radaszkiewicz, T., Kosco, M., Melchers, F., and Rolink, A.G. (1992). Development of autoimmune disease in SCID mice populated with long-term "*in vitro*" proliferating (NZB × NZW)F1 pre-B cells. *J. Exp. Med. 176*, 1343–1353.

Roark, J.H., Kuntz, C.L., Nguyen, K.A., Caton, A.J., and Erikson, J. (1995a). Breakdown of B cell tolerance in a mouse model of systemic lupus erythematosus. *J. Exp. Med. 181*, 1157–1167.

Roark, J.H., Kuntz, C.L., Nguyen, K.A., Mandik, L., Cattermole, M., and Erikson, J. (1995b). B cell selection and allelic exclusion of an anti-DNA Ig transgene in MRL-lpr/lpr mice. *J. Immunol. 154*, 4444–4455.

Ronnblom, L. and Alm, G.V. (2001a). An etiopathogenic role for the type I IFN system in SLE. *Trends Immunol. 22*, 427–431.

Ronnblom, L. and Alm, G.V. (2001b). A pivotal role for the natural interferon alpha-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. *J. Exp. Med. 194*, F59–63.

Ronnelid, J., Tejde, A., Mathsson, L., Nilsson-Ekdahl, K., and Nilsson, B. (2003). Immune complexes from SLE sera induce IL10 production from normal peripheral blood mononuclear cells by an FcgammaRII dependent mechanism: implications for a possible vicious cycle maintaining B cell hyperactivity in SLE. *Ann. Rheum. Dis. 62*, 37–42.

Rozzo, S.J., Drake, C.G., Chiang, B.L., Gershwin, M.E., and Kotzin, B.L. (1994). Evidence for polyclonal T cell activation in murine models of systemic lupus erythematosus. *J. Immunol. 153*, 1340–1351.

Rui, L., Vinuesa, C.G., Blasioli, J., and Goodnow, C.C. (2003). Resistance to CpG DNA-induced autoimmunity through tolerogenic B cell antigen receptor ERK signaling. *Nat. Immunol. 4*, 594–600.

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*, 1901–1910.

Sano, H., and Morimoto, C. (1981). Isolation of DNA from DNA/anti-DNA antibody immune complexes in systemic lupus erythematosus. *J. Immunol. 126*, 538–539.

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*, 777–788.

Santoro, T.J., Portanova, J.P., and Kotzin, B.L. (1988). The contribution of L3T4+ T cells to lymphoproliferation and autoantibody production in MRL-lpr/lpr mice. *J. Exp. Med. 167*, 1713–1718.

Schmiedeke, T.M., Stockl, F.W., Weber, R., Sugisaki, Y., Batsford, S.R., and Vogt, A. (1989). Histones have high affinity for the glomerular basement membrane. Relevance for immune complex formation in lupus nephritis. *J. Exp. Med. 169*, 1879–1894.

Shevach, E.M. (2000). Regulatory T cells in autoimmmunity. *Annu. Rev. Immunol. 18*, 423–449.

Shlomchik, M.J., Craft, J.E., and Mamula, M.J. (2001). From T to B and back again: positive feedback in systemic autoimmune disease. *Nat. Rev. Immunol. 1*, 147–153.

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*, 1295–1306.

Shlomchik, M.J., Marshak-Rothstein, A., Wolfowicz, C.B., Rothstein, T.L., and Weigert, M.G. (1987). The role of clonal selection and somatic mutation in autoimmunity. *Nature 328*, 805–811.

Shlomchik, M.J., 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*, 265–273.

Singer, G.G. and Abbas, A.K. (1994). The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity 1*, 365–371.

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.

Sobel, E.S., Morel, L., Baert, R., Mohan, C., Schiffenbauer, J., and Wakeland, E.K. (2002). Genetic dissection of systemic lupus erythematosus pathogenesis: evidence for functional expression of Sle3/5 by non-T cells. *J. Immunol. 169*, 4025–4032.

Steinberg, A.D., Roths, J.B., Murphy, E.D., Steinberg, R.T., and Raveche, E.S. (1980). Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. *J. Immunol. 125*, 871–873.

Stohl, W. (1995). Impaired polyclonal T cell cytolytic activity. A possible risk factor for systemic lupus erythematosus. *Arthritis Rheum. 38*, 506–516.

Tan, E.M. (1989). Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol. 44*, 93–151.

Tax, W.J., Kramers, C., van Bruggen, M.C., and Berden, J.H. (1995). Apoptosis, nucleosomes, and nephritis in systemic lupus erythematosus [editorial]. *Kidney Int. 48*, 666–673.

Tsokos, G.C., Nambiar, M.P., Tenbrock, K., and Juang, Y.T. (2003). Rewiring the T-cell: signaling defects and novel prospects for the treatment of SLE. *Trends Immunol. 24*, 259–263.

Tsokos, G.C., Smith, P.L., Christian, C.B., Lipnick, R.N., Balow, J.E., and Djeu, J.Y. (1985). Interleukin-2 restores the depressed allogeneic cell-mediated lympholysis and natural killer cell activity in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol. 34*, 379–386.

Tyrrell-Price, J., Lydyard, P.M., and Isenberg, D.A. (2001). The effect of interleukin-10 and of interleukin-12 on the *in vitro* production of anti-double-stranded DNA antibodies from patients with systemic lupus erythematosus. *Clin. Exp. Immunol. 124*, 118–125.

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.

Vassilopoulos, D., Kovacs, B., and Tsokos, G.C. (1995). TCR/CD3 complex-mediated signal transduction pathway in T cells and T cell lines from patients with systemic lupus erythematosus. *J. Immunol. 155*, 2269–2281.

Viglianti, G.A., Lau, C.M., Hanley, T.M., Miko, B.A., Shlomchik, M.J., and Marshak-Rothstein, A. (2003). Activation of autoreactive B cells by CpG dsDNA. *Immunity 19*, 837–847.

Vlahakos, D., Foster, M.H., Ucci, A.A., Barrett, K.J., Datta, S.K., and Madaio, M.P. (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.

Vratsanos, G., Kang, I., and Craft, J. (2001a). Systemic lupus erythematosus. In K.F. Austen, M.M. Frank, J.P. Atkinson, and H. Cantor (eds.), *Samter's Immunologic Diseases.* (pp. 475–495). Philadelphia, Lippincott Williams & Wilkins.

Vratsanos, G.S., Jung, S., Park, Y.M., and Craft, J. (2001b). 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*, 329–338.

Warrington, R.J. and Rutherford, W.J. (1990). Normal mitogen-induced suppression of the interleukin-6 (IL-6) response and its deficiency in systemic lupus erythematosus. *J. Clin. Immunol. 10*, 52–60.

Wofsy, D., Ledbetter, J.A., Hendler, P.L., and Seaman, W.E. (1985). Treatment of murine lupus with monoclonal anti-T cell antibody. *J. Immunol. 134*, 852–857.

Wong, H.K., Kammer, G.M., Dennis, G., and Tsokos, G.C. (1999). Abnormal NF-kappa B activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-RelA protein expression. *J. Immunol. 163*, 1682–1689.

Xaio, H., Banks, W.A., Niehoff, M.L., and Morley, J.E. (2001). Effect of LPS on the permeability of the blood–brain barrier to insulin. *Brain Res. 896*, 36–42.

Xu, L., Zhang, L., Yi, Y., Kang, H.K., and Datta, S.K. (2004). Human lupus T cells resist inactivation and escape death by upregulating COX-2. *Nat. Med. 10*, 411–415.

Yan, J. and Mamula, M.J. (2002). Autoreactive T cells revealed in the normal repertoire: escape from negative selection and peripheral tolerance. *J. Immunol. 168*, 3188–3194.

Yi, Y., McNerney, M., and Datta, S.K. (2000). Regulatory defects in Cbl and mitogen-activated protein kinase (extracellular signal-related kinase) pathways cause persistent hyperexpression of CD40 ligand in human lupus T cells. *J. Immunol. 165*, 6627–6634.

Zhao, Z., Weinstein, E., Tuzova, M., Davidson, A., Mundel, P., Marambio, P., and Putterman, C. (2005). Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. *Arthritis Rheum. 52*, 522–530.

Zielinski, C.E., Jacob, S.N., Bouzahzah, F., Ehrlich, B.E., and Craft, J. (2005). Naive CD4+ T cells from lupus-prone Fas-intact MRL mice display TCR-mediated hyper-proliferation due to intrinsic threshold defects in activation. *J. Immunol. 174*, 5100–5109.

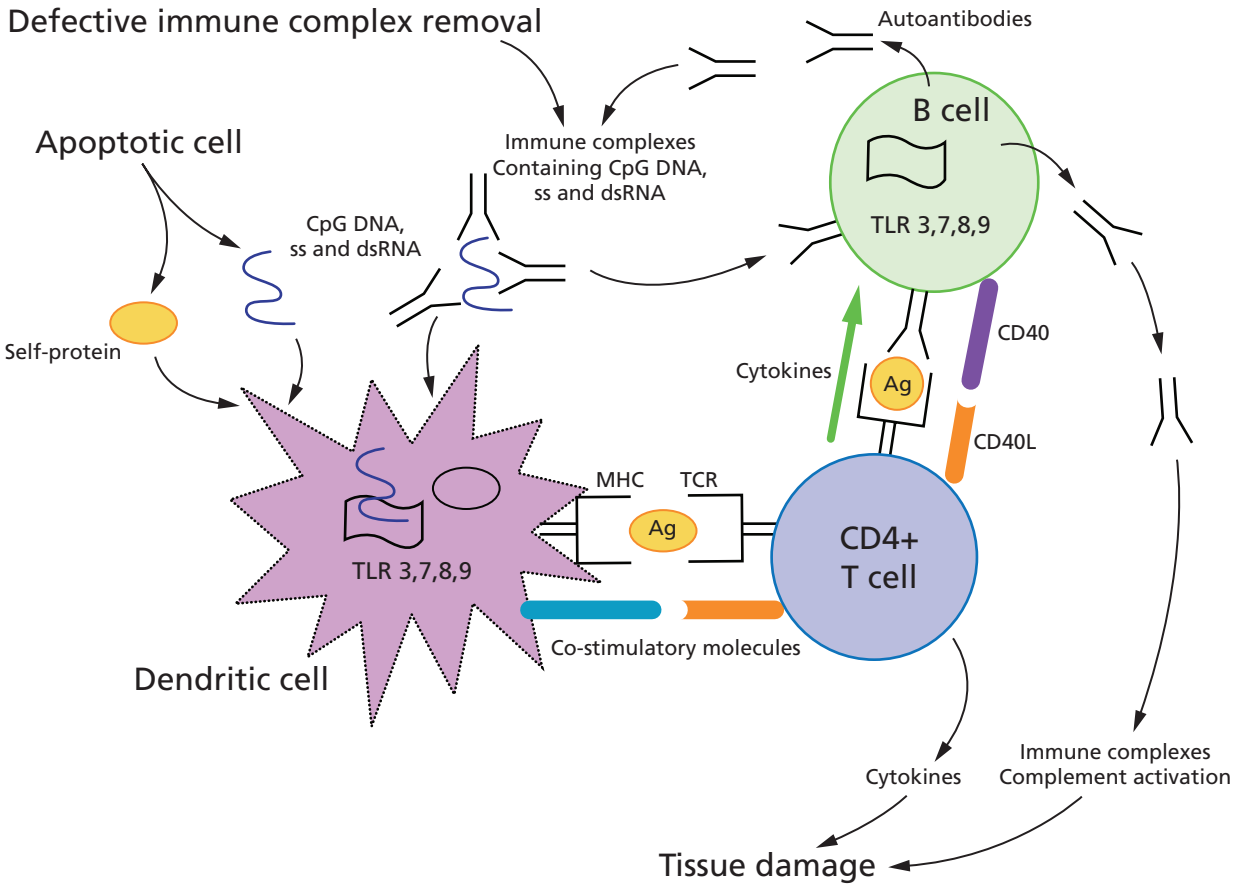


FIGURE 28.1 Immunologic pathogenesis of systemic lupus erythematosus (SLE). The figure depicts potential immunologic mechanisms involved in the development of SLE. These include defects in immune complex removal, apoptosis, and antigen presentations by antigen presenting cells (APCs) such as dendritic cells, as well as altered T- and B-cell interactions and their cytokine and autoantibody production. Nucleic acids (CpG DNA and ssRNA) from self and from microorganisms can also participate in stimulating APCs and B cells through Toll-like receptors (TLRs). Ag, antigen; ds, double stranded; MHC, major histocompatibility complex; ss, single stranded; TCR, T-cell receptor.

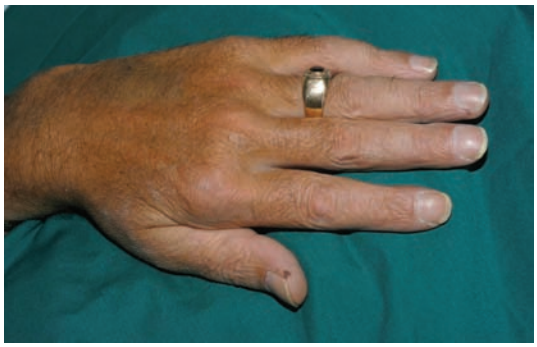


FIGURE 32.1 Rheumatoid arthritis with nailfold vasculitis.

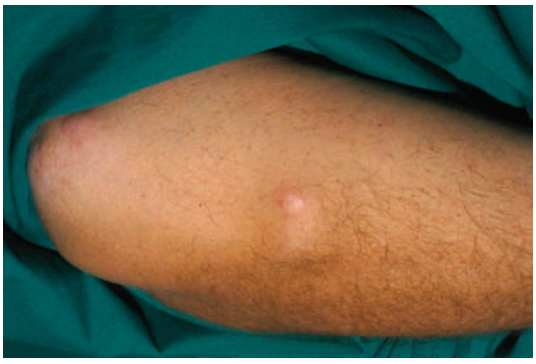


FIGURE 32.2 Rheumatoid nodule on extensor aspect of forearm.

CHAPTER

29

Systemic Sclerosis, Scleroderma

CHRISTOPHER P. DENTON AND CAROL M. BLACK

Centre for Rheumatology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, U.K.

The first convincing description of systemic sclerosis (SSc) was a report of Carlo Curzio in 1753, although the term scleroderma was not applied until a century later (Gintrac, 1847). These early cases focused upon the diffuse cutaneous subset, recognition of limited cutaneous SSc was much later, with the definition of CREST (Calcinosis, Raynaud phenomenon, Esophageal involvement, Sclerodactyly, Telangiectasis) syndrome by Winterbauer in 1964. As outlined below, the terms CREST and progressive systemic sclerosis (PSS) are generally avoided as they are potentially misleading. Patients with CREST represent a subset of cases of limited cutaneous SSc, and other complications such as lung fibrosis, gut disease, and pulmonary hypertension are not highlighted. Progressive SSc is misleading as not all cases of diffuse cutaneous SSc are progressive. Regression of skin involvement for example is common.

Systemic sclerosis, although uncommon, has the highest case-specific mortality of any of the autoimmune rheumatic diseases. This is largely as a consequence of organ-based vascular and fibrotic complications. In fact SSc can be regarded as a prototypic fibrotic disease and serves as a reminder that much of the morbidity of autoimmune and inflammatory disease occurs due to fibrosis, or scarring. Autoimmunity is nevertheless a hallmark of the condition; evidenced by the near universal occurrence of antinuclear antibodies (ANA) and the association of a number of hallmark ANA reactivities with SSc. In addition, there is evidence of immunologic reactivity to extracellular matrix components and early-stage skin and lung involvement is characterized by a T-lymphocyte-rich mononuclear inflammatory cell infiltrate.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Clinical Features

Systemic sclerosis is a multisystem disease, and the clinical features can be divided into those that are generally present in all cases, to a greater or lesser degree, and the features only present in a minority of affected individuals (Figure 29.1). The former include skin and musculoskeletal involvement leading to sclerodactyly; Raynaud phenomenon, the absence of which in established SSc should lead to the diagnosis being questioned, and esophageal dysmotility and reflux. Changes in the skin usually proceed through three phases: early, established, and late. The early stage can be difficult to diagnose, and a high level of suspicion is needed in the edematous phase when the only feature may be puffiness of the hands and feet, most marked in the morn-

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

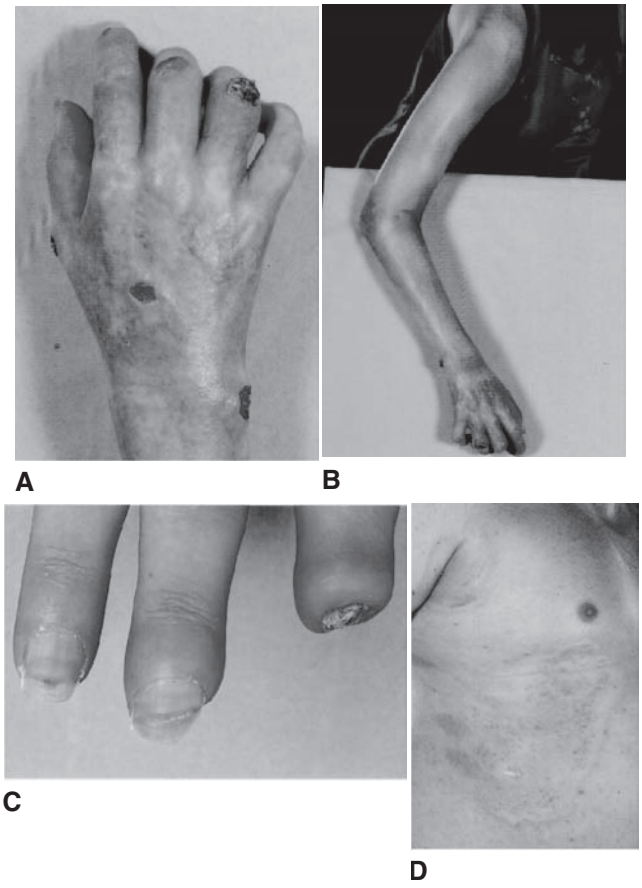


FIGURE 29.1 Clinical features of scleroderma. Skin involvement is widespread in diffuse cutaneous systemic sclerosis *(A,B)* but much more restricted in limited cutaneous systemic sclerosis *(C)*. Digital loss from severe vascular disease is common. *D,* Morphea plaque: although histologically similar to systemic sclerosis, skin involvement in localized scleroderma has a distinctive clinical appearance and distribution. See color plate section.

ings. Edema may be dependent and can lead to symptoms of neural compression including carpal tunnel syndrome. The face may feel slightly taut at this stage and Raynaud phenomenon may be present. On examination there is a nonpitting edema with intact epidermal and dermal appendages. The subsequent, often abrupt, development of firm, taut, hidebound skin proximal to the metacarpophalangeal joints, adherent to deeper structures such as tendons and joints, causing limitation of their movement and subsequent contractures, permits a definitive diagnosis in over 90% of patients. The skin may be coarse, pigmented, and dry at this stage. The epidermis thins, hair growth ceases, sweating is impaired, and skin creases disappear.

A consensus classification for scleroderma spectrum disorders has been developed that separates localized from systemic disease (Table 29.1). Localized scleroderma encompasses the various forms of morphea and linear scleroderma occurring in adults or children. In its classical forms, it is not associated with internal-organ disease. The traditional boundaries between different clinical subgroups of the scleroderma spectrum are being blurred although the basic principles of classification remain (Anonymous, 1980). It is now appreciated that there are some cases of SSc that develop one or more areas of localized scleroderma, generally plaque morphea, and conversely it is reported that some patients with typical localized scleroderma develop serologic features of systemic disease or other autoimmune rheumatic disorders such as systemic lupus erythematosus (SLE). Also, a subset of patients with clinical features of isolated Raynaud phenomenon have evidence of microvasculopathy based upon nailfold capillaroscopy, or have serum autoantibodies against nuclear antigens. Such cases may progress to develop a defined multisystem autoimmune disease, with best estimates suggesting between 10 and 15% of cases evolving in this way within 5 years of presentation. Also, presence of scleroderma-related capillaroscopic findings or ANAs against hallmark scleroderma antigens may identify a subset of patients who will develop features of limited cutaneous SSc. Based upon the absence of skin changes at presentation, the term “limited systemic sclerosis” has been proposed for this group (LeRoy et al., 2001). Further, there is a small number of patients who have

TABLE 29.1 Classification of Scleroderma and Systemic Sclerosis

Classification	Description
Localized scleroderma	
Plaque morphea	Fewer than four patches of localized scleroderma not following a linear distribution. Face generally spared.
Generalized morphea	More than four patches of localized scleroderma. May be very extensive but spares the hands, and Raynaud usually absent.
Linear scleroderma	Localized scleroderma in a linear distribution. Often affects the face and scalp. Usually asymmetrical even when bilateral. Most common form of childhood-onset scleroderma. Can lead to major growth deficit of underlying connective tissues. Variants include *en coup de sabre* and hemifacial atrophy.
Systemic sclerosis	
Diffuse cutaneous systemic sclerosis	Skin involvement proximal to knees, elbows or neck. Approximately 30% of SSc cases. Inflammatory features prominent in first 3 years of disease. Renal and interstitial lung involvement more common. Raynaud phenomenon may not be the first manifestation.
Limited cutaneous systemic sclerosis	Distal skin involvement. Prominent vascular rather than inflammatory features. Raynaud phenomenon invariably the first manifestation. Around 60% of SSc cases. Severe bowel involvement and isolated pulmonary hypertension are common. Lung fibrosis and renal involvement also occur.
Overlap syndromes	Cases of SSc but also manifestations of another rheumatic disease such as SLE, myositis, or polyarthritis. Approximately 15–20% cases of SSc have overlap features.
Systemic sclerosis sine scleroderma	Vascular, serologic, and organ-based manifestations of SSc but without definite skin sclerosis. A rare subset, less than 5% of cases.

SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

vascular and serologic features of diffuse cutaneous SSc (dcSSc) but lack significant skin sclerosis, designated as systemic sclerosis sine scleroderma (Poomoghim et al., 2000).

Pathologic Features

In the early phase, there are collections of mononuclear cells in the dermis, particularly around blood vessels. The soluble products of these monocytes and lymphocytes may have pathogenetic significance in the disease process. Later, fibrosis replaces the cellular infiltrate and may extend deep into the connective tissue to surround tendons, nerves, muscle bundles, and joint capsules. In the final stage, the fibrosis may be less evident, with epidermal thinning and loss of appendages a prominent feature.

The pathology of SSc involves vascular change with endothelial cell activation and microvascular damage. Later, there is an inflammatory phase with mononuclear cell infiltration. Overexpression of a number of key growth factors and cytokines is common to both pathologies including transforming growth factor-β (TGF-β) isoforms and connective tissue growth factor. Later, the sclerotic phase of localized scleroderma resembles the established fibrotic lesion of SSc, and activated fibroblasts and myofibroblasts predominate. There are serologic features in common between the two conditions, including the presence of ANA reactivity or the hallmark scleroderma autoantibodies (Marzano et al., 2003) (and see later). In addition, minor reactivities against extracellular matrix components including fibrillin 1 have been demonstrated in localized as well as systemic forms of scleroderma (Tan et al., 1999). These shared features suggest that there are similar pathogenic mechanisms for these diseases and that genetic or environmental predisposition in common may underlie them.

Epidemiologic Features

Scleroderma is an uncommon disorder and most of the descriptive epidemiology is derived from retrospective or prospective reviews of patients attending hospitals or institutions serving a defined denominator population: there is only one true population-based study (Maricq et al., 1989). Although there are apparently different disease frequencies between populations, some of these may be accounted for by methodologic factors. Interestingly, although the prevalence in the UK was previously estimated in a large West Midlands population as several-fold lower than in the study of Maricq (1989), a smaller but thorough examination has recently been reported (Allcock et al., 2004) that finds higher frequency, in line with that in North America (Mayes et al., 2003) and Australia (Roberts-Thomson et al., 2001). Currently, the best estimate for UK prevalence overall is approximately 12 in 100,000 of the population (Allcock et al., 2004), which is lower than reported for North America, but not the order of magnitude lower that was suggested in earlier work. The demographic conclusions that may be drawn from these data are that the disease is rare in childhood, and that its incidence increases steadily with age among adults. Its rarity suggests that the genetic and/or

environmental exposures necessary for disease susceptibility occur infrequently in the population. Systemic sclerosis is a disorder with a female to male excess (4:1 male to female overall).

A number of geographical clusters of SSc have been reported, including clustering close to international airports in the UK (Silman, 1995). A cluster of patients with a variety of autoimmune rheumatic diseases is reported from The Republic of Georgia (Freni-Titulaer, 1989), and a clearer cluster of SSc in a region of Italy close to Rome (Valesini, 1993). In North America a high prevalence has been observed among Choctaw Native Americans in Oklahoma. There is increasing evidence for a genetic basis for this high prevalence and on-going studies are defining this in detail (Tan and Arnett, 2000). Finally, in common with some other autoimmune rheumatic diseases, various environmental agents have been implicated in SSc, including increased silica exposure.

AUTOIMMUNE FEATURES

Some of the clinical and immunologic features of SSc resemble those of other autoimmune disorders such as SLE, dermatomyositis, and rheumatoid arthritis, and there are also patients who have overlap syndromes or who have sequential development of more than one autoimmune rheumatic disease. Cases of familial SSc and familial associations of SSc with other autoimmune diseases such as rheumatoid arthritis and SLE occur and have already been mentioned. Abnormalities in both humoral and cell-mediated immunity occur in SSc, although the importance of these immunologic events in the pathogenesis remains uncertain. The lack of a generalized immune dysfunction in SSc suggests that the derangement of immune-cell dysfunction may be specific to certain antigens or cell types (Lupoli et al., 1990).

T-Cell Abnormalities

The association of SSc with particular HLA alleles and the close association of certain HLA alleles with SSc-specific antibodies is indirect evidence for T-cell involvement in SSc. Evidence for T-cell activation in SSc includes an increased ratio of circulating $CD4^+$:$CD8^+$ cells, reflecting an increased number of $CD4^+$ and/or a reduced number of $CD8^+$ lymphocytes. A particular role for $\gamma\delta$ T-cells has been suggested (White, 1994; Tiev et al., 2005), including a potential predisposition to microchimerism (Giacomelli et al., 2004), and others have reported increased numbers of lymphokine-activated killer and natural killer cells in blood samples from patients with SSc. Furthermore, several studies have found increased soluble interleukin 2 (IL-2) receptor in serum in SSc, sometimes appearing to correlate with disease activity. Support for the possibility that activated T-cells are important in pathogenesis is provided by the presence of infiltrates in lesions of $\alpha\beta$ T-cells that are $CD3^+$, $CD4^+$, $CD450^+$, interleukin 2-producing, HLA-DR^+, and leukocyte function-associated antigen-1-positive (Prescott et al., 1992). Also, in humans, chronic graft-versus-host disease (GvHD) shows several histologic and clinical similarities with SSc, and is known to be a T-cell-mediated process. Although circulating immune complexes have been reported in SSc, most studies have not found functional complement abnormalities, probably because most SSc-associated autoantibodies do not activate the complement cascade (White, 1994).

B-Cell Abnormalities

Humoral abnormalities in SSc are most clearly reflected by the presence of autoantibodies with well-defined target specificities. Autoantibody production is an early and almost universal feature of SSc, and the number of autoantibody targets identified in SSc continues to increase. The major reactants are topoisomerase 1, centromeric proteins, and RNA polymerases I, II, and III. Virtually all patients have antinuclear antibodies when HEp-2 lines are used in indirect immunofluorescence (IIF) tests. Characteristic staining patterns for ANAs within the nuclear structures are relatively specific and can be confirmed by more refined tests such as counterimmunoelectrophoresis (CIE) and Western immunoblotting. A diffusely grainy pattern of ANA staining is associated with the presence of antibodies to topoisomerase 1 (Topo 1, Scl-70), a nuclear enzyme required for the unwinding of DNA for replication and RNA transcription. Topo 1 to Scl-70 occur in up to 40% of patients with diffuse SSc and 15% of those with limited disease (Bunn and Black, 1999). The frequency of occurrence of this antibody in SSc varies between laboratories; the immunoblot technique is more sensitive than CIE and by this technique the positivity rate is 30–40% in dcSSc.

Anticentromere staining by IIF occurs in some 80% of patients with the limited form of SSc, although it is also sometimes present in other diseases including primary biliary cirrhosis and in otherwise healthy subjects. The antigens recognized by positive sera are CENP-A, CENP-B, and CENP-C, with molecular weights of 19, 80, and 140 kDa, respectively (Earnshaw et al., 1986). Since a correlation has been shown (Jabs et al., 1993) between anticentromere antibodies and aneuploidy in patients with SSc, anticentromere antibodies could disrupt centromere function and allow chromosomes to segregate inappropriately during mitosis, leading to a high rate of chromosomal breakage and sister chromatid exchange. However, this would assume that antibody can penetrate cells and, to date, no correlation has been found between the presence of anticentromere antibody and chromosomal changes.

Antibodies against RNA polymerase (RNAP) occur mainly in patients with diffuse disease, and antibodies

TABLE 29.2 Autoantibodies Associated with Systemic Sclerosis

Antigen	Frequency	Immunofluorescence pattern	Clinical association*
Centromere	60% lcSSc up to 25% in primary biliary cirrhosis, often with features of lcSSc	Centromere	Almost restricted to lcSSc subset. These patients are at risk of isolated pulmonary hypertension and severe gut disease but relatively protected from lung fibrosis
Topoisomerase 1 (Scl 70)	35% dcSSc 10–15% lcSSc	Nuclear (diffuse fine speckles)	Associated with diffuse skin involvement and especially with lung fibrosis in both subsets of SSc
RNA polymerases I and III	20% dcSSc	Nucleolar (punctate)	Associated with diffuse skin involvement and especially with renal involvement in both subsets
Fibrillarin	5%	Nucleolar (clumpy) staining of coilin bodies	Occurs in both major subsets. Associated with poor outcome in dcSSc with cardiac disease, pulmonary hypertension, renal involvement, and myositis
Polymyositis-scleroderma PM-Scl	3%	Nucleolar (homogeneous)	High frequency of myositis. In childhood may associate with milder disease but renal involvement increased in adult SSc
U1 RNP	10% SSc 5% dcSSc 14% lcSSc	Speckled antinuclear	Higher frequency in black patients with SSc. Associated with joint involvement and lung fibrosis in SSc. Also frequent in overlap syndromes

*For further details see Bunn et al., 1998; Bunn and Black 1999; Tormey et al., 2001.
dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; RNP, ribonuclear protein; SSc, systemic sclerosis.

against RNAPI, II, and III have been described (Bunn et al., 1998). The RNAPs are multiprotein complexes and are components of the transcription complex (Reeves et al., 1994). Each RNAP is composed of collections of smaller proteins shared by other RNAPs and two large distinct proteins: RNAPI synthesizes ribosomal RNA precursors in nucleoli, whereas RNAPs II and III are found in nuclei. Most of the small nuclear RNAs found in ribonucleoprotein particles that mediate pre-mRNA splicing and synthesize precursors of mRNA are synthesized by RNAPII, and RNAPIII synthesizes small RNAs including single-strand ribosomal RNA and transfer RNA. Anti-RNAP antibodies target both the smaller shared subunits and the larger distinct proteins, which explains antibody reactivity against several RNAPs in one serum sample. Radioprecipitation assays are currently the only way of identifying anti-RNAPs, and require the facilities of specialized laboratories. Autoantibodies associated with SSc are detailed in Table 29.2.

Anti-fibrillarin (U3-RNP) antibodies produce a typical clumpy nucleolar staining pattern by IIF with coilin bodies appearing as bright nuclear dots. Several studies have associated this reactivity with a poor outcome, particularly in dcSSc (Tormey et al., 2001). Initial reports suggested a high frequency of isolated pulmonary hypertension, myositis, and cardiac disease in African-American SSc patients (Arnett et al., 1996).

The antinuclear antibody response is not as clear-cut in the juvenile as in the adult form of SSc, although some trends are emerging. Serum ANAs have been reported in 25–55% of juveniles (onset under 16 years of age) with localized scleroderma, the association being most marked in the linear group and in patients with extensive cutaneous lesions. Antibodies to single-stranded DNA also appear to be correlated with the extent of localized disease, whereas antibodies to double-stranded DNA are rarely found. Notably, in the generalized form of childhood scleroderma, anticentromere antibodies have not been reported, even in those children with disease identical to that found in the adult.

A direct pathogenetic role for various autoantibodies in SSc has long been sought. There are defined epitopes for several of the autoantibodies (Bunn and Black, 1999) and recent work has shown homology between target autoantigens in SSc and retroviral proteins (Prokop et al., 2004), suggesting molecular mimicry, which may have significance in disease pathogenesis. There are reports that some of the antibodies present in SSc are also able to enter intracellular compartments (Ma et al., 1991) and, thereby, to mediate intracellular events, such as the reported ability of anticentromeric antibodies to disrupt the centromere, although this remains very controversial. In addition, autoantibodies might be able to activate cells that bear the target autoantigens; for example, patients with SSc produce antibodies that bind FcγRI (CD64), FcγRII (CD32: activating), and FcγRIII (CD16: inhibitory) (Boros et al., 1993). It has been suggested that some of the autoantibodies in serum from SSc may mediate antibody-dependent cytotoxicity, and potential effector cells have been found in the skin of some patients (White et al., 1994). Another speculation is that these antibodies might contribute to the pathology if they mediated complement-dependent cellular lysis or

phagocytosis. However, these ideas must be kept in perspective balanced against the lack of correlation of antibody titer with disease duration or activity.

Other Autoantibodies

Anti-endothelial cell antibodies (AECA) have been recognized in SSc sera for many years, but their significance remains uncertain. Cross-reactivity with viral antigens has been reported, and this may provide a clue concerning the origin of these antibodies and possibly implicate viral infection in endothelial cells as a pathogenic mechanism in SSc (Lunardi et al., 2000). More recently, functional effects of AECA in SSc have been explored in detail and the ability of AECA to induce endothelial cell apoptosis has been demonstrated (Worda et al., 2003). It has been demonstrated that AECA may also react with other cell types, including fibroblasts (Renaudineau et al., 2001) and that some AECA recognize hallmark SSc-associated antigens (Garcia de la Pena-Lefebvre et al., 2004).

Other autoantibodies have also been detected in SSc as well as localized scleroderma, such as those reacting against the microfibrillar protein fibrillin 1 and antibodies against a number of different cytokines (White, 1994). These reactivities appear more likely to be related to abnormal protein expression or processing for these antigens and may provide insight into the biochemical processes that are perturbed in scleroderma spectrum disorders, although it seems unlikely that they are directly involved in disease pathogenesis.

Circulating antibodies to the extracellular matrix proteins, collagens I, III, IV and VI, and laminin and fibrillin have also been found in SSc, but their role is undetermined (Mackel et al., 1982; Tan et al., 1999).

GENETIC FEATURES

Certain of the SSc autoantibodies are closely related to particular HLA alleles. For example, the class II major histocompatibility complex (MHC) haplotype is an important factor determining *in vitro* responsiveness to topoisomerase antigen, both in patients with SSc and in healthy control individuals (Kuwana et al., 1995). Moreover, there may be racial differences in HLA associations for the various autoantibodies. These antibodies not only mark out certain subsets of patients with SSc but are of increasing importance in defining that subgroup of patients with isolated Raynaud phenomenon likely to develop SSc.

It is likely that new approaches to SSc genetics will identify additional susceptibility and severity genes. For example, gene-profiling experiments that have been fruitful in identifying over- or underexpressed genes that are associated with lesional SSc skin or fibroblasts. Interestingly, many of these genes are regulated by TGF-β, providing additional evidence that this is a key cytokine in pathogenesis of SSc (Kissin and Korn, 2002). Association studies have also been performed including a recent genome-wide screen of Choctaw Native American patients (Zhou et al., 2003). There has been recent interest in comparing gene expression in non-involved skin sites with healthy controls to determine potential susceptibility loci (Whitfield et al., 2003). Recent reports have highlighted several genes that may underlie fibroblast dysfunction in SSc through genetic linkage or association studies, and by differential display methodologies including cDNA based transcriptional profiling and polymerase chain reaction (PCR) based subtractive hybridization methods. Candidate genes include protease nexin 1 (Strehlow et al., 1999), connective tissue growth factor (Shi-wen et al., 2000), TGF-β2, -β3, and TIMP1 (Susol et al., 2000). A major challenge arising from such studies is confirmation that overexpression of these genes leads to fibrosis.

ENVIRONMENTAL INFLUENCES

A range of agents can induce a SSc-like disease and certain chemically induced SSc-like disorders tend to be associated with males, partly due to an occupational bias. It is almost certain that sporadic cases can follow certain occupational exposures, but that both the absolute and attributable risks are low (see Chapter 23). There are some MHC associations with the environmentally induced cases, for example, toxic oil syndrome is characterized by a raised incidence of HLA-DR4, while vinyl chloride disease is primarily associated with HLA-DR5 and HLA-DR3 being a marker of severity. Another environmentally induced, SSc-like condition is the eosinophilic-myalgic syndrome associated with the oral ingestion of certain preparations of the essential amino acid L-tryptophan. Although there has been much speculation about a possible association of silicone-containing cosmetic prostheses and the development of multisystem autoimmune disease, several well-conducted studies and reviews have failed to show any statistically significant association between these and SSc. Much of the work in this area has been generated by the need to obtain accurate data because of considerable litigation, particularly in the United States. Hochberg (1994) has carefully reviewed the epidemiologic aspects of the literature and has reasonably concluded that none of the available studies has demonstrated a statistical association between augmentation mammoplasty with silicone gel-filled prostheses and SSc.

ANIMAL MODELS

There are several established animal models for scleroderma, each demonstrating some features of the disease

(Christner and Jimenez, 2004). The early vascular events of scleroderma, including endothelial cell apoptosis, are demonstrated in the UCD2000 tight-skin chicken model. Fibrosis in this condition remains localized although scleroderma-associated hallmark autoantibodies have been reported at later stages. The two tight-skin mouse models for scleroderma, types 1 and 2 designated Tsk1 and Tsk2, respectively, are both genetically determined. Tsk1 is a spontaneous mutant strain demonstrating fibrosis in the skin and some internal organs (though not the lungs) from around 10 days old. It has been mapped to a partial reduplication at the fibrillin 1 gene locus on chromosome 2. This leads to secretion of a larger-than-normal fibrillin 1 protein although the mechanism by which this leads to generalized connective tissue fibrosis is unclear. Recent association of human SSc with microsatellite marker haplotypes near the fibrillin 1 locus, and detection of anti-fibrillin autoantibodies in scleroderma-spectrum disorders have revived interest in the Tsk1 model. In contrast, the precise genetic basis for Tsk2 has not yet been determined, although it has been mapped to a 25 kb region of chromosome 1. There are reports that an inflammatory cell infiltrate may precede the development of skin fibrosis and the induced nature of this strain by an environmental mutagen (ethylnitrosourea) similar to some agents associated with development of human disease, and in these respects it is possible that Tsk2 is a more complete, though less well-characterized animal model.

Chronic murine GvHD occurs after bone marrow engraftment in susceptible strains across certain H-2 differences. This model is clearly immunologically determined and has prominent mononuclear cell infiltrates in the skin and viscera. It has been shown to be treatable using agents that neutralize TGF-β. Other models that have recently been described include induction of skin sclerosis by bleomycin in certain mouse strains that are resistant to lung disease. This process has also been demonstrated to be TGF-β dependent, as it is absent in Smad3-deficient animals. A hybrid mouse strain in which the IFN-γ receptor knockout line is back-crossed with a pro-inflammatory line has also been described. Other genetically modified mice are now being characterized, which may further illuminate pathogenic processes *in vivo* and allow the role of key pathways and gene products in the development of scleroderma to be assessed.

Lineage-specific gene expression can be used to develop novel genetic models for SSc (Denton et al., 2003). For example, transgenic mice expressing a mutant kinase-deficient type II TGF-β receptor provide an exciting and novel genetically determined model for SSc. Paradoxically, although this construct sometimes operates as a dominant negative inhibitor of TGF-β signaling, *in vivo* under the control of a fibroblast-specific expression cassette this construct appears to result in facilitation of basal TGF-β signaling activity. Transgenic mice develop dermal and sporadic pulmonary fibrosis, recapitulating two of the key pathologic features of SSc (Denton and Abraham, 2004).

PATHOGENIC MECHANISMS

There have been intensive efforts over the past two decades to define the key pathogenetic components that drive the scleroderma process. These efforts have been hampered by disease heterogeneity, which implies that some events occur to a different extent in the two major subsets, and also by the necessity to explain different patterns of organ-based disease. As with many complex diseases, it is accepted that there are important host (genetic) and environmental factors involved in pathogenesis, and for many patients it seems likely that a triggering event occurring in the context of a susceptible host is a key process.

Macrophages, mast cells, eosinophils, and basophils are found in increased numbers and in an activated state in tissues of patients with SSc. These cells are capable of producing soluble mediators and can thereby modify endothelial and fibroblast function; for example, mast cells produce histamine, which stimulates both proliferation and matrix synthesis by fibroblasts and causes retraction of endothelial cells.

The initiating stimulus in idiopathic scleroderma is unknown, although the identification of chemical precipitants for environmentally induced SSc as discussed above (e.g., vinyl chloride and epoxy resin) may provide some clues to the processes involved, particularly in view of the similar immunogenetic associations for both idiopathic and chemically induced disease (Black et al., 1983). The most obvious major targets for the immune response in SSc are endothelial cells and fibroblasts. Stimulation of collagen synthesis could involve an increasing number of cytokines known to modulate the properties of fibroblasts. It is possible that cascades of such cytokines or autocrine/paracrine loops stimulate or maintain the disease process. It is now appreciated that the repertoire of mediators and cytokines produced by immune cells, fibroblasts, and endothelial cells is large. It is possible that the aberrant properties of connective tissue cells (e.g., excess synthesis of collagen, fibronectin, and glycosaminoglycans) and the endothelial-cell damage and vasculopathy, are consequences of the immunological events in SSc.

The ultimate effector cell of scleroderma is a matrix synthesizing fibroblastic cell. These cells often express markers and phenotypic properties of myofibroblasts (Kissin and Korn, 2002). There may well be phenotypic fluidity between fibroblasts and myofibroblasts and also between components of the vasculature such as microvascular pericytes. It has also been suggested that circulating progenitor cells may be deposited at sites of fibrosis; in line with this, there are recent reports of circulating endothelial cells in SSc (Del Papa

et al., 2004). Embryonically defined regulatory elements within extracellular matrix genes have recently been identified as potential targets for activation in a mouse model of scleroderma (see earlier), and these elements are also present in human matrix genes. A persistent population of cells in which embryonic regulatory pathways of gene regulation are active and susceptible to upregulation is intriguing.

It suggests that there may be a resident population of progenitor fibroblasts that can be activated in fibrosis (Denton et al., 2001).

There has been recent interest in the potential role of microchimerism in the pathogenesis of SSc (Evans et al., 1999). The observation that fetal cells and even naked fetal DNA may persist in the maternal circulation after pregnancy has fueled the hypothesis that some of these fetal cells may become reactivated, and that scleroderma could represent a graft-versus-host disease. Indeed it has even been suggested that maternal cells passed to the fetus may persist, allowing an alloreactive process to be implicated in male patients with scleroderma. Although the concept is attractive, there is only weak evidence to support it and recent data have suggested that differences between scleroderma and control levels of foreign DNA are at most quantitative (Jimenez and Artlett, 2005).

There is considerable evidence that oxidant stress may play a role in pathogenesis of SSc. It is potentially involved in the fragmentation of autoantigens to expose cryptic epitopes and facilitate the development of autoantibodies (see Chapter 15). This has been shown for RNA polymerases and topoisomerase 1 and may be catalyzed by heavy metal ions. There are some data to support an additional association between heavy metal exposure and the development of autoantibodies. The combination of an appropriate HLA haplotype and exposure to appropriate immunogenic epitopes offers a unifying hypothesis to link different hallmark events in scleroderma. Since tissue hypoxia may occur secondary to Raynaud phenomenon and the vasculopathy of scleroderma, perhaps in concert with the relative tissue hypoxia of the established lesional tissue, it is possible that oxidant stress may promote disease development. Moreover, there is additional evidence that oxidative modification of proteins may also facilitate the development of scleroderma. Antioxidant strategies for therapy offer an exciting possibility for treatment and are being pursued.

IMMUNOLOGIC MARKERS IN DISEASE

Serologic associations are now much better understood. It has been clearly shown that the ability of an individual to respond to immunogenic determinants of hallmark scleroderma autoantigens is associated with certain class II HLA alleles. This is consistent with current understanding of antigen presentation and accessory/costimulatory signals. It is less certain whether such associations are held across different ethnic or geographic boundaries, although an ongoing study in three different North American groups can be expected to specifically address this (Reveille et al., 2001). Intriguingly, the hallmark autoantibodies associated with SSc have proven to be associated with different patterns of disease (Bunn et al., 1998; Tormey et al., 2001). This suggests that the antibodies themselves, or more likely the host factors that determine the development of these antibodies, influence the interplay between other key events in disease progression. Within single center cohorts, associations are now well established and are listed in Table 29.2. It appears that anti-RNA polymerase I or III reactivity is an independent risk factor for scleroderma renal crisis although anti-topoisomerase 1 is not. There is increased frequency of anti-topoisomerase 1 with interstitial pulmonary fibrosis. Pulmonary hypertension occurs commonly in SSc, and is associated with anticentromere antibody reactivity and also with anti-U3-RNP reactivity. In the latter case, it occurs typically in the absence of major lung fibrosis. Ultimately, serologically defined subsets may be helpful in risk stratification, used in conjunction with other clinical features so that protocols for investigation, monitoring, and treating SSc patients can be individualized. Eventually, genetic markers and other soluble serum factors are likely to be used in a similar way.

TREATMENT AND OUTCOME

The principles of effective management of these diseases include establishment of a robust diagnosis, determining the clinical subset and stage of SSc, and confirming the presence or absence of overlap features that might require specific therapy such as arthritis or myositis. In dcSSc it is generally considered appropriate to attempt to treat active skin disease, whereas vascular therapy is the major focus for limited cutaneous SSc (lcSSc). In all SSc it is critical that internal organ involvement is identified early and managed appropriately.

Many treatments for skin involvement have been assessed and, although a number of studies have been encouraging, none of the major controlled trials has been positive. This is important since the extent and severity of skin involvement has been shown in these same trials to associate with outcome and the occurrence of major organ-based complications. It has, therefore, been suggested that if a treatment was developed that modified skin involvement it could be expected, based upon a common pathogenic process, to influence other disease outcome and might be defined a true disease-modifying therapy. At present, many strategies are under evaluation or suggested ranging from oral tolerization to type I collagen to targeted

biological therapies aimed at putative key cytokines driving the disease. Intensive immunosuppression with autologous peripheral stem cell rescue has also been used in a number of centers and is the focus of two major international controlled trials (Binks et al., 2001; Farge et al., 2002).

There are many potential treatments for SSc, and it should no longer be regarded as untreatable even if a cure is certainly not possible. Our approach is to treat the vascular and inflammatory components of dcSSc and enroll patients vigorously into controlled trials or the ongoing observational study of protocolized therapy. In this way, treatments shown to be ineffective can be avoided and much more information obtained about clinical progression and potential response to current best practice treatment. Early dcSSc is treated with immunosuppression generally using cyclophosphamide, mycophenolate mofetil or antithymocyte globulin. Other possible agents include azathioprine or methotrexate. The rationale and experience of these agents is reviewed elsewhere (Denton and Black, 2004). All dcSSc cases should be closely followed and screened for major complications. Vascular treatments including vasodilators for Raynaud phenomenon and drugs such as bosartan with potential remodeling effects are used. There are clinical trials underway to examine whether endothelin receptor blockade with bosentan, an ET-A and ET-B receptor blocking drug, may have beneficial effects on SSc vasculopathy. Initial data suggest a possible beneficial effect for bosentan treatment in preventing new ischemic digital ulceration (Korn et al., 2004). Since there is some evidence implicating endothelin 1 as a mediator of fibrosis in SSc, there is also interest in the potential beneficial effect of bosentan in lung fibrosis in SSc and clinical trials are currently underway. Intermittent prostacyclin may be beneficial and undoubtedly benefits digital ulceration and Raynaud phenomenon. There are studies ongoing to evaluate biological therapies for dcSSc. These include evaluation of anti-TNF-α strategies as well as agents targeting other potential mediators. One of the most exciting approaches has been the development of fully human recombinant antibodies specific for TGF-β isoforms. So far only one study has been reported in dcSSc. Antibodies specific for TGF-β1 were shown to be safe and well tolerated in a study cohort of early stage dcSSc cases (Denton et al., 2004). No data regarding efficacy of such novel treatments are yet available.

CONCLUDING REMARKS—FUTURE PROSPECTS

Systemic sclerosis has an increasingly high profile as a result of advances in the understanding of organ-based disease and an appreciation of the importance of regular follow-up and screening for treatable complications. However, progress is hampered by its relative rarity and the clinical heterogeneity. Strong clinical and research collaborative links are being established internationally and a number of data collection initiatives across centers are underway. These should provide the tools for future clinical trials and epidemiological studies that will shed light upon this challenging autoimmune rheumatic disease.

References

Allcock, R.J., Forrest, I., Corris, P.A., Crook, P.R., and Griffiths, I.D. (2004). A study of the prevalence of systemic sclerosis in northeast England. *Rheumatology 43,* 596–602.

Anonymous. (1980). Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum. 23,* 581–590.

Arnett, F.C., Reveille, J.D., Goldstein, R., Pollard, K.M., Leaird, K., Smith, E.A., Leroy, E.C., and Fritzler, M.J. (1996). Autoantibodies to fibrillarin in systemic sclerosis (scleroderma). An immunogenetic, serologic, and clinical analysis. *Arthritis Rheum. 39,* 1151–1160.

Arnett, F.C., Fritzler, M.J., Ahn, C., and Holian, A. (2000). Urinary mercury levels in patients with autoantibodies to U3-RNP (fibrillarin). *J. Rheumatol. 27,* 405–410.

Binks, M., Passweg, J.R., Furst, D., McSweeney, P., Sullivan, K., Besenthal, C., Finke, J., Peter, H.H., van Laar, J., Breedveld, F.C., Fibbe, W.E., Farge, D., Gluckman, E., Locatelli, F., Martini, A., van den Hoogen, F., van de Putte, L., Schattenberg, A.V., Arnold, R, Bacon, P.A., Emery, P., Espigado, I., Hertenstein, B., Hiepe, F., Kashyap, A., Kotter, I., Marmont, A., Martinez, A., Pascual, M.J., Gratwohl, A., Prentice, H.G., Black, C., and Tyndall, A. (2001). Phase I/II trial of autologous stem cell transplantation in systemic sclerosis: procedure related mortality and impact on skin disease. *Ann. Rheum. Dis. 60,* 577–584.

Black, C.M., Welsh, K.I., Walker, A.E., Bernstein, R.M., Catoggio, L.J., McGregor, A.R., and Jones, J.K. (1983). Genetic susceptibility to scleroderma-like syndrome induced by vinyl chloride. *Lancet 1,* 53–55.

Boros, P., Muryoi, T., Spiera, H., Bona, C., and Unkeless, J.C. (1993). Autoantibodies directed against different classes of FcR are found in sera of autoimmune patients. *J. Immunol. 50,* 2018–2024.

Bunn C.C. and Black C.M. (1999). Systemic sclerosis: an autoantibody mosaic. *Clin. Exp. Immunol. 117,* 207–208.

Bunn, C.C., Denton, C.P., Shi-Wen, X., Knight, C., and Black, C.M. (1998). Anti-RNA polymerases and other autoantibody specificities in systemic sclerosis. *Br. J. Rheumatol. 37,* 15–20.

Christner, P.J. and Jimenez, S.A. (2004). Animal models of systemic sclerosis: insights into systemic sclerosis pathogenesis and potential therapeutic approaches. *Curr. Opin. Rheumatol. 16,* 746–752.

Curzio C. (1753). *Discussioni Anatomico-pratiche Di un raro, e stravagante morbo cutaneou in una giovane donna felicement curato in questo grande ospedale degl'incurabili.* Napoli: Presso Giovanni di Simone.

Del Papa, N., Colombo, G., Fracchiolla, N., Moronetti, L.M., Ingegnoli, F., Maglione, W., Comina, D.P., Vitali, C., Fantini, F., and Cortelezzi, A. (2004). Circulating endothelial cells as a marker of ongoing vascular disease in systemic sclerosis. *Arthritis Rheum. 50,* 1296–1304.

Denton, C.P. and Abraham, D.J. (2004). Transgenic analysis of scleroderma: understanding key pathogenic events *in vivo*. *Autoimmun. Rev. 3,* 285–293.

Denton C.P., Zheng B., Evans, L.A., Shi-wen, X., Ong, V.H., Fisher, I., Lazaridis, K., Abraham, D.J., Black, C.M., de Crombrugghe, B. (2003). Fibroblast-specific expression of a kinase-deficient type II transforming growth factor beta (TGFbeta) receptor leads to paradoxical activa-

tion of TGFbeta signaling pathways with fibrosis in transgenic mice. *J Biol Chem*, *278*, 25109–25119.

Denton, C.P., Merkel, P.A., Furst, D.E. Khanna, D., Emery, P., Hsu, V.M., Silliman, N., Streisand, J., Powell, J., Korn, J.H., Black, C.M., and Seibold, J.R. (2004). Anti-TGFbeta1 therapy for dcSSc: a multicenter randomized placebo controlled trial of CAT-192. *Arthritis Rheum. 50S,* 1852 [abstract].

Denton, C.P., Zheng, B., Shiwen, X., Zhang, Z., Bou-Gharios, G., Eberspaecher, H., Black, C.M., and de Crombrugghe, B. (2001). Activation of a fibroblast-specific enhancer of the proα2(I) collagen gene in tight-skin mice. *Arthritis Rheum. 44,* 712–722.

Evans, P.C., Lambert, N., Maloney, S., Furst, D.E., Moore, J.M., Nelson, J.L. (1999). Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. *Blood*, *93*, 2033–2037.

Farge, D., Marolleau, J.P., Zohar, S., Marjanovic, Z., Cabane, J., Mounier, N., Hachulla, E., Philippe, P., Sibilia, J., Rabian, C., Chevret, S., and Gluckman, E. (2002). Intensification et Autogreffe dans les Maladies Auto Immunes Resistantes (ISAMAIR) Study Group. Autologous bone marrow transplantation in the treatment of refractory systemic sclerosis: early results from a French multicentre phase I-II study. *Br. J. Haematol. 119,* 726–739.

Freni-Titulaer, L.W., Kelley, D.B., Grow, A.G., McKinley, T.W., Arnett, F.C., and Hochberg, M.C. (1989). Connective tissue disease in south eastern Georgia: a case-control study. *Am. J. Epidemiol. 129,* 404–409.

Garcia de la Pena-Lefebvre, P., Chanseaud, Y., Tamby, M.C., Reinbolt, J., Batteux, F., Allanore, Y., Kahan, A., Meyer, O., Benveniste, O., Boyer, O., Guillevin, L., Boissier, M.C., and Mouthon, L. (2004). IgG reactivity with a 100-kDa tissue and endothelial cell antigen identified as topoisomerase 1 distinguishes between limited and diffuse systemic sclerosis patients. *Clin. Immunol. 111,* 241–251.

Giacomelli, R., Cipriani, P., Fulminis, A., Nelson, J.L., and Matucci-Cerinic, M. (2004). Gamma/delta T cells in placenta and skin: their different functions may support the paradigm of microchimerism in systemic sclerosis. *Clin. Exp. Rheumatol. 22*(3 Suppl 33), S28–30.

Gintrac, M. (1847). Note sur la sclerodermie. *Rev. Med. Chir. 2,* 263–281.

Hochberg, M.C., Perlmutter, D.L., Medsger, T.A. Jr., Nguyen, K., Steen, V., Weisman, M.H., White, B., and Wigley, F.M. (1996). Lack of association between augmentation mammoplasty and systemic sclerosis (scleroderma). *Arthritis Rheum. 39,* 1125–1131.

Jimenez, S.A. and Artlett, C.M. (2005). Microchimerism and systemic sclerosis [Review]. *Curr. Opin. Rheumatol. 17,* 86–90.

Kissin, E. and Korn, J.H. (2002). Apoptosis and myofibroblasts in the pathogenesis of systemic sclerosis. *Curr. Rheumatol. Rep. 4,* 129–135.

Korn, J.H., Mayes, M., Matucci Cerinic, M., Rainisio, M., Pope, J., Hachulla, E., Rich, E., Carpentier, P., Molitor, J., Seibold, J.R., Hsu, V., Guillevin, L., Chatterjee, S., Peter, H.H., Coppock, J., Herrick, A., Merkel, P.A., Simms, R., Denton, C.P., Furst, D., Nguyen, N., Gaitonde, M., and Black, C. (2004). Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist. *Arthritis Rheum. 50,* 3985–3993.

Kuwana, M., Medsger, T.A., and Wright, T.M. (1995). T cell proliferative response induced by DNA topoisomerase 1 in patients with systemic sclerosis and healthy donors. *J. Clinical Invest. 96,* 586–596.

LeRoy, E.C. and Medsger, T.A. Jr. (2001). Criteria for the classification of early systemic sclerosis. *J. Rheumatol. 28,* 1573–1576.

Lunardi, C., Bason, C., Navone, R., Millo, E., Damonte, G., Corrocher, R., and Puccetti, A. (2000). Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. *Nat. Med. 6*(10), 1183–1186.

Lupoli, S., Amlot, P., and Black, C.M. (1990). Normal immune responses in systemic sclerosis. *J. Rheumatol. 17,* 323–337.

Ma, J., Chapman, G. V., Chen, S.L., Melick, G., Penny, R., and Briet, S.N. (1991). Antibody penetration of viable human cells: I. Increased penetration of human lymphocytes by anti-RNP IgG. *Clin. Exp. Immunol. 84,* 83–91.

Mackel, A.M., De Lustro, F., Harper, F.E., and LeRoy, E.C. (1982). Antibodies to collagen in scleroderma. *Arthritis Rheum. 25,* 522–531.

Maricq, H.R., Weinrich, M.C., Keil, J.E., Smith, E.A., Harper, F.E., Nussbaum, A.I., LeRoy, E.C., McGregor, A.R., Diat, F., and Rosal, E.J. (1989). Prevalence of scleroderma spectrum disorders in the general population of South Carolina. *Arthritis Rheum. 32,* 998–1006.

Marzano, A.V., Menni, S., Parodi, A., Borghi, A., Fuligni, A., Fabbri, P., and Caputo, R. (2003). Localized scleroderma in adults and children. Clinical and laboratory investigations on 239 cases. *Eur. J. Dermatol. 13,* 171–176.

Mayes, M.D., Lacey, J.V. Jr., Beebe-Dimmer, J., Gillespie, B.W., Cooper, B., Laing, T.J., and Schottenfeld, D. (2003). Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum. 48,* 2246–2255.

Poormoghim, H., Lucas, M., Fertig, N., and Medsger, T.A. Jr. (2000). Systemic sclerosis sine scleroderma: demographic, clinical, and serologic features and survival in forty-eight patients. *Arthritis Rheum. 43,* 444–451.

Prokop, J. and Jagodzinski, P.P. (2004). Identification of retroviral conserved pol sequences in serum of mixed connective tissue disease and systemic sclerosis patients. *Biomed. Pharmacother. 58,* 61–64.

Reeves, W.H., Satih, M., Wang, J., Chou, C.-H., and Ajmani, A.K. (1994). Antibodies to DNA, DNA binding proteins, and histones. *Rheum. Dis. Clin. N. Am. 20,* 1–28.

Renaudineau, Y., Grunebaum, E., Krause, I., Praprotnik, S., Revelen, R., Youinou, P., Blanks, M., Gilburd, B., Sherer, Y., Luderschmidt, C., Eldor, A., Weksler, B., Gershwin, E.M., and Shoenfeld, Y. (2001). Anti-endothelial cell antibodies (AECA) in systemic sclerosis-increased sensitivity using different endothelial cell substrates and association with other autoantibodies. *Autoimmunity 33,* 171–179.

Reveille, J.D., Fischbach, M., McNearney, T., Friedman, A.W., Aguilar, M.B., Lisse, J., Fritzler, M.J., Ahn, C., and Arnett, F.C. (2001). GENISOS Study Group. Systemic sclerosis in 3 US ethnic groups: a comparison of clinical, sociodemographic, serologic, and immunogenetic determinants. *Semin. Arthritis Rheum. 30,* 332–246

Roberts-Thomson, P.J., Jones, M., Hakendorf, P., Kencana Dharmapatni, A.A., Walker, J.G., MacFarlane, J.G., Smith, M.D., and Ahern, M.J. (2001). Scleroderma in South Australia: epidemiological observations of possible pathogenic significance. *Intern. Med. J. 31,* 220–229.

Shi-wen, X., Pennington, D., Holmes, A., Leask, A., Bradham, D., Beauchamp, J.R., Fonseca, C., du Bois, R.M., Martin, G.R., Black, C.M., and Abraham, D.J. (2000). Autocrine overexpression of CTGF maintains fibrosis: RDA analysis of fibrosis genes in systemic sclerosis. *Exp. Cell Res. 259,* 213–224.

Silman, A.J. (1995). Scleroderma. *Ballières Clin. Rheumatol. 9,* 471–478.

Strehlow, D., Jelaska, A., Strehlow, K., and Korn, J.H. (1999). A potential role for protease nexin 1 overexpression in the pathogenesis of scleroderma. *J. Clin. Invest. 103,* 1179–1190.

Susol, E., Rands, A.L., Herrick, A., McHugh, N., Barrett, J.H., Ollier, W.E., and Worthington, J. (2000). Association of markers for TGFbeta3, TGFbeta2 and TIMP1 with systemic sclerosis. *Rheumatology 39,* 1332–1336.

Tan, F.K., Arnett, F.C., Antohi, S., Saito S., Mirarchi, A., Spiera, H., Sasaki, T., Shoichi, O., Takeuchi, K., Pandey, J.P., Silver, R.M., LeRoy, C., Postlethwaite, A.E., and Bona, C.A. (1999). Autoantibodies to the extracellular matrix microfibrillar protein, fibrillin-1, in patients with scleroderma and other connective tissue diseases. *J. Immunol. 163,* 1066–1072.

Tan, F.K. and Arnett, F.C. (2000). Genetic factors in the etiology of systemic sclerosis and Raynaud phenomenon. *Curr. Opin. Rheumatol. 12,* 511–519.

Tiev, K.P., Abriol, J., Burland, M.C., Antonelli, D., Klatzmann, D., Cabane, J., and Boyer, O. (2005). T cell repertoire in patients with stable scleroderma. *Clin. Exp. Immunol. 139*(2), 348–354.

Tormey, V.J., Bunn, C.C., Denton, C.P., and Black, C.M. (2001). Anti-fibrillarin antibodies in systemic sclerosis. *Rheumatology 40,* 1157–1162.

Valesini, G., Litta, A., Bonavita, M.S., Luan, F.L., Purpura, M., Mariani, M., and Balsano, F. (1993). Geographical clustering of scleroderma in a rural area in the province of Rome. *Clin. Exp. Rheumatol. 11,* 41–47.

White, B. (1994). Immunologic aspects of scleroderma. *Curr. Opin. Rheumatol. 6,* 612–615.

Whitfield, M.L., Finlay, D.R., Murray, J.I., Troyanskaya, O.G., Chi, J.T., Pergamenschikov, A., McCalmont, T.H., Brown, P.O., Botstein, D., and Connolly, M.K. (2003). Systemic and cell type-specific gene expression patterns in scleroderma skin. *Proc. Natl. Acad. Sci. U.S.A. 100,* 12319–12324.

Winterbauer, R.H. (1964). Multiple telangiectasia, Raynaud's phenomenon, sclerodactyly and subcutaneous calcinosis: a syndrome mimicking hereditary hemorrhagic telangiectasis. *Bull, Johns Hopkins Hospital 114,* 361–383.

Worda, M., Sgonc, R., Dietrich, H., Niederegger, H., Sundick, R.S., Gershwin, M.E., and Wick, G. (2003). *In vivo* analysis of the apoptosis-inducing effect of anti-endothelial cell antibodies in systemic sclerosis by the corionallantoic membrane assay. *Arthritis Rheum. 48,* 2605–2614.

Zhou, X., Tan, F.K., Wang, N., Xiong, M., Maghidman, S., Reveille, J.D., Milewicz, D.M., Chakraborty, R., and Arnett, F.C. (2003). Genome-wide association study for regions of systemic sclerosis susceptibility in a Choctaw Indian population with high disease prevalence. *Arthritis Rheum. 48,* 2585–2592.

CHAPTER

30

Antiphospholipid Syndrome

ROBERT A.S. ROUBEY

Division of Rheumatology and Immunology, University of North Carolina, CB 7280 Thurston Building/Rm 3330, Chapel Hill, NC 27599-7280, USA

The antiphospholipid syndrome (APS) is the association of autoantibodies having an apparent specificity for anionic phospholipids with one or more clinical manifestations that include venous and arterial thrombosis and pregnancy loss and morbidity.

HISTORICAL BACKGROUND

The history of antiphospholipid antibodies (aPL) dates to the early twentieth century and the development of serologic tests for syphilis. It was not until 1941, however, that Pangborn identified the essential antigenic component of the tissue extracts used in these tests as cardiolipin, a novel phospholipid isolated from heart tissue (Pangborn, 1941). Widespread screening for syphilis, beginning in the late 1930s, led to the identification of individuals with false-positive serologic tests (Moore and Mohr, 1952; Moore and Lutz, 1955). Transiently false-positive tests were found to be associated with various infections, while chronically false-positive tests were associated with the subsequent development of systemic lupus erythematosus (SLE) and similar conditions. In the early 1950s, acquired inhibitors that blocked phospholipid-dependent coagulation reactions *in vitro* were found in a number of patients with SLE, some of whom had false-positive syphilis serology (Conley and Hartmann, 1952; Frick, 1955; Laurell and Nilsson, 1957). These inhibitors came to be known as *lupus anticoagulants (LA)* (Feinstein and Rapaport, 1972), although they occur in many individuals without SLE and are not typically associated with bleeding (Margolius et al., 1961).

The first case report of APS was probably that of a 35-year-old woman with five spontaneous abortions, an episode of thrombophlebitis, and an anticoagulant acting in the middle stages of coagulation (Alagille et al., 1956). In the English literature, Bowie et al. (1963) were the first to report

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

the association of lupus anticoagulants with thrombosis. Lechner, in a review, described the association of chronic false-positive syphilis serology, LA, thrombosis, and thrombocytopenia in patients with SLE (Lechner, 1974). In addition to Alagille's case, several other early reports associated pregnancy loss with LA (Nilsson et al., 1975; Firkin et al., 1980; Soulier and Boffa, 1980; Carreras et al. 1981). Of note, a number of these cases occurred in patients without SLE. Much of the current awareness of APS derives from the work of Hughes and colleagues in the 1980s (Boey et al., 1983; Hughes, 1983), including the development of immunoassays for anticardiolipin antibodies (Harris et al., 1983; Loizou et al., 1985). The term *primary APS* to refer to APS occurring in the absence of SLE or related autoimmune disease was introduced by Alarcon-Segovia's and Hughes' groups (Alarcon-Segovia and Sanchez-Guerrero, 1989a; Asherson et al., 1989a) In contrast, the term *secondary APS* refers to APS in the setting of SLE or related conditions. More recently, a multicenter study indicates that there is little fundamental difference between the primary and secondary syndromes (Vianna et al., 1994).

Through the 1980s, it was generally thought that aPL were directed against anionic phospholipids, e.g., cardiolipin and phosphatidylserine. In 1990, however, three groups independently discovered that anticardiolipin antibodies in most patients with APS did not bind to cardiolipin, but rather were directed against the phospholipid-binding plasma protein, β2-glycoprotein I (β2GPI) (Galli et al., 1990; Matsuura et al., 1990; McNeil et al., 1990). It was subsequently found that most LAs were directed against either β2GPI or prothrombin (Bevers et al., 1991; Galli et al., 1992; Oosting et al., 1992b; Roubey et al., 1992). In retrospect, a number of early reports suggested that certain plasma proteins played an important role in the expression of LA activity. Loeliger was among the first to describe the LA "cofactor" phenomenon (normal plasma contains a cofactor that potentiates lupus anticoagulant activity), and concluded that the cofactor was most likely prothrombin itself (Loeliger, 1959). In 1965, Yin and Gaston purified a LA antibody and demonstrated that its anticoagulant activity required a protein cofactor distinct from prothrombin (Yin and Gaston, 1965). Although the cofactor was not completely characterized, it may well have been β2GPI.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Thrombosis

Thrombosis is a major clinical manifestation of APS, and has been reported in nearly all sites of the vascular tree. The most common sites of venous thrombosis are the deep and superficial veins of the lower extremities (Alarcon-Segovia et al., 1992; Asherson et al., 1989b; Vianna et al., 1994). Pulmonary embolism may occur in up to half the cases of deep venous thrombosis. The most common form of arterial thrombosis in APS is stroke (Asherson et al., 1989b; Levine et al., 1990). Thrombosis may be the underlying pathophysiological process in a number of other clinical manifestations of APS. Examples include placental thrombosis and infarction leading to pregnancy loss (Magid et al., 1998; Sebire et al., 2003), cutaneous ulcers (thrombosis of dermal blood vessels) (Grattan and Burton, 1991), certain forms of neurological disease (multi-infarct dementia) (Chapman et al., 2003), pulmonary hypertension (recurrent pulmonary emboli) (Asherson et al., 1990), and renal dysfunction (thrombosis of intrarenal blood vessels) (Nochy et al., 1999).

Pregnancy Mortality and Morbidity

Fetal deaths occurring from the late first trimester onward are strongly associated with aPL (Branch and Khamashta, 2003). The association of aPL with losses at less than 10 weeks gestation is somewhat less well established due to the high incidence of such losses in the general population (Branch, 1994). Various types of pregnancy morbidity are also associated with aPL. Most cases of fetal death related to aPL are preceded by fetal growth impairment and oligohydramnios. Other obstetric complications include preeclampsia, fetal distress, fetal growth impairment, premature delivery, and maternal thrombotic events in the postpartum period.

Skin Manifestations

Livedo reticularis, a latticework of blue-to red subcutaneous mottling, is often present in patients with aPL (Englert et al., 1989). Sneddon syndrome, the association of livedo reticularis and stroke, is associated with aPL in many instances (Frances et al., 1999). Cutaneous ulcers, typically involving the legs, have been reported in several series (Johansson et al., 1977; Grattan and Burton, 1991; Naldi et al., 1993). Skin necrosis may also occur (Paira et al., 1999; Sharkey et al., 2002).

Thrombocytopenia

Thrombocytopenia in SLE patients is associated with aPL, occurring in approximately 40% of patients with aPL and only 10% of patients without aPL (McNeil et al., 1991). Between 70 and 80% of lupus patients with thrombocytopenia have aPL (Diz-Kucukkaya et al., 2001), and aPL are also present in nearly one-third of patients with chronic autoimmune thrombocytopenia. Thrombocytopenia in APS is usually moderate and not associated with hemorrhage. Low platelet counts do not appear to protect APS patients

from the risk of thrombosis (Alarcón-Segovia and Sanchez-Guerrero, 1989b).

Heart Valve Disease

Valvular heart disease is associated with aPL in patients with and without SLE (Khamashta et al., 1990; Leung et al., 1990; Galve et al., 1992; Vianna et al., 1994). Non-infective verrucous vegetations (Libman–Sacks endocarditis) are characteristic. These vegetations may embolize, causing stroke and other ischemic events (Bulckaen et al., 2003).

Neurologic Syndromes

The major neurologic manifestations of APS are cerebrovascular thrombosis and embolic stroke. Transient ischemic attacks may also occur (Tietjen et al., 1993). Recurrent strokes may lead to multi-infarct dementia (Coull et al. 1987; Asherson et al., 1989a). A variety of non-stroke neurologic events have been reported in patients with aPL including migraine headache (Brey et al., 1993), transverse myelitis (Lavalle et al., 1990), Guillain–Barré syndrome, chorea (Cervera et al., 1997), and syndromes resembling multiple sclerosis (Tourbah et al., 1998; Ijdo et al., 1999). The strength of these associations is controversial, however (Chapman et al., 2003). There is growing evidence that aPL are associated with cognitive dysfunction (Denburg et al., 1997; Menon et al. 1999; McLaurin et al., 2005).

Catastrophic Antiphospholipid Syndrome

Catastrophic APS is a syndrome of widespread, multiple vascular occlusions associated with aPL (Asherson et al., 2003). It has been reported in over 130 patients (Asherson et al., 1998; 2001). Common features include multiple vascular occlusive events presenting over a short period of time (days to weeks). Thrombosis typically affects small vessels supplying organs (e.g., brain, lungs, kidney, heart, liver). Large vessel occlusions such as deep vein thromboses are less common than in typical APS. Many patients with catastrophic APS have a history of SLE, a lupus-like disease, or primary APS; however, the catastrophic syndrome was the first manifestation of APS in about half of the reported cases. Apparent precipitating events, identifiable in 40–50% of cases, include infections, surgery, trauma, neoplasia, and withdrawal of anticoagulant medications. Catastrophic APS was fatal in nearly 50% of cases. Cardiac involvement (myocardial infarction, heart failure due to myocardial microthrombi, heart block) and pulmonary involvement (acute respiratory distress syndrome, pulmonary embolism) were the major causes of death. A long term outcome study of 58 catastrophic APS survivors found that 66% of patients subsequently did well, without any further manifestations of APS (on anticoagulation) over an average follow-up period of 5–6 years (Erkan et al., 2003). In contrast, 26% of patients experienced additional APS-related events, approximately one-quarter of which were fatal. In the remainder of cases, the patients did not develop further APS manifestations, but died either from late complications of the initial catastrophic illness or from apparently unrelated causes.

Prevalence of Antiphospholipid Antibodies and Antiphospholipid Syndrome

In cross-sectional studies, anticardiolipin antibodies or LA are present in approximately one-third of SLE patients (Petri, 2000). A similar prevalence of anticardiolipin antibodies was observed in a community-based cohort of newly diagnosed SLE patients (Cooper et al., 2002). Roughly 30–50% of patients with antibodies (i.e., 10–20% of all SLE patients) have one or more clinical manifestation of APS. The prevalence of the primary APS is more difficult to ascertain. In retrospective studies, aPL are present in 5–30% of patients with thrombosis in the absence of SLE (Exner and Koutts, 1988; Petri, 2000). In a large prospectively followed cohort, medium-to-high levels of anticardiolipin antibodies were found in approximately 20% of patients who subsequently had deep venous thrombosis and/or pulmonary embolism (Ginsburg et al. 1992). No association with ischemic stroke was observed in this study. In contrast, other studies indicate that aPL may account for about one-third of strokes in relatively young patients (under the age of 50 years) (Kittner and Gorelick, 1992). Among women with recurrent pregnancy losses, it is estimated that 10–20% are attributable to APS (Yetman and Kutteh, 1996; Branch and Khamashta, 2003). Antiphospholipid antibodies are present in approximately 2% (range 1–5%) of healthy controls, and this frequency may increase with age (Petri, 2000).

Classification Criteria

International consensus criteria for the classification of definite APS have been proposed (Box 30.1) (Wilson et al., 1999). It should be noted these criteria include only thrombosis and pregnancy morbidity/mortality as clinical manifestations of APS, and are designed for use in research studies, not for the diagnosis of individual patients.

AUTOIMMUNE FEATURES

Autoantibodies

As mentioned above, most autoantibodies associated with APS and detected in routine anticardiolipin and LA assays are not directed against cardiolipin or other anionic

Box 30.1
Preliminary Classification Criteria for Definite Antiphospholipid Syndrome*

Clinical criteria (at least 1 must be present)

1. Vascular thrombosis

One or more clinical episodes of arterial, venous, or small vessel thrombosis in any tissue or organ, confirmed by imaging or Doppler studies, or histopathology (except for superficial venous thrombosis). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. Pregnancy Morbidity

- One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or direct examination, or
- 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
- Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and parental chromosomal causes excluded.

Laboratory criteria (at least 1 must be present)

1. Anticardiolipin antibody

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 (ELISA) for β2-glycoprotein I-dependent anticardiolipin 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 Haemostasis Scientific Standardization Subcommittee on Lupus Anticoagulants/Phospholipid-Dependent Antibodies.†

*Wilson et al., 1999. †Brandt et al., 1995.

phospholipids. At present the most common and best-characterized antigenic targets are β2GPI and prothrombin.

β2-Glycoprotein I

β2-glycoprotein I is a 50 kDa glycoprotein present in normal plasma. Structurally, it is a member of the complement control protein family of molecules (Reid et al., 1986), although it has no known function in the complement system at this time. It has five of the consensus repeats, or so-called "sushi domains," characteristic of such proteins. The fifth domain, with a concentration of positively charged amino acids, contains a major phospholipid-binding region (Steinkasserer et al., 1992; Hunt et al., 1993). The fifth domain also contains a plasmin cleavage site; when cleaved by plasmin, β2GPI loses its affinity for anionic phospholipids (Matsuura et al., 2000). Although it has no structural similarity to other apolipoproteins, β2GPI has also been termed apolipoprotein H (Lee et al., 1983). The plasma level of β2GPI is under genetic control (Mehdi et al., 2003). Approximately 94% of the population is homozygous for the normal allele, with a mean plasma concentration of approximately 200 μg/mL, whereas about 6% are heterozygous with a concentration of approximately 100 μg/mL. Homozygous deficiency of β2GPI is rare (<1 in 1000). Neither homozygous deficiency nor heterozygosity is clearly associated with a clinical phenotype (Takeuchi et al., 2000; Yasuda et al., 2000).

The physiological role of β2GPI is unknown. It binds to anionic phospholipids under certain conditions (Wurm, 1984), although binding to phospholipid membranes under physiological conditions is relatively weak, compared with coagulation factors (Harper et al., 1998). Some *in vitro* data suggest that β2GPI could function as a physiological anticoagulant (Schousboe, 1985; Nimpf et al., 1986; 1987), however, this is unlikely based on it phospholipid-binding properties. A novel role of β2GPI is suggested by the recent observation that β2GPI binds to factor XI and inhibits activation of factor XI by thrombin (Shi et al., 2004).

The specificity for β2GPI distinguishes the "anticardiolipin" antibodies associated with APS from those that are associated with syphilis and certain other infectious diseases (Matsuura et al., 1990; 1992; Roubey et al., 1992; McNally et al., 1995). Anticardiolipin antibodies associated with syphilis bind to cardiolipin in the absence of β2GPI.

Autoimmune "anticardiolipin" antibodies recognize epitopes expressed on β2GPI and bind to it in the absence of phospholipid under appropriate conditions (Arvieux et al., 1991; Roubey et al., 1995; Tincani et al., 1996; Iverson et al., 1998; Giles et al., 2003). Certain human monoclonal "anticardiolipin" antibodies derived from APS patient B cells similarly bind to β2GPI (Ichikawa et al., 1994).

Most anti-β2GPI autoantibodies in APS patient sera recognize epitopes expressed on native β2GPI (Roubey et al., 1995; Tincani et al., 1996; Giles et al., 2003), with the major autoepitopes residing on domain I (Iverson et al., 1998; 2002). These autoantibodies typically have low intrinsic affinity, with high-avidity binding dependent upon multivalent attachment to immobilized antigen (Giles et al., 1995; Tincani et al., 1996). Other data suggest that certain anti-β2GPI autoantibodies may be specific for conformational epitopes of β2GPI, formed when the protein binds to

anionic phospholipids or other negatively-charged surfaces (Matsuura et al., 1994; Pengo et al., 1995). IgG, IgM, and IgA isotypes of anti-β2GPI are all associated with APS. Among IgG antibodies, the response is skewed toward the IgG2 subclass.

Autoantibodies to β2GPI may have LA activity (Galli et al., 1992; Oosting et al., 1992b; Roubey et al., 1992; Keeling et al., 1993), as do certain anti-β2GPI monoclonal and polyclonal antibodies (Arvieux et al., 1992; Roubey et al., 1992; Arnout et al., 1998a). Lupus anticoagulant activity is due to antibody cross-linking of surface-bound β2GPI (Willems et al., 1996; Arnout et al., 1998b; Lutters et al., 2001). Cross-linked antibody–β2GPI complexes bind to phospholipid membranes with very high avidity, effectively decreasing the amount of phospholipid surface available to participate in the coagulation reaction. Why some anti-β2GPI antibodies have LA activity and others do not is unclear, but may be related to antibody titer, differences in affinity/avidity, and/or epitope specificity.

Prothrombin

Antiprothrombin autoantibodies are the other major specificity of LAs in APS patients (Bevers et al., 1991; Galli et al., 1997; Horbach et al., 1998; Amengual et al., 2003). Similar to anti-β2GPI antibodies, many antiprothrombin antibodies are of relatively low affinity. A small subset of patients with LA has hypoprothrombinemia, probably due to high-affinity antiprothrombin antibodies and the clearance of antibody–antigen complexes (these patients are at risk for bleeding, not APS). Some antiprothrombin antibodies in APS patients may recognize conformational epitopes formed when prothrombin binds to anionic phospholipids, such as phosphatidylserine (Amengual et al., 2003). The mechanism of LA activity appears to be analogous to that of anti-β2GPI antibodies, i.e., by cross-linking of membrane-bound prothrombin. Antibody–prothrombin complexes bind to phospholipid membrane with very high avidity, thereby decreasing the amount of catalytic phospholipid surface available to bind other coagulation factors (Simmelink et al., 2001).

Other Phospholipid-Binding Proteins

Autoantibodies to other phospholipid-binding proteins, although not detectable in standard aPL assays, may also be associated with APS. Antigenic targets include protein C and protein S (Pengo et al., 1996; Song et al., 2000; Nojima et al., 2001), annexin A5 (Matsuda et al., 1994; Lakos et al., 2000; Nojima et al., 2001), and tissue factor pathway inhibitor (TFPI) (Forastiero et al., 2003). Autoantibodies to factor V (Ortel, 1999), factor XI (Sugi and McIntyre, 2001) and factor XII (Åberg and Nilsson, 1972; Jones et al., 2001) have also been reported in small numbers of patients. Rarely, anticardiolipin assays may detect autoantibodies recognizing complement factor H, complement C4b-binding protein, thrombin-modified antithrombin, or lipopolysaccharide binding protein, although it is not clear whether such antibodies are associated with clinical manifestations of APS (Arvieux et al., 1999).

Autoantibodies with an apparent specificity for phosphatidylethanolamine, a zwitterionic phospholipid, have been reported in certain patients with clinical manifestations of APS (Karmochkine et al., 1992; Berard et al., 1996; McIntyre and Wagenknecht, 2000). These antibodies do not recognize phosphatidylethanolamine itself, but are directed against phosphatidylethanolamine-binding plasma proteins, including high- and/or low-molecular-weight kininogens, prekallikrein, and factor XI (Sugi and McIntyre, 1995; Boffa, et al. 1996; Sugi and McIntyre, 2001).

Phospholipids

It is unclear whether antibodies that bind directly to phospholipids are associated with APS. Older studies suggesting these specificities are difficult to interpret due to the fact that serum or plasma was present in most or all of these experiments and the key role of β2GPI and other proteins may have been missed. Data clearly demonstrating antibody specificity for cardiolipin or other phospholipids in the absence of any plasma or serum proteins is scant. In one study, a human monoclonal antibody to cardiolipin has been shown to induce pregnancy loss in an animal model (Ikematsu et al., 1998).

Anticardiolipin assays may detect antibodies directed against oxidized lipids. The fatty acid chains of cardiolipin purified from natural sources are readily oxidizable, and some oxidation is likely under the conditions used in anticardiolipin ELISAs (Hörkkö et al., 1997). Some reports suggest a cross-reactivity between anticardiolipin antibodies and antibodies to oxidized lipoproteins (the latter are thought to play a role in the pathophysiology of atherosclerosis) (Hörkkö et al., 1996). However, β2GPI, binds covalently to certain oxidized phospholipids (Kobayashi et al., 2001) confounding this interpretation.

Cell Surface Proteins

Antiplatelet and anti-endothelial cell antibodies have been detected in patients with APS (Harris et al., 1985; Jouhikainen et al., 1990). The relationship of these autoantibodies to those detected in LA and anticardiolipin assays is not entirely clear. In some cases, antibody binding to endothelial cells (Del Papa, et al. 1995; Simantov et al., 1995) and to platelets (Shi et al., 1993) is dependent upon β2GPI, which may bind to these cells via membrane phospholipids; however, several cell surface receptors have been implicated, e.g., annexin A2 on endothelial cells (Ma et al.,

TABLE 30.1 Reported HLA associations in antiphospholipid syndrome

Study	Patient group	Clinical or laboratory feature	HLA association
Hartung et al., 1992	European SLE	aCL	DR4, DR7, DRw53
Granados et al., 1997	Mexican SLE	aCL	DR7
Hashimoto et al., 1998	Japanese SLE	aCL	DRB1*0901
Galeazzi et al., 2000	European SLE	aCL	DRB1*04, DRB1*0402, DRB1*0403, DRB1*07, DRB3*0301, DQA1*0201, DQA1*0301, DQB1*0302
Sebastiani et al., 2003	European SLE	aCL	DPB1*1501, DPB1*2301
Arnett et al., 1999	Mixed, SLE, PAPS, other CTD	aβ2GPI	DQB1*0302, DRB1*1302; DQB1*0604/5 in blacks; DRB4*0101 in Mexican Americans
Ioannidis et al., 1999	Greek APS	aβ2GPI	HLA-DQA1*03 in particular *0301, HLA-DRB1*1302-DQB1*0604
Galeazzi et al., 2000	European SLE	aβ2GPI	DRB1*0402, DRB1*0403, DQB1*0302.
Sebastiani et al., 2003	European SLE	aβ2GPI	DPB1*0301, DPB1*1901
Arnett et al., 1991	Mixed, LA^+	LA	DQB1*0301
Panzer et al., 1997	LA^+	LA, aPlt	HLA-DQB1*06
Bertolaccini et al., 2000	British aPL^+	aPS/PT	DQB1*0301/4,
Sanchez et al., 2004	Caucasian, PAPS, SLE	aPL	HLA-DMA*0102
Caliz et al., 2001	British Caucasian SAPS, PAPS	APS	DQB1*0604/5/6/7/9-DQA1*0102-DRB1*1302 haplotype DQB1*0303-DQA1*0201-DRB1*0701 haplotype

aβ2GPI, anti-β2GPI antibodies; aCL, anticardiolipin antibodies; CTD, connective tissue diseases; aPlt, anti-platelet antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; aPS/PT, anti-phosphatidylserine/prothrombin antibodies; LA, lupus anticoagulant; PAPS, primary APS; SAPS, secondary APS; SLE, systemic lupus erythematosus.

2000) and the apolipoprotein E receptor 2′ on platelets (Lutters et al., 2003).

Cellular Autoimmunity

Until recently relatively little was known about the cellular autoimmune response in APS. Initial reports on T-cell reactivity towards β2GPI differed significantly among research groups (Visvanathan and McNeil, 1999; Hattori et al., 2000; Ito et al., 2000). Subsequently, consistent data from Kuwana and colleagues suggest that β2GPI-reactive $CD4^+$ cells are present in both normal individuals and APS patients, but activated *in vivo* only the latter (Hattori et al., 2000; Kuwana, 2004). These cells preferentially recognize peptides that include amino acids 276–290 (which encompasses the major phospholipid-binding site) in the context of DR53 (Arai et al., 2001), and preferential usage of $V\beta7^+$ and $V\beta8^+$ T-cell receptors (Yoshida et al., 2002). Interestingly, antigen-presenting cells pulsed with phospholipid-bound β2GPI, but not with phospholipid or β2GPI alone, stimulated p276–290-specific $CD4^+$ T-cell lines generated from the APS patients (Kuwana et al., 2004). This continues to be an exciting area for future research.

GENETIC FEATURES

Familial occurrences of APS have been reported by several groups. Goel et al. studied seven families, in which 30 of 101 family members met diagnostic criteria for APS (Goel et al., 1999) . Segregation analysis strongly supported a genetic basis for the disease that was best explained by a dominant or co-dominant model. Interestingly, in these families APS was not linked with HLA genes.

At least a dozen studies have investigated the HLA associations of aPL and APS (Domenico et al., 2003). Data from these studies are briefly summarized in Table 30.1.

There are a number of polymorphisms of β2GPI, and several groups have investigated whether these are associated with anti-β2GPI antibodies and APS. The valine/

leucine polymorphism at position 247 has been the focus of several reports (Atsumi et al., 1999; Hirose et al., 1999; Camilleri et al., 2003; Prieto et al., 2003). An increased frequency of valine at this position was found in white patients with primary APS and anti-β2GPI antibodies (Atsumi et al., 1999). Hirose et al. studied this polymorphism in APS patients and healthy controls of various racial backgrounds (Hirose et al., 1999). Among controls, the valine allele and the valine/valine genotype were more common in white than in African-American or Asian patients. There were no significant differences in allele or genotype frequencies between white or African-American patients and racially matched controls. In contrast, the valine allele and valine/valine genotype were significantly more frequent among Asian APS patients with anti-β2GPI antibodies than among Asian controls. In a relatively small group of Mexican patients with primary APS, the valine allele and valine/valine genotype were associated with anti-β2GPI antibodies (Prieto et al., 2003). Another study, however, found no association of this polymorphism with aPL in a white patient population (Camilleri et al., 2003). Two polymorphisms in the fifth domain of β2GPI, at codons 306 and 316, affect phospholipid binding (Sanghera et al., 1997), and β2GPI from homozygotes with either mutation or the compound heterozygote does not bind to anionic phospholipids. Data on whether either of these polymorphisms offers protection against developing aPL and APS are equivocal (Gushiken et al. 1999; Kamboh et al. 1999).

ANIMAL MODELS

Animal models of APS are important tools for investigating the cellular basis of the autoimmune response, the contribution of genetic factors, and mechanisms by which aPL contribute to thrombosis and pregnancy loss (Radway-Bright et al., 1999).

The MRL/lpr and NZW × BXSB F1 strains of autoimmune mice are putative spontaneous models of APS (Smith et al. 1990; Hashimoto et al., 1992). Although MRL/lpr mice have antibodies detectable in anticardiolipin assays, the exact nature of these anticardiolipin antibodies is controversial. Stroke, decreased fecundity, and thrombocytopenia have been reported in these animals. NZW × BXSB F1 mice develop true anti-β2GPI autoantibodies (Monestier et al., 1996). In addition to features of lupus, these mice develop a degenerative coronary vasculopathy with myocardial infarction.

Induced animal models of APS include passive transfer of antibodies and various forms of active immunization. Passive transfer models evaluate the short-term effects of antibodies on coagulation and/or pregnancy in recipient animals. Pierangeli and colleagues have used this model extensively to examine the effects of a "pinch" injury to an isolated blood vessel on thrombus size and kinetics (Pierangeli et al., 1994), and to study leukocyte adhesion in the microvasculature (Pierangeli et al., 2001). Active immunization models can be divided into two groups based on the nature of the immunizing agent—APS-associated antigens and aPL. Experiments in which normal mice are immunized with APS-associated antigens generally confirm the observation that phospholipids themselves are poor antigens. In contrast, immunization with heterologous (human) β2GPI and an adjuvant induces an initial antibody to respond to the foreign protein. Over time tolerance is broken with the development of anti-murine β2GPI autoantibodies (Tincani et al., 2002). Mice immunized with human β2GPI develop clinical features of APS in approximate temporal association with the appearance of autoreactive anti-murine β2GPI antibodies (Blank et al., 1994). Immunization of mice with heterologous β2GPI bound to apoptotic cells, in the absence of adjuvant, also breaks tolerance (Levine et al., 1998). The second type of active immunization model has been developed by Shoenfeld and colleagues (Blank et al., 1991). This is an idiotype/anti-idiotype model in which mice immunized with an anti-cardiolipin (anti-β2GPI) antibody produce antibodies of the same specificity (an anti-anti-Id response). While this model may be useful for studying antibody effects, it is not known whether it is relevant to the immunopathogenesis of human APS.

PATHOGENIC MECHANISMS

It is widely hypothesized that aPL play a direct role in the pathogenesis of APS, contributing to hypercoagulability and possibly other mechanisms. This hypothesis is generally supported by the association of aPL titer with the risk of developing clinical manifestations of APS (Harris et al., 1986; Ginsburg et al., 1992), and by the passive-transfer animal models described above (Branch et al., 1990; Blank et al., 1991; Pierangeli et al., 1994; Radway-Bright et al., 1999). A wide variety of specific mechanisms of aPL-mediated hypercoagulability and pregnancy loss have been proposed.

Dysregulation of Hemostatic Reactions

Autoantibodies to β2GPI, prothrombin, and perhaps other phospholipid-binding plasma proteins may cross-link membrane-bound antigens leading to a very high avidity between the antibody–antigen complex and the vesicle or cell surface. This is the mechanism by which certain antibodies express LA activity *in vitro*. It is likely that similar high avidity interactions could also occur *in vivo*, leading to the dysregulation of phospholipid-dependent hemostatic reactions.

Inhibition of the Protein C Pathway

The protein C pathway is an important natural anticoagulant mechanism. Genetic abnormalities of this pathway (deficiencies of protein C and protein S, factor V_{Leiden}) are common causes of inherited thrombophilia. Antiphospholipid antibodies may inhibit phospholipid-dependent reactions of the protein C anticoagulant pathway: 1) the activation of protein C by thrombin bound to thrombomodulin; and/or 2) the inactivation of factors Va and VIIIa by activated protein C and its cofactors protein S and factor V (Comp et al., 1983; Cariou et al., 1988; Marciniak and Romond, 1989; Malia et al., 1990; Oosting et al., 1993; Izumi et al., 2002; Hwang et al., 2003). Anti-β2GPI autoantibodies may be involved. Autoantibodies directed against protein-C-pathway components, e.g., protein C and protein S, may also play a role (Oosting et al., 1993; Nojima et al., 2002). Some APS patients are reported to have decreased levels of protein S, although the data are equivocal (Ruiz-Argüelles et al., 1991; Bertolaccini et al., 2003; Song et al., 2000).

Displacement of Annexin A5

Rand and colleagues have suggested that annexin A5 bound to the luminal surface of vascular endothelial cells functions as a physiological anticoagulant, and that aPL (e.g., β2GPI/anti-β2GPI complexes) are procoagulant because they displace annexin A5 from the endothelial cell surface (Rand, 2002). Other data do not support this hypothesis (Lakasing et al., 1999; Bevers et al., 2000; Donohoe et al., 2000; Willems et al., 2000). Further, while annexin A5 may play an important thrombomodulatory role in the placental circulation (Krikun et al., 1994; Wang et al., 1999), at the present time there is little direct evidence that it functions as an anticoagulant throughout the systemic vasculature.

Inhibition of β2-Glycoprotein I

It has been suggested that β2GPI may function as an anticoagulant, although the phospholipid-binding properties of β2GPI (Harper et al., 1998) and studies of β2GPI-deficient individuals (Takeuchi et al., 2000) do not clearly support such a function. However, β2GPI may play such a role if recent observations that β2GPI binds to factor XI and is cleaved by plasmin are shown to be physiologically important. Anti-β2GPI antibodies could, in theory, interfere with these interactions.

Impaired Fibrinolysis

Impairment of fibrinolysis has been observed in patients with APS (Sanfelippo and Drayna, 1982; Killeen et al., 1987; Kolev et al., 2002) and anti-β2GPI antibodies have been implicated (Takeuchi et al., 2002; Yasuda et al., 2004).

Cellular Effects

Although the mechanisms listed above involve reactions on cell surface, activation or inhibition of cellular function is not primarily involved. There is growing evidence, however, that aPL may stimulate or activate various types of cells (monocytes, endothelial cells, platelets), leading to a procoagulant phenotype. Antiphospholipid antibodies may do this via cross-linking of antigens, such as β2GPI, bound to cell surface receptors.

Monocytes

Tissue factor (TF) is the major initiator of normal and pathologic coagulation and is not normally expressed by cells in contact with blood. A number of studies suggest that increased expression of TF on circulating blood monocytes is an important mechanism of hypercoagulability in APS (Cuadrado et al., 1997; Amengual et al., 1998; Dobado-Berrios et al., 1999; Zhou et al., 2004). Anti-β2GPI antibodies from patient sera and patient-derived monoclonal anti-β2GPI autoantibodies induce TF expression on normal blood monocytes *in vivo*, and TF expression is increased on monocytes from patients with IgG anticardiolipin antibodies and a history of thrombosis.

Endothelial Cells

Antiphospholipid antibodies may stimulate or increase the procoagulant activity of vascular endothelial cells via a number of mechanisms (Riboldi et al., 2003). Sera and IgG fractions from certain APS patients increase TF expression (Oosting et al., 1992a), the production of endothelin 1 (Atsumi et al., 1998), and the expression of the adhesion molecules E-selectin, VCAM-1, and ICAM-1 (Simantov et al., 1995; Del Papa et al., 1995). The latter effects are dependent on anti-β2GPI antibodies and β2GPI. Upregulation of adhesion molecules has been shown to mediate the thrombogenic effects of aPL in animal models (Pierangeli et al., 2001; Espinola et al., 2003).

Antiphospholipid syndrome autoantibodies may cause dysregulation of eicosanoid metabolism, inhibiting the production of endothelial cell prostacyclin (PGI_2), a potent vasodilator and platelet inhibitor (Carreras et al., 1996). Antiphospholipid antibodies may also affect platelet eicosanoids, as discussed below.

Platelets

Autoimmune thrombocytopenia is a manifestation of APS and thought to be due to the anti-platelet reactivity of

certain aPL. Potential target antigens that may be involved include β2GPI bound to platelet membranes or receptors (Shi et al., 1993), CD36, and several of the major platelet membrane glycoproteins (Rock et al., 1994; Macchi et al., 1997; Pelegri et al., 2003). Dysregulation of eicosanoids by aPL increases platelet thromboxane A_2 production (Martinuzzo et al., 1993; Forastiero et al., 1998). Anti-β2GPI autoantibodies are associated with increased levels of platelet-derived thromboxane urinary metabolites. Several studies demonstrate that aPL induce platelet aggregation (Wiener et al., 2001; Lutters et al., 2003). Monoclonal anti-β2GPI antibodies bind to platelets in a β2GPI-dependent fashion and lead to platelet activation in the presence of sub-threshold concentrations of weak agonists (Arvieux et al., 1993).

Pregnancy Loss

The proximate cause of fetal death in APS is probably hypoxia due to insufficient uteroplacental blood flow (Branch, 1994). Pathophysiological findings in the placenta of women with pregnancy loss include maternal spiral artery vasculopathy, placental infarction, chronic villitis, atherosis, decreased number of syncitio-vascular membranes, increased number of syncitial knots, and fetal thrombi (Magid et al., 1998; Ogishima et al., 2000; Locatelli et al., 2002; Sebire et al., 2002). Infarctions in the placenta may be associated with decreased amounts of annexin A5 on placental villi (Rand, 2002), although some investigators report normal amounts of annexin A5 are present (Donohoe et al., 2000; Lakasing et al., 1999).

Recently the work of Salmon and colleagues has highlighted the role of the complement activation in mediating pregnancy loss in a murine model of aPL-induced pregnancy loss (Salmon et al., 2003). Mice genetically deficient in complement components and mice treated with complement inhibitors were protected from fetal resorption (Holers et al., 2002; Girardi et al., 2003; Thurman et al., 2005). Heparin, a complement inhibitor as well as an anticoagulant, prevented fetal resorption, whereas fondaparinux and hirudin, anticoagulants that do not inhibit complement, did not (Girardi et al., 2004)

IMMUNOLOGIC MARKERS IN DIAGNOSIS

The two standard clinical laboratory tests for aPL are anticardiolipin ELISAs and LA. In general, both tests may need to be performed as the assays are discordant in up to 35% of APS patients (Petri, 1994). The presence of aPL should be confirmed by repeating positive tests in 6 to 8 weeks. The significance of transiently positive tests is unclear.

Tests are available for IgG, IgM, and IgA anticardiolipin antibodies. Although the strongest clinical associations are with IgG anticardiolipin antibodies, isolated IgM or IgA antibodies are also associated with APS. Anticardiolipin ELISAs are reasonably well-standardized, and positive reference standards for IgG, IgM, IgA isotypes are available (Harris et al., 1987; Harris, 1990; Tincani et al., 2001). Interlaboratory variation remains somewhat of a problem, however (Reber et al., 1995). Practically speaking, there is generally good agreement on most specimens, with most variability occurring with results that fall close to the upper limit of normal or in the low end of the positive range. Care should be taken in interpreting results in this range.

Anti-β2GPI ELISAs have been developed and are clinically available (Arvieux et al., 1991; Matsuura et al., 1994; Roubey et al., 1995). Compared with anticardiolipin assays, anti-β2GPI assays may be more specific for clinical manifestations of APS and may detect species-specific autoantibodies that recognize human β2GPI, but not bovine β2GPI (present in most anticardiolipin assays) (Arvieux et al., 1996). Currently, most experienced clinicians in this field consider the anti-β2GPI assay as a second-line test. It may provide useful information in situations where there is a high index of suspicion for APS and the standard aPL tests are negative. Additional interlaboratory standardization efforts and prospective clinical studies will help define the role of anti-β2GPI assays in clinical practice. Immunoassays for antiprothrombin antibodies are currently in development (Atsumi et al., 2000; Amengual et al., 2003).

The first step in LA testing is an appropriately sensitive screening test, e.g., lupus-activated partial thromboplastin time (aPTT), dilute Russell viper venom time, and the kaolin clotting time (Brandt et al., 1995a; 1995b). A routine aPTT is generally not adequate for LA screening. If the screening test is prolonged, confirmation of a positive LA requires 1) demonstration that prolongation of the screening test is due to an inhibitor rather than a factor deficiency (failure of the abnormal screening test to correct when patient plasma is mixed with normal plasma); and 2) demonstration of phospholipid-dependence (the inverse relationship of phospholipid concentration to prolongation of the coagulation test). Phospholipid-dependence may be demonstrated in an assay in which reduced phospholipid accentuates the anticoagulant activity, or an assay in which excess phospholipid neutralizes the anticoagulant activity (e.g., platelet-neutralization procedure, hexagonal-phase phospholipid-neutralization procedure).

Testing for antibodies to multiple phospholipids is generally not recommended (Bertolaccini et al., 1998). These panels of tests can be expensive, lack standardization, and have little proven diagnostic value. An exception might be antibodies to phosphatidylethanolamine. Clinical manifestations of APS have been reported in a number of patients with

anti-phosphatidylethanolamine antibodies in the absence of other aPL (Berard et al., 1996; Boffa et al., 1996). The test is not widely available or standardized, and is best considered as a research tool at this time.

TREATMENT AND OUTCOME

Thrombosis

Medium-to-high levels of aPL are associated with a substantial risk for thromboembolic disease. The relative risk of deep venous thrombosis, pulmonary embolism, or stroke is approximately 7–8 (Ginsburg et al., 1992). Patients with medium to high aPL levels and a history of arterial or venous thrombosis have a significant risk of recurrent thrombosis (Rosove and Brewer, 1992; Khamashta et al., 1995; Ruiz-Irastorza et al., 2002). After initial treatment with heparin followed by warfarin, long-term warfarin is recommended to prevent recurrent thrombotic events. Retrospective studies suggest that a relatively high level of anticoagulation, international normalized ratio (INR) of more than 3.0, is necessary. A recent randomized controlled trial, however, found that high-intensity warfarin (target INR 3.1–4.0) was not superior to moderate-intensity warfarin (target INR 2.0–3.0) (Crowther et al., 2003). Many experienced clinicians in the field now recommend the lower INR target. Lifelong anticoagulation may be necessary. The decision regarding long-term anticoagulation should be individualized, given the risks of warfarin, and various factors should be considered (age and reliability of the patient, level of aPL, the type of thrombotic event, temporal distance from the event). In certain patients with LA, these autoantibodies may interfere with accurate determination of the INR, requiring other tests to assess the level of anticoagulation (Della Valle et al., 1996; Moll and Ortel, 1997).

In general, immunosuppression is not thought to be effective in preventing thrombosis, and is not recommended. Anecdotally, rituximab has been tried in a few severe, refractory cases of APS with variable results.

Pregnancy Loss

Women with moderate-to-high levels of aPL and a history of one or more otherwise unexplained fetal deaths (>10 weeks gestation) or recurrent pre-embryonic or embryonic spontaneous abortions are candidates for treatment to prevent loss and morbidity in subsequent pregnancies. Currently, most experts recommend treatment with heparin and low-dose aspirin (Kutteh, 1996; Rai et al., 1997; Branch and Khamashta, 2003). Prednisone and aspirin appear to be equally efficacious, but prednisone is associated with a higher frequency of adverse side effects, including infection, pre-eclampsia, gestational diabetes, and osteonecrosis (Cowchock et al., 1992). With treatment, the chance of a successful pregnancy is 70–80%. Women with APS who are considering pregnancy should have preconception consultations with their obstetrician and rheumatologist and/or hematologist. Low-dose aspirin is often initiated (or continued) at this time. Treatment with heparin is begun as soon as pregnancy is diagnosed, usually at 5–7 weeks gestation. Either unfractionated or low-molecular-weight heparin (enoxaparin, dalteparin) may be used. For women without a history of thrombosis, a typical regimen is subcutaneous heparin, 5000–10,000 units every 12 hours, and aspirin, 75 or 81 mg daily. The low end of the heparin-dose range appears to be as efficacious as the high end (Kutteh and Ermel, 1996; Rai et al., 1997). For women with prior thrombosis, higher doses of heparin are recommended. Specific heparin regimens for prevention of APS-related pregnancy loss have recently been reviewed (Branch and Khamashta, 2003). Heparin is held at the time of delivery, and then reinstituted for 4–6 weeks after delivery, due to the risk of thrombosis in the postpartum period. Intravenous immunoglobulin (IVIg) therapy is an option if heparin and prednisone fail (Scott et al., 1988; Spinnato et al., 1995; Clark et al., 1999), although a small randomized controlled trial did not show an increased benefit of IVIG over heparin and aspirin (Branch et al., 2000). In a randomized trial of low-molecular-weight heparin and aspirin versus IVIG, the live birth rate was higher in the heparin/aspirin group (16/19 or 84% vs 12/21 or 57% for IVIG) but the difference did not reach statistical significance (Triolo et al., 2003). It is unclear whether women with low levels of aPL and recurrent pregnancy loss require treatment. A randomized controlled trial, in which a majority of subjects had low titer antibodies, observed successful pregnancy rates of 80–85% in patients receiving low-dose aspirin or placebo (Pattison et al., 2000).

Catastrophic APS

Due to the severe nature of catastrophic APS, most patients receive a combination of therapies, including anticoagulants, corticosteroids, plasmapheresis, cyclophosphamide, and IVIG (Asherson et al., 1998; Asherson et al., 2001). Anticoagulation appears to be particularly useful; survival was 62% in patients receiving anticoagulation versus 23% in those who were not anticoagulated (Asherson et al., 2001). In individual cases plasmapheresis (Neuwelt et al., 1997) and defibrotide (Burcoglu-O'Ral et al., 2002) have each been helpful.

Thrombocytopenia

Thrombocytopenia in APS is usually moderate and does not require treatment. Lower counts may require treatment with corticosteroids.

Asymptomatic Patients

Low-dose aspirin is generally recommended for asymptomatic patients with aPL but without a history of thrombosis (Erkan et al., 2001; 2002; Alarcon-Segovia et al., 2003). These patients are usually identified in three situations, i.e., patients with SLE found to have aPL on routine screening, women with a diagnosis of APS based on pregnancy loss or morbity, and LA^+ individuals discovered during coagulation screening. Hydroxychloroquine may help prevent thrombosis in patients with aPL and SLE (Petri et al., 1994; Erkan et al., 2002).

CONCLUDING REMARKS— FUTURE PROSPECTS

APS is an under-recognized autoimmune disease that accounts for a significant proportion of thromboembolic disease and recurrent pregnancy loss. Anticoagulation rather than immunosuppression is the current mainstay of therapy. Although effective in most cases, full anticoagulation carries a significant risk of adverse side effects. Future research characterizing the underlying autoimmune response, the spectrum of APS autoantibodies, and the relevant mechanisms of hypercoagulability will lead to the identification of novel therapeutic targets and approaches. These new approaches raise hope for safer and more effective treatment of APS.

References

Åberg, H. and Nilsson, I.M. (1972). Recurrent thrombosis in a young woman with a circulating anticoagulant directed against factors XI and XII. *Acta. Med. Scand. 192,* 419–425.

Alagille, D., Crosnier, J., and Soulier, J.P. (1956). Anticoagulant circulant á activité antithromboplastique. *Rev. Franç. Et. Clin. Biol. 1,* 335–345.

Alarcon-Segovia, D., Boffa, M.C., Branch, W., Cervera, R., Gharavi, A., Khamashta, M., Shoenfeld, Y., Wilson, W., and Roubey, R. (2003). Prophylaxis of the antiphospholipid syndrome: a consensus report. *Lupus 12*(7), 499–503.

Alarcon-Segovia, D., Perez-Vazquez, 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*(5), 275–286.

Alarcon-Segovia, D. and Sanchez-Guerrero, J. (1989a). Primary antiphospholipid syndrome. *J. Rheumatol. 16*(4), 482–488.

Alarcón-Segovia, D. and Sanchez-Guerrero, J. (1989b). Correction of thrombocytopenia with small dose aspirin in the primary antiphospholipid syndrome. *J. Rheumatol. 16,* 1359–1361.

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*(2), 276–281.

Amengual, O., Atsumi, T., and Koike, T. (2003). Specificities, properties, and clinical significance of antiprothrombin antibodies. *Arthritis Rheum. 48*(4), 886–895.

Arai, T., Yoshida, K., Kaburaki, J., Inoko, H., Ikeda, Y., Kawakami, Y., and Kuwana, M. (2001). Autoreactive CD4(+) T-cell clones to beta2-glycoprotein I in patients with antiphospholipid syndrome: preferential recognition of the major phospholipid-binding site. *Blood 98*(6), 1889–1896.

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.

Arnett, F.C., Thiagarajan, P., Ahn, C., and Reveille, J.D. (1999). Associations of anti-β_2-glycoprotein I autoantibodies with HLA class II alleles in three ethnic groups. *Arthritis Rheum. 42*(2), 268–274.

Arnout, J., Vanrusselt, M., Wittevrongel, C., and Vermylen, J. (1998a). Monoclonal antibodies against beta-2-glycoprotein I: use as reference material for lupus anticoagulant tests. *Thromb. Haemost. 79*(5), 955–958.

Arnout, J., Wittevrongel, C., Vanrusselt, M., Hoylaerts, M., and Vermylen, J. (1998b). Beta-2-glycoprotein I dependent lupus anticoagulants form stable bivalent antibody beta-2-glycoprotein I complexes on phospholipid surfaces. *Thromb. Haemost. 79*(1), 79–86.

Arvieux, J., Roussel, B., Jacob, M.C., and Colomb, M.G. (1991). Measurement of anti-phospholipid antibodies by ELISA using β_2-glycoprotein I as an antigen. *J. Immunol. Methods* 143, 223–229.

Arvieux, J., Pouzol, P., Roussel, B., Jacob, M.C., and Colomb, M.G. (1992). Lupus-like anticoagulant properties of murine monoclonal antibodies to β_2-glycoprotein I. *Br. J. Haematol.* 81, 568–573.

Arvieux, J., Roussel, B., Pouzol, P., and Colomb, M.G. (1993). Platelet activating properties of murine monoclonal antibodies to β_2-glycoprotein I. *Thromb. Haemost. 70,* 336–341.

Arvieux, J., Darnige, L., Hachulla, E., Roussel, B., Bensa, J.C., and Colomb, M.G. (1996). Species specificity of anti-β_2-glycoprotein I autoantibodies and its relevance to anticardiolipin antibody quantitation. *Thromb. Haemost. 75*(5), 725–730.

Arvieux, J., Pernod, G., Regnault, V., Darnige, L., and Garin, J. (1999). Some anticardiolipin antibodies recognize a combination of phospholipids with thrombin-modified antithrombin, complement C4b-binding protein, and lipopolysaccharide binding protein. *Blood 93*(12), 4248–4255.

Asherson, R.A., Cervera, R., De Groot, P.G., Erkan, D., Boffa, M.C., Piette, J.C., Khamashta, M.A., and Shoenfeld, Y. (2003). Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus 12*(7), 530–534.

Asherson, R.A., Cervera, R., Piette, J.C., Font, J., Lie, J.T., Burcoglu, A., Lim, K., Munoz-Rodriguez, F.J., Levy, R.A., Boue, F., Rossert, J., and Ingelmo, M. (1998). Catastrophic antiphospholipid syndrome. Clinical and laboratory features of 50 patients. *Medicine 77*(3), 195–207.

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 (Baltimore) 80*(6), 355–377.

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.

Asherson, R.A., Khamashta, M.A., Gil, A., Vazquez, J.J., Chan, O., Baguley, E., and Hughes, G.R. (1989a). Cerebrovascular disease and antiphospholipid antibodies in systemic lupus erythematosus, lupus-like disease, and the primary antiphospholipid syndrome. *Am. J. Med. 86,* 391–399.

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. (1989b). The "primary" antiphospholipid syndrome:

major clinical and serological features. *Medicine (Baltimore). 68,* 366–374.

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*(9), 1982–1993.

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*(5), 800–807.

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 (Oxford) 38*(8), 721–723.

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*(8), 1369–1374.

Bertolaccini, M.L., Atsumi, T., Caliz, A.R., Amengual, O., Khamashta, M.A., Hughes, G.R., and Koike, T. (2000). Association of antiphosphatidylserine/prothrombin autoantibodies with HLA class II genes. *Arthritis Rheum. 43*(3), 683–688.

Bertolaccini, M.L., Roch, B., Amengual, O., Atsumi, T., Khamashta, M.A., and Hughes, G.R. (1998). Multiple antiphospholipid tests do not increase the diagnostic yield in antiphospholipid syndrome. *Br. J. Rheumatol. 37*(11), 1229–1232.

Bertolaccini, M.L., Sanna, G., Ralhan, S., Gennari, L.C., Merrill, J.T., Khamashta, M.A., and Hughes, G.R. (2003). Antibodies directed to protein S in patients with systemic lupus erythematosus: prevalence and clinical significance. *Thromb. Haemost. 90*(4), 636–641.

Bevers, E.M., Galli, M., Barbui, T., Comfurius, P., and Zwaal, R.F.A. (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.

Bevers, E.M., Janssen, M.P., Willems, G.M., and Zwaal, F.A. (2000). No evidence for enhanced thrombin formation through displacement of annexin V by antiphospholipid antibodies [letter] [In Process Citation]. *Thromb. Haemost. 83*(5), 792–794.

Blank, M., Cohen, J., Toder, V., and Shoenfeld, Y. (1991). Induction of antiphospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti-cardiolipin antibodies. *Proc. Natl. Acad. Sci. U.S.A. 88,* 3069–3073.

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(4), 441–455.

Boey, M.L., Colaco, C.B., Gharavi, A.E., Elkon, K.B., Loizou, S., and Hughes, G.R.V. (1983). Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. *Br. Med. J. 287,* 1021–1023.

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*(8), 1375–1379.

Bowie, E.J.W., Thompson, J.H., Jr., Pascuzzi, C.A., and Owen, C.A., Jr. (1963). Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. *J. Lab. Clin. Med. 62,* 416–430.

Branch, D.W. (1994). Thoughts on the mechanism of pregnancy loss associated with the antiphospholipid syndrome. *Lupus 3,* 275–280.

Branch, D.W., Dudley, D.J., and Mitchell, M.D. (1990). IgG fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss. *Am. J. Obstet. Gynecol. 163,* 210–216.

Branch, D.W. and Khamashta, M.A. (2003). Antiphospholipid syndrome: obstetric diagnosis, management, and controversies. *Obstet. Gynecol. 101*(6), 1333–1344.

Branch, D.W., Peaceman, A.M., Druzin, M., Silver, R.K., El-Sayed, Y., Silver, R.M., Esplin, M.S., Spinnato, J., and Harger, J. (2000). A multicenter, placebo-controlled pilot study of intravenous immune globulin treatment of antiphospholipid syndrome during pregnancy. The Pregnancy Loss Study Group. *Am. J. Obstet. Gynecol. 182*(1 Pt 1), 122–127.

Brandt, J.T., Barna, L.K., and Triplett, D.A. (1995a). Laboratory identification of lupus anticoagulants: results of the Second International Workshop for Identification of Lupus Anticoagulants. On behalf of the Subcommittee on Lupus Anticoagulants/Antiphospholipid Antibodies of the ISTH. *Thromb. Haemost. 74*(6), 1597–1603.

Brandt, J.T., Triplett, D.A., Alving, B., and Scharrer, I. (1995b). Criteria for the diagnosis of lupus anticoagulants: an update. *Thromb. Haemost. 74*(4), 1185–1190.

Brey, R.L., Gharavi, A.E., and Lockshin, M.D. (1993). Neurologic complications of antiphospholipid antibodies. *Rheum. Dis. Clin. N. Am. 19,* 833–850.

Bulckaen, H.G., Puisieux, F.L., Bulckaen, E.D., Di Pompeo, C., Bouillanne, O.M., Watel, A. A., Fauchais, A.L., De Groote, P., and Millaire, A. (2003). Antiphospholipid antibodies and the risk of thromboembolic events in valvular heart disease. *Mayo Clin. Proc. 78*(3), 294–298.

Burcoglu-O'Ral, A., Erkan, D., and Asherson, R. (2002). Treatment of catastrophic antiphospholipid syndrome with defibrotide, a proposed vascular endothelial cell modulator. *J. Rheumatol. 29*(9), 2006–2011.

Caliz, R., Atsumi, T., Kondeatis, E., Amengual, O., Khamashta, M.A., Vaughan, R.W., Lanchbury, J.S., and Hughes, G.R. (2001). HLA class II gene polymorphisms in antiphospholipid syndrome: haplotype analysis in 83 Caucasoid patients. *Rheumatology (Oxford) 40*(1), 31–36.

Camilleri, R.S., Mackie, I.J., Humphries, S.E., Machin, S.J., and Cohen, H. (2003). Lack of association of beta2-glycoprotein I polymorphisms Val247Leu and Trp316Ser with antiphospholipid antibodies in patients with thrombosis and pregnancy complications. *British J. Haematol. 120*(6), 1066–1072.

Cariou, R., Tobelem, G., Bellucci, S., Soria, J., Soria, C., Maclouf, J., and Caen, J. (1988). Effect of lupus anticoagulant on antithrombogenic properties of endothelial cells—inhibition of thrombomodulin-dependent protein C activation. *Thromb. Haemost. 60,* 54–58.

Carreras, L.O., DeFreyn, G., Machin, S.J., Vermylen, J., Deman, R., Spitz, B., and Van Assche, A. (1981). Arterial thrombosis, intrauterine death and "lupus" anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. *Lancet* i, 244–246.

Carreras, L.O., Martinuzzo, M.E., and Maclouf, J. (1996). Antiphospholipid antibodies, eicosanoids and expression of endothelial cyclooxygenase-2. *Lupus 5*(5), 494–497.

Cervera, R., Asherson, R.A., Font, J., Tikly, M., Pallarés, 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*(3), 203–212.

Chapman, J., Rand, J.H., Brey, R.L., Levine, S.R., Blatt, I., Khamashta, M.A., and Shoenfeld, Y. (2003). Non-stroke neurological syndromes associated with antiphospholipid antibodies: evaluation of clinical and experimental studies. *Lupus 12*(7), 514–517.

Clark, A.L., Branch, D.W., Silver, R.M., Harris, E.N., Pierangeli, S., and Spinnato, J.A. (1999). Pregnancy complicated by the antiphospholipid syndrome: outcomes with intravenous immunoglobulin therapy. *Obstet. Gynecol. 93*(3), 437–441.

Comp, P.C., DeBault, L.E., Esmon, N.L., and Esmon, C.T. (1983). Human thrombomodulin is inhibited by IgG from two patients with nonspecific anticoagulants [abstract]. *Blood 62* (Suppl. 1), 299a.

Conley, C.L., and Hartmann, R.C. (1952). A hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J.Clin.Invest. 31,* 621–622.

Cooper, G.S., Parks, C.G., Treadwell, E.L., St Clair, E.W., Gilkeson, G.S., Cohen, P.L., Roubey, R.A., and Dooley, M.A. (2002). Differences by race, sex and age in the clinical and immunologic features of recently diagnosed systemic lupus erythematosus patients in the southeastern United States. *Lupus 11*(3), 161–167.

Coull, B.M., Bourdette, D.N., Goodnight, S.H.J., Briley, D.P., and Hart, R. (1987). Multiple cerebral infarctions and dementia associated with anticardiolipin antibodies. *Stroke 18,* 1107–1112.

Cowchock, F.S., Reece, E.A., Balaban, D., Branch, D.W., and Plouffe, L. (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.

Crowther, M.A., Ginsberg, J.S., Julian, J., Denburg, J., Hirsh, J., Douketis, J., Laskin, C., Fortin, P., Anderson, D., Kearon, C., Clarke, A., Geerts, W., Forgie, M., Green, D., Costantini, L., Yacura, W., Wilson, S., Gent, M., and Kovacs, M.J. (2003). A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. *N. Engl. J. Med. 349*(12), 1133–1138.

Cuadrado, M.J., López-Pedrera, C., Khamashta, M.A., Camps, M.T., Tinahones, F., Torres, A., Hughes, G.R.V., and Velasco, F. (1997). Thrombosis in primary antiphospholipid syndrome: a pivotal role for monocyte tissue factor expression. *Arthritis Rheum. 40*(5), 834–841.

Del Papa, N., Guidali, L., Spatola, L., Bonara, P., Borghi, M.O., Tincani, A., Balestrieri, G., and Meroni, P.L. (1995). Relationship between antiphospholipid and anti-endothelial cell antibodies III: β_2-glycoprotein I mediates the antibody binding to endothelial membranes and induces the expression of adhesion molecules. *Clin. Exp. Rheumatol. 13,* 179–185.

Della Valle, P., Crippa, L., Safa, O., Tomassini, L., Pattarini, E., Vigano'D'Angelo, S., Sabbadini, M.G., and D'Angelo, A. (1996). Potential failure of the international normalized ratio (INR) system in the monitoring of oral anticoagulation in patients with lupus anticoagulants. *Ann. Med. Interne 147* (Suppl 1), 10–14.

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*(4), 377–386.

Diz-Kucukkaya, R., Hacihanefioglu, A., Yenerel, M., Turgut, M., Keskin, H., Nalcaci, M., and Inanc, M. (2001). Antiphospholipid antibodies and antiphospholipid syndrome in patients presenting with immune thrombocytopenic purpura: a prospective cohort study. *Blood* 98(6), 1760–1764.

Dobado-Berrios, P.M., López-Pedrera, C., Velasco, F., Aguirre, M.A., Torres, A., and Cuadrado, M.J. (1999). Increased levels of tissue factor mRNA in mononuclear blood cells of patients with primary antiphospholipid syndrome. *Thromb. Haemost. 82,* 1578–1582.

Domenico, S.G., Minisola, G., and Galeazzi, M. (2003). HLA class II alleles and genetic predisposition to the antiphospholipid syndrome. *Autoimmun. Rev.* 2(6), 387–394.

Donohoe, S., Kingdom, J.C., Mackie, I.J., Burrell, S., Quenby, S., Jauniaux, E., and Machin, S.J. (2000). Ontogeny of beta 2 glycoprotein I and annexin V in villous placenta of normal and antiphospholipid syndrome pregnancies. *Thromb. Haemost. 84*(1), 32–38.

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.

Erkan, D., Asherson, R.A., Espinosa, G., Cervera, R., Font, J., Piette, J.C., and Lockshin, M.D. (2003). Long term outcome of catastrophic antiphospholipid syndrome survivors. *Ann. Rheum. Dis. 62*(6), 530–533.

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(6), 1466–1467.

Erkan, D., Yazici, Y., Peterson, M.G., Sammaritano, L., and Lockshin, M.D. (2002). A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. *Rheumatology 41*(8), 924–929.

Espinola, R.G., Liu, X., Colden-Stanfield, M., Hall, J., Harris, E.N., and Pierangeli, S.S. (2003). E-Selectin mediates pathogenic effects of antiphospholipid antibodies. *J. Thromb. Haemost. 1*(4), 843–848.

Exner, T., and Koutts, J. (1988). Autoimmune cardiolipin-binding antibodies in oral anticoagulant patients. *Aust. N.Z. J. Med. 18,* 669–673.

Feinstein, D.I., and Rapaport, S.I. (1972). Acquired inhibitors of blood coagulation. *Prog. Hemost. Thromb. 1,* 75–95.

Firkin, B.G., Howard, M.A., and Radford, N. (1980). Possible relationship between lupus inhibitor and recurrent abortion in young women. *Lancet 2,* 366.

Forastiero, R., Martinuzzo, M., Carreras, L.O., and Maclouf, J. (1998). Anti-β_2-glycoprotein I antibodies and platelet activation in patients with antiphospholipid antibodies: association with increased excretion of platelet-derived thromboxane urinary metabolites. *Thromb. Haemost. 79*(1), 42–45.

Forastiero, R.R., Martinuzzo, M.E., and Broze, G.J. (2003). High titers of autoantibodies to tissue factor pathway inhibitor are associated with the antiphospholipid syndrome. *J. Thromb. Haemost. 1*(4), 718–724.

Frances, C., Papo, T., Wechsler, B., Laporte, J.L., Biousse, V., and Piette, J.C. (1999). Sneddon syndrome with or without antiphospholipid antibodies. A comparative study in 46 patients.. *Medicine 78*(4), 209–219.

Frick, P.G. (1955). Acquired circulating anticoagulants in systemic "collagen disease". *Blood 10,* 691–706.

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*(1), 47–55.

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*(3), 486–491.

Galli, M., Comfurius, P., Barbui, T., Zwaal, R.F.A., and Bevers, E.M. (1992). Anticoagulant activity of β_2-glycoprotien I is potentiated by a distinct subgroup of anticardiolipin antibodies. *Thromb. Haemost. 68,* 297–300.

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., and Bevers, E.M. (1990). Anticardiolipin antibodies directed not to cardiolipin but to a plasma protein cofactor. *Lancet 335,* 1544–1547.

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.

Giles, I.P., Isenberg, D.A., Latchman, D.S., and Rahman, A. (2003). How do antiphospholipid antibodies bind beta2-glycoprotein I?. *Arthritis Rheum. 48*(8), 2111–2121.

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.

Girardi, G., Berman, J., Redecha, P., Spruce, L., Thurman, J.M., Kraus, D., Hollmann, T.J., Casali, P., Caroll, M.C., Wetsel, R.A., Lambris, J.D., Holers, V.M., and Salmon, J.E. (2003). Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J. Clin. Invest 112*(11), 1644–1654.

Girardi, G., Redecha, P., and Salmon, J.E. (2004). Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nat. Med. 10*(11), 1222–1226.

Goel, N., Ortel, T.L., Bali, D., Anderson, J., Gourley, I.S., Smith, H., Morris, C., Branch, D.W., Ford, P., Berdeaux, D., Roubey, R.A.S., Kingsmore, S.F., Thiel, T., Amos, C., and Seldin, M.F. (1999). Familial antiphospholipid antibody syndrome: criteria for disease and evidence for autosomal dominant inheritance. *Arthritis Rheum. 42*(2), 318–327.

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*(1), 57–62.

Grattan, C.E., and Burton, J.L. (1991). Antiphospholipid syndrome and cutaneous vasoocclusive disorders. *Semin. Dermatol. 10,* 152–159.

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*(6), 1189–1193.

Harper, M.F., Hayes, P.M., Lentz, B.R., and Roubey, R.A.S. (1998). Characterization of β_2-glycoprotein I binding to phospholipid membranes. *Thromb. Haemost. 80*(4), 610–614.

Harris, E.N. (1990). Special report. The Second International Anticardiolipin Standardization Workshop/the Kingston Anti-Phospholipid Antibody Study (KAPS) group. *Am. J. Clin. Pathol. 94,* 476–484.

Harris, E.N., Asherson, R.A., Gharavi, A.E., Morgan, S.H., Derue, G., and Hughes, G.R.V. (1985). Thrombocytopenia in SLE and related autoimmune disorders: association with anticardiolipin autoantibody. *Br. J. Haematol. 59,* 227–230.

Harris, E.N., Chan, J.K.H., Asherson, R.A., Aber, V.A., Gharavi, A.E., and Hughes, G.R.V. (1986). Thrombosis, recurrent fetal loss, thrombocytopenia: predictive value of IgG anticardiolipin antibodies. *Arch. Intern. Med. 146,* 2153–2156.

Harris, E.N., Gharavi, A.E., Boey, M.L., Patel, B.M., Mackworth-Young, C.G., Loizou, S., and Hughes, G.R.V. (1983). Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet ii,* 1211–1214.

Harris, E.N., Gharavi, A.E., Patel, S.P., and Hughes, G.R.V. (1987). Evaluation of the anti-cardiolipin test: report of an international workshop held 4 April 1986. *Clin. Exp. Immunol. 68,* 215–222.

Hartung, K., Coldewey, R., Corvetta, A., Deicher, H., Kalden, J.R., Krapf, F., Lang, B., Lakomek, H.J., Liedvogel, B., and Peter, H.H. (1992). MHC gene products and anticardiolipin antibodies in systemic lupus erythematosus results of a multicenter study. SLE Study Group. *Autoimmunity 13*(2), 95–99.

Hashimoto, H., Yamanaka, K., Tokano, Y., Iida, N., Takasaki, Y., Kabasawa, K., and Nishimura, Y. (1998). HLA-DRB1 alleles and beta 2 glycoprotein I-dependent anticardiolipin antibodies in Japanese patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol. 16*(4), 423–427.

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 antibody syndrome. *J. Immunol. 149,* 1063–1068.

Hattori, N., Kuwana, M., Kaburaki, J., Mimori, T., Ikeda, Y., and Kawakami, Y. (2000). T cells that are autoreactive to beta2-glycoprotein I in patients with antiphospholipid syndrome and healthy individuals. *Arthritis Rheum. 43*(1), 65–75.

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 β_2-glycoprotein I gene in the generation of anti-β_2-glycoprotein I antibodies in the antiphospholipid syndrome. *Arthritis Rheum. 42*(8), 1655–1661.

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(2), 211–220.

Horbach, D.A., van Oort, E., Derksen, R.H., and De Groot, P.G. (1998). The contribution of anti-prothrombin-antibodies to lupus anticoagulant activity—discrimination between functional and non-functional anti-prothrombin-antibodies. *Thromb. Haemost. 79*(4), 790–795.

Hörkkö, 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 β_2-glycoprotein I (and other proteins). *Proc. Natl. Acad. Sci. U.S.A. 94,* 10356–10361.

Hörkkö, S., Miller, E., Dudl, E., Reaven, P., Curtiss, L.K., Zvaifler, N.J., Terkeltaub, R., Pierangeli, S.S., Branch, D.W., Palinski, W., and Witztum, J.L. (1996). Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. *J. Clin. Invest. 98,* 815–825.

Hughes, G.R.V. (1983). Thrombosis, abortion, cerebral disease and the lupus anticoagulant. *Br. Med. J. 287,* 1088–1089.

Hunt, J.E., Simpson, R.J., and Krilis, S.A. (1993). Identification of a region of β_2-glycoprotein I critical for lipid binding and anti-cardiolipin cofactor activity. *Proc. Natl. Acad. Sci. U.S.A. 90,* 2141–2145.

Hwang, K.K., Yang, C.D., Yan, W., Grossman, J.M., Hahn, B.H., and Chen, P.P. (2003). A thrombin-cross-reactive anticardiolipin antibody binds to and inhibits the anticoagulant function of activated protein C. *Arthritis Rheum. 48*(6), 1622–1630.

Ichikawa, K., Khamashta, M.A., Koike, T., Matsuura, E., and Hughes, G.R.V. (1994). β_2-glycoprotein I reactivity of monoclonal anticardiolipin antibodies from patients with the antiphospholipid syndrome. *Arthritis Rheum. 37*(10), 1453–1461.

Ijdo, J.W., Conti-Kelly, A.M., Greco, P., Abedi, M., Amos, M., Provenzale, J.M., and Greco, T.P. (1999). Anti-phospholipid antibodies in patients with multiple sclerosis and MS-like illnesses: MS or APS?. *Lupus 8*(2), 109–115.

Ikematsu, W., Luan, F.L., La Rosa, L., Beltrami, B., Nicoletti, F., Buyon, J.P., Meroni, P.L., Balestrieri, G., and Casali, P. (1998). Human anticardiolipin monoclonal autoantibodies cause placental necrosis and fetal loss in BALB/c mice. *Arthritis Rheum. 41*(6), 1026–1039.

Ioannidis, J.P., Tektonidou, M.G., Vlachoyiannopoulos, P.G., Stavropoulos-Giokas, C., Spyropoulou-Vlachou, M., Reveille, J.D., Arnett, F.C., and Moutsopoulos, H.M. (1999). HLA associations of anti-beta2 glycoprotein I response in a Greek cohort with antiphospholipid syndrome and meta-analysis of four ethnic groups. *Hum. Immunol. 60*(12), 1274–1280.

Ito, H., Matsushita, S., Tokano, Y., Nishimura, H., Tanaka, Y., Fujisao, S., Mitsuya, H., Hashimoto, H., and Nishimura, Y. (2000). Analysis of T cell responses to the β_2-glycoprotein I-derived peptide library in patients with anti-β_2-glycoprotein I antibody-associated autoimmunity. *Hum. Immunol. 61*(4), 366–377.

Iverson, G.M., Reddel, S., Victoria, E.J., Cockerill, K.A., Wang, Y.X., Marti-Renom, M.A., Sali, A., Marquis, D.M., Krilis, S.A., and Linnik, M.D. (2002). Use of single point mutations in domain I of beta 2-glycoprotein I to determine fine antigenic specificity of antiphospholipid autoantibodies. *J. Immunol. 169*(12), 7097–7103.

Iverson, G.M., Victoria, E.J., and Marquis, D.M. (1998). Anti-β_2-glycoprotein I (β2GPI) autoantibodies recognize an epitope on the first domain of β2GPI. *Proc. Natl. Acad. Sci. U.S.A. 95,* 15542–15546.

Izumi, T., Pound, M.L., Su, Z., Iverson, G.M., and Ortel, T.L. (2002). Anti-beta(2)-glycoprotein I antibody-mediated inhibition of activated protein C requires binding of beta(2)-glycoprotein I to phospholipids. *Thromb. Haemost. 88*(4), 620–626.

Johansson, E.A., Niemi, K.M., and Mustakallio, K.K. (1977). A peripheral vascular syndrome overlapping with systemic lupus erythematosus. *Dermatologica 155,* 257–267.

Jones, D.W., Mackie, I.J., Gallimore, M.J., and Winter, M. (2001). Antibodies to factor XII and recurrent fetal loss in patients with the antiphospholipid syndrome. *Br. J. Haematol. 113*(2), 550–552.

Jouhikainen, T., Kekomaki, R., Leirisalo Repo, M., Backlund, T., and Myllyla, G. (1990). Platelet autoantibodies detected by immunoblotting in systemic lupus erythematosus: association with the lupus anticoagulant, and with history of thrombosis and thrombocytopenia. *Eur. J. Haematol. 44,* 234–239.

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 (β_2-glycoprotein I) affects the occurrence of antiphospholipid antibodies and apolipoprotein H concentrations in systemic lupus erythematosus. *Lupus 8*(9), 742–750.

Karmochkine, M., Cacoub, P., Piette, J.C., Godeau, P., and Boffa, M.C. (1992). Antiphosphatidylethanolamine antibody as the sole antiphospholipid antibody in systemic lupus erythematosus with thrombosis. *Clin. Exp. Rheumatol. 10*(6), 603–605.

Keeling, D.M., Wilson, A.J.G., Mackie, I.J., Isenberg, D.A., and Machin, S.J. (1993). Lupus anticoagulant activity of some antiphospholipid antibodies against phospholipid-bound β_2 glycoprotein I. *J. Clin. Pathol. 46,* 665–667.

Khamashta, M.A., Cervera, R., Asherson, R.A., Font, J., Gil, A., Coltart, D.J., Vázquez, J.J., Paré, C., Ingelmo, M., Oliver, J., and Hughes, G.R.V. (1990). Association of antibodies against phospholipids with heart valve disease in systemic lupus erythematosus. *Lancet 335,* 1541–1544.

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.

Killeen, A.A., Meyer, K.C., Vogt, J.M., and Edson, J.R. (1987). Kallikrein inhibition and C_1-esterase inhibitor levels in patients with lupus inhibitor. *Am. J. Clin. Pathol. 88,* 223–228.

Kittner, S.J. and Gorelick, P.B. (1992). Antiphospholipid antibodies and stroke: an epidemiological perspective. *Stroke 23* (Suppl. 1), 19–22.

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*(5), 697–709.

Kolev, K., Gombas, J., Varadi, B., Skopal, J., Mede, K., Pitlik, E., Nagy, Z., and Machovich, R. (2002). Immunoglobulin G from patients with antiphospholipid syndrome impairs the fibrin dissolution with plasmin. *Thromb. Haemost. 87*(3), 502–508.

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,* 601–612.

Kutteh, W.H. (1996). Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *American J. Obstet. Gynecol. 174*(5), 1584–1589.

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*(4), 402–407.

Kuwana, M. (2004). Beta2-glycoprotein I: antiphospholipid syndrome and T-cell reactivity. *Thromb. Res. 114*(5–6), 347–355.

Kuwana, M., Matsuura, E., Kobayashi, K., Okazaki, Y., Kaburaki, J., Ikeda, Y., and Kawakami, Y. (2004). Binding of β2-Glycoprotein I to anionic phospholipids facilitates processing and presentation of a cryptic epitope that activates pathogenic autoreactive T cells. *Blood 105*(4), 1552–1557.

Lakasing, L., Campa, J.S., Poston, R., Khamashta, M.A., and Poston, L. (1999). Normal expression of tissue factor, thrombomodulin, and annexin V in placentas from women with antiphospholipid syndrome. *American Journal of Obstetrics and Gynecology* 181(1), 180–189.

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*(4), 924–929.

Laurell, A.-B., and Nilsson, I.M. (1957). Hypergammaglobulinemia, circulating anticoagulant, and biologic false positive Wassermann reaction. *J. Lab. Cli. Med. 49,* 694–707.

Lavalle, C., Pizarro, S., Drenkard, C., Sanchez-Guerrero, J., and Alarcón-Segovia, D. (1990). Transverse myelitis: a manifestation of systemic lupus erythematosus strongly associated with antiphospholipid antibodies. *J. Rheumatol. 17,* 34–37.

Lechner, K. (1974). Acquired inhibitors in nonhemophilic patients. *Haemostasis 3,* 65–93.

Lee, N.S., Brewer, H.B. Jr., and Osborne, J.C. Jr. (1983). Beta-2-glycoprotein I: molecular properties of an unusual apolipoprotein, apolipoprotein H. *J. Biol. Chem. 258,* 4765–4770.

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.

Levine, J.S., Subang, R., Koh, J. S., and Rauch, J. (1998). Induction of antiphospholipid autoantibodies by β_2-glycoprotein I bound to apoptotic thymocytes. *J. Autoimmun. 11*(5), 413–424.

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.

Locatelli, A., Patane, L., Ghidini, A., Marinetti, E., Zagarella, A., Pezzullo, J.C., and Cappellini, A. (2002). Pathology findings in preterm placentas of women with autoantibodies: a case-control study. *J. Matern. Fetal Neonatal Med. 11*(5), 339–344.

Loeliger, A. (1959). Prothrombin as co-factor of the circulating anticoagulant in systemic lupus erythematosus?. *Thromb. Diath. Haemorrh. 3,* 237–256.

Loizou, S., McCrea, J.D., Rudge, A.C., Reynolds, R., Boyle, C.C., and Harris, E.N. (1985). Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. *Clin. Exp. Immunol. 62,* 738–745.

Lutters, B.C., Derksen, R.H., Tekelenburg, W.L., Lenting, P.J., Arnout, J., and De Groot, P.G. (2003). Dimers of beta 2-glycoprotein I increase platelet deposition to collagen via interaction with phospholipids and the apolipoprotein E receptor 2′. *J. Biol. Chem. 278*(36), 33831–33838.

Lutters, B.C., Meijers, J.C., Derksen, R.H., Arnout, J., and De Groot, P.G. (2001). Dimers of beta 2-glycoprotein I mimic the *in vitro* effects of beta 2-glycoprotein I-anti-beta 2-glycoprotein I antibody complexes. *J. Biol. Chem. 276*(5), 3060–3067.

Ma, K., Simantov, R., Zhang, J.-C., Silverstein, R., Hajjar, K.A., and McCrae, K.R. (2000). High affinity binding of β_2-glycoprotein I to human endothelial cells is mediated by annexin II. *J. Biol. Chem. 275*(20), 15541–15548.

Macchi, L., Rispal, P., Clofent-Sanchez, G., Pellegrin, J.L., Nurden, P., Leng, B., and Nurden, A.T. (1997). Anti-platelet antibodies in patients with systemic lupus erythematosus and the primary antiphospholipid

antibody syndrome: their relationship with the observed thrombocytopenia. *Br. J. Haematol. 98*(2), 336–341.

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*(1), 226–234.

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,* 101–107.

Marciniak, E. and Romond, E.H. (1989). Impaired catalytic function of activated protein C: a new *in vitro* manifestation of lupus anticoagulant. *Blood 74,* 2426–2432.

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,* 145–202.

Martinuzzo, M.E., Maclouf, J., Carreras, L.O., and Lévy-Toledano, S. (1993). Antiphospholipid antibodies enhance thrombin-induced platelet activation and thromboxane formation. *Thromb. Haemost. 70,* 667–671.

Matsuda, J., Gotoh, M., Saitoh, N., Gohchi, K., Tsukamoto, M., and Yamamoto, T. (1994). Anti-annexin antibody in the sera of patients with habitual fetal loss or preeclampsia. *Thromb. Res. 75,* 105–106.

Matsuura, E., Igarashi, Y., Fujimoto, M., Ichikawa, K., and Koike, T. (1990). Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. *Lancet 336,* 177–178.

Matsuura, E., Igarashi, Y., Fujimoto, M., Ichikawa, K., Suzuki, T., Sumida, T., Yasuda, T., and Koike, T. (1992). Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. *J. Immunol.* 148, 3885–3891.

Matsuura, E., Igarashi, Y., Yasuda, T., Koike, T., and Triplett, D.A. (1994). Anticardiolipin antibodies recognize β_2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J. Exp. Med. 179,* 457–462.

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 beta(2)-glycoprotein I: reduction of antigenicity and the structural relationship. *Intl Immunol. 12*(8), 1183–1192.

McIntyre, J.A. and Wagenknecht, D.R. (2000). Anti-phosphatidylethanolamine (aPE) antibodies: a survey. *J. Autoimmun. 15*(2), 185–193.

McLaurin, E.Y., Holliday, S.L., Williams, P., and Brey, R.L. (2005). Predictors of cognitive dysfunction in patients with systemic lupus erythematosus. *Neurology 64*(2), 297–303.

McNally, T., Purdy, G., Mackie, I.J., Machin, S.J., and Isenberg, D.A. (1995). The use of an anti-β_2-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. *Br. J. Haematol. 91,* 471–473.

McNeil, H.P., Chesterman, C.N., and Krilis, S.A. (1991). Immunology and clinical importance of antiphospholipid antibodies. *Adv. Immunol. 49,* 193–280.

McNeil, H.P., Simpson, R.J., Chesterman, C.N., and Krilis, S.A. (1990). Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: β_2-glycoprotein I (apolipoprotein H). *Proc. Natl. Acad. Sci. U.S.A. 87,* 4120–4124.

Mehdi, H., Manzi, S., Desai, P., Chen, Q., Nestlerode, C., Bontempo, F., Strom, S.C., Zarnegar, R., and Kamboh, M.I. (2003). A functional polymorphism at the transcriptional initiation site in beta2-glycoprotein I (apolipoprotein H) associated with reduced gene expression and lower plasma levels of beta2-glycoprotein I. *Eur. J. Biochem. 270*(2), 230–238.

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*(4), 735–741.

Moll, S., and Ortel, T.L. (1997). Monitoring warfarin therapy in patients with lupus anticoagulants. *Ann. Intern. Med. 127*(3), 177–185.

Monestier, M., Kandiah, D.A., Kouts, S., Novick, K.E., Ong, G.L., Radic, M.Z., and Krilis, S.A. (1996). Monoclonal antibodies from NZW × BXSB F_1 mice to β_2-glycoprotein I and cardiolipin. *J. Immunol. 156,* 2631–2641.

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,* 297–316.

Moore, J.E., and Mohr, C.F. (1952). Biologically false positive serologic tests for syphilis. *JAMA 150,* 467–473.

Naldi, L., Locati, F., Marchesi, L., Cortelazzo, S., Finazzi, G., Galli, M., Brevi, A., Cainelli, T., and Barbui, T. (1993). Cutaneous manifestations associated with antiphospholipid antibodies in patients with suspected primary antiphospholipid syndrome: a case-control study. *Ann. Rheum. Dis. 52,* 219–222.

Neuwelt, C.M., Daikh, D.I., Linfoot, J.A., Pfister, D.A., Young, R.G., Webb, R.L., London, S.S., and Asherson, R.A. (1997). Catastrophic antiphospholipid syndrome: response to repeated plasmapheresis over three years [see comments]. *Arthritis Rheum. 40*(8), 1534–1539.

Nilsson, I.M., Åstedt, B., Hedner, U., and Berezin, D. (1975). Intrauterine death and circulating anticoagulant ("antithromboplastin"). *Acta. Med. Scand. 197,* 153–159.

Nimpf, J., Bevers, E.M., Bomans, P.H.H., Till, U., Wurm, H., Kostner, G.M., and Zwaal, R.F. A. (1986). Prothrombinase activity of human platelets is inhibited by β_2-glycoprotein I. *Biochim. Biophys. Acta 884,* 142–149.

Nimpf, J., Wurm, H., and Kostner, G.M. (1987). β_2-glycoprotein-I (apo H) inhibits the release reaction of human platelets during ADP-induced aggregation. *Atherosclerosis 63,* 109–114.

Nochy, D., Daugas, E., Droz, D., Beaufils, H., Grunfeld, J.P., Piette, J.C., Bariety, and Hill, G. (1999). The intrarenal vascular lesions associated with primary antiphospholipid syndrome. *J. Am. So. Nephrol. 10*(3), 507–518.

Nojima, J., Kuratsune, H., Suehisa, E., Futsukaichi, Y., Yamanishi, H., Machii, T., Iwatani, Y., and Kanakura, Y. (2001). Association between the prevalence of antibodies to beta(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*(6), 1008–1015.

Nojima, J., Kuratsune, H., Suehisa, E., Kawasaki, T., Machii, T., Kitani, T., Iwatani, Y., and Kanakura, Y. (2002). Acquired activated protein C resistance associated with anti-protein S antibody as a strong risk factor for DVT in non-SLE patients. *Thromb. Haemost. 88*(5), 716–722.

Ogishima, D., Matsumoto, T., Nakamura, Y., Yoshida, K., and Kuwabara, Y. (2000). Placental pathology in systemic lupus erythematosus with antiphospholipid antibodies. *Pathol. Int. 50*(3), 224–229.

Oosting, J.D., Derksen, R.H.W.M., Blokzijl, L., Sixma, J.J., and De Groot, P.G. (1992a). Antiphospholipid antibody positive sera enhance endothelial cell procoagulant activity—studies in a thrombosis model. *Thrombosis Haemost. 68,* 278–284.

Oosting, J.D., Derksen, R.H.W.M., Entjes, H.T.I., Bouma, B.N., and De Groot, P.G. (1992b). Lupus anticoagulant activity is frequently dependent on the presence of β_2-glycoprotein I. *Thromb. Haemost. 67,* 499–502.

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,* 2618–2625.

Ortel, T.L. (1999). Clinical and laboratory manifestations of anti-factor V antibodies. *J. Lab. Clin. Med. 133*(4), 326–334.

Paira, S., Roverano, S., Zunino, A., Oliva, M.E., and Bertolaccini, M.L. (1999). Extensive cutaneous necrosis associated with anticardiolipin antibodies. *J. Rheumatol. 26*(5), 1197–2000.

Pangborn, M.C. (1941). A new serologically active phospholipid from beef heart. *Proc. Soc. Exp. Biol. Med. 48,* 484–486.

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*(2), 342–345.

Pattison, N.S., Chamley, L.W., Birdsall, M., Zanderigo, A.M., Liddell, H.S., and McDougall, J. (2000). Does aspirin have a role in improving pregnancy outcome for women with the antiphospholipid syndrome? A randomized controlled trial. *Am. J. Obstet. Gynecol. 183*(4), 1008–1012.

Pelegri, Y., Cerrato, G., Martinuzzo, M.E., Carreras, L.O., and Forastiero, R.R. (2003). Link between anti-CD36 antibodies and thrombosis in the antiphospholipid syndrome. *Clin. Exp. Rheumatol. 21*(2), 221–224.

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*(5), 721–724.

Pengo, V., Biasiolo, A., and Fior, M.G. (1995). Autoimmune antiphospholipid antibodies are directed against a cryptic epitope expressed when β2-glycoprotein I is bound to a suitable surface. *Thromb. Haemost. 73*(1), 29–34.

Petri, M. (1994). Diagnosis of antiphospholipid antibodies. *Rheum. Dis. Clin. N. Am. 20,* 443–469.

Petri, M. (2000). Epidemiology of the antiphospholipid antibody syndrome. *J. Autoimmun. 15*(2), 145–151.

Petri, M., Hellman, D., Hochberg, M., Meyerhoff, J., Bell, W., and Goldman, D. (1994). Arterial thrombotic events in SLE: The Baltimore Lupus Cohort Study [abstract]. *Arthritis Rheum. 37,* S297.

Pierangeli, S.S., Barker, J.H., Stikovac, D., Ackerman, D., Anderson, G., Barquinero, J., Acland, R., and Harris, E.N. (1994). Effect of human IgG antiphospholipid antibodies on an *in vivo* thrombosis model in mice. *Thromb. Haemost. 71*(5), 670–674.

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*(2), 245–250.

Prieto, G.A., Cabral, A.R., Zapata-Zuniga, M., Simon, A.J., Villa, A.R., Alarcon-Segovia, D., and Cabiedes, J. (2003). Valine/valine genotype at position 247 of the beta2-glycoprotein I gene in Mexican patients with primary antiphospholipid syndrome: association with anti-beta2-glycoprotein I antibodies. *Arthritis Rheum. 48*(2), 471–474.

Radway-Bright, E.L., Inanc, M., and Isenberg, D.A. (1999). Animal models of the antiphospholipid syndrome. *Rheumatology (Oxford) 38*(7), 591–601.

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. *Br. Med. J. 314*(7076), 253–257.

Rand, J.H. (2002). Molecular pathogenesis of the antiphospholipid syndrome. *Circ. Res. 90*(1), 29–37.

Reber, G., Arvieux, J., Comby, E., Degenne, D., De Moerloose, P., Sanmarco, M., and Potron, G. (1995). Multicenter evaluation of nine commercial kits for the quantitation of anticardiolipin antibodies. The Working Group on Methodologies in Haemostasis from the GEHT (Groupe d'Etudes sur l'Hemostase et la Thrombose). *Thromb. Haemost. 73*(3), 444–452.

Reid, K.B.M., Bentley, D.R., Campbell, R.D., Chung, L.P., Sim, R.B., Kristensen, T., and Tack, B.F. (1986). Complement system proteins which interact with C3b or C4b: a superfamily of structurally related proteins. *Immunol. Today 7,* 230–234.

Riboldi, P., Gerosa, M., Raschi, E., Testoni, C., and Meroni, P.L. (2003). Endothelium as a target for antiphospholipid antibodies. *Immunobiology 207*(1), 29–36.

Rock, G., Chauhan, K., Jamieson, G.A., and Tandon, N.N. (1994). Anti-CD36 antibodies in patients with lupus anticoagulant and thrombotic complications. *Br. J. Haematol. 88,* 878–880.

Rosove, M.H., and Brewer, P.M.C. (1992). Antiphospholipid thrombosis: clinical course after the first thrombotic event in 70 patients. *Ann. Intern. Med. 117,* 303–308.

Roubey, R.A.S., Eisenberg, R.A., Harper, M.F., and Winfield, J.B. (1995). "Anticardiolipin" autoantibodies recognize β_2-glycoprotein I in the absence of phospholipid. Importance of antigen density and bivalent binding. *J. Immunol. 154,* 954–960.

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 β_2-glycoprotein I. *J. Clin. Invest. 90,* 1100–1104.

Ruiz-Argüelles, G.J., Ruiz-Argüelles, A., Alarcón-Segovia, D., Drenkard, C., Villa, A., Cabiedes, J., Presno-Bernal, M., Deleze, M., Ortiz-Lopez, R., and Vazquez-Prado, J. (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. Rheum. 18,* 552–558.

Ruiz-Irastorza, G., Khamashta, M.A., Hunt, B.J., Escudero, A., Cuadrado, M.J., and Hughes, G.R. (2002). Bleeding and recurrent thrombosis in definite antiphospholipid syndrome: analysis of a series of 66 patients treated with oral anticoagulation to a target international normalized ratio of 3.5. *Arch. Intern. Med. 162*(10), 1164–1169.

Salmon, J.E., Girardi, G., and Holers, V.M. (2003). Activation of complement mediates antiphospholipid antibody-induced pregnancy loss. *Lupus 12*(7), 535–538.

Sanchez, M.L., Katsumata, K., Atsumi, T., Romero, F.I., Bertolaccini, M.L., Funke, A., Amengual, O., Kondeatis, E., Vaughan, R.W., Cox, A., Hughes, G.R., and Khamashta, M.A. (2004). Association of HLA-DM polymorphism with the production of antiphospholipid antibodies. *Ann. Rheum. Dis. 63*(12), 1645–1648.

Sanfelippo, M.J., and Drayna, C.J. (1982). Prekallikrein inhibition associated with the lupus anticoagulant: a mechanism of thrombosis. *Am. J. Clin. Pathol. 77,* 275–279.

Sanghera, D.K., Wagenknecht, D.R., McIntyre, J.A., and Kamboh, M.I. (1997). Identification of structural mutations in the fifth domain of apolipoprotein H (beta 2-glycoprotein I) which affect phospholipid binding. *Hum. Mol. Genet. 6*(2), 311–316.

Schousboe, I. (1985). β_2-glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. *Blood 66,* 1086–1091.

Scott, J.R., Branch, D.W., Kochenour, N.K., and Ward, K. (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.

Sebastiani, G.D., Galeazzi, M., Tincani, A., Scorza, R., Mathieu, A., Passiu, G., Morozzi, G., Piette, J.C., Cervera, R., Houssiau, F., Smolen, J., Fernandez, N.A., de Ramon, E., Goral, A.J., Papasteriades, C., Ferrara, G.B., Carcassi, C., Bellisai, F., and Marcolongo, R. (2003). HLA-DPB1 alleles association of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. *Lupus 12*(7), 560–563.

Sebire, N.J., Backos, M., El Gaddal, S., Goldin, R.D., and Regan, L. (2003). Placental pathology, antiphospholipid antibodies, and pregnancy outcome in recurrent miscarriage patients. *Obstet. Gynecol. 101*(2), 258–263.

Sebire, N.J., Regan, L., and Rai, R. (2002). Biology and pathology of the placenta in relation to antiphospholipid antibody-associated pregnancy failure. *Lupus 11*(10), 641–643.

Sharkey, M.P., Daryanani, I.I., Gillett, M.B., and Jones, S.K. (2002). Localized cutaneous necrosis associated with the antiphospholipid syndrome. *Australas. J. Dermatol. 43*(3), 218–220.

Shi, T., Iverson, G.M., Qi, J.C., Cockerill, K.A., Linnik, M.D., Konecny, P., and Krilis, S.A. (2004). Beta 2-Glycoprotein I binds factor XI and inhibits its activation by thrombin and factor XIIa: loss of inhibition by clipped beta 2-glycoprotein I. *Proc. Natl. Acad. Sci. U.S.A 101*(11), 3939–3944.

Shi, W., Chong, B.H., and Chesterman, C.N. (1993). β_2-Glycoprotein I is a requirement for anticardiolipin antibodies binding to activated platelets: differences with lupus anticoagulants. *Blood 81,* 1255–1262.

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.

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 antiprothrombin antibodies and prothrombin cause lupus anticoagulant activity by competing with the binding of clotting factors for catalytic phospholipid surfaces. *Br. J. Haematol. 113*(3), 621–629.

Smith, H.R., Hansen, C.L., Rose, R., and Canoso, R.T. (1990). Autoimmune MRL-lpr/lpr mice are an animal model for the secondary APS. *J. Rheumatol. 17,* 911–915.

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*(3), 557–560.

Soulier, J.P. and Boffa, M.C. (1980). Avortements à répétition, thrombose et anticoagulant circulant anti-thromboplastine. *Nouv. Presse. Med. 9,* 859–864.

Spinnato, J.A., Clark, A.L., Pierangeli, S.S., and Harris, E.N. (1995). Intravenous immunoglobulin therapy for the antiphospholipid syndrome in pregnancy. *Am. J. Obstet. Gynecol. 172,* 690–694.

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 β_2-glycoprotein I. *FEBS Lett. 313,* 193–197.

Sugi, T., and McIntyre, J.A. (1995). Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen-PE complex. *Blood 86*(8), 3083–3089.

Sugi, T., and McIntyre, J.A. (2001). Certain autoantibodies to phosphatidylethanolamine (aPE) recognize factor XI and prekallikrein independently or in addition to the kininogens. *J. Autoimmun. 17*(3), 207–214.

Takeuchi, R., Atsumi, T., Ieko, M., Amasaki, Y., Ichikawa, K., and Koike, T. (2002). Suppressed intrinsic fibrinolytic activity by monoclonal anti-beta-2 glycoprotein I autoantibodies: possible mechanism for thrombosis in patients with antiphospholipid syndrome. *Br. J. Haematol. 119*(3), 781–788.

Takeuchi, R., Atsumi, T., Ieko, M., Takeya, H., Yasuda, S., Ichikawa, K., Tsutsumi, A., Suzuki, K., and Koike, T. (2000). Coagulation and fibrinolytic activities in 2 siblings with β_2-glycoprotein I deficiency. *Blood 96*(4), 1594–1595.

Thurman, J.M., Kraus, D.M., Girardi, G., Hourcade, D., Kang, H.J., Royer, P.A., Mitchell, L.M., Giclas, P.C., Salmon, J., Gilkeson, G., and Holers, V.M. (2005). A novel inhibitor of the alternative complement pathway prevents antiphospholipid antibody-induced pregnancy loss in mice. *Mol. Immunol. 42*(1), 87–97.

Tietjen, G.E., Levine, S.R., Brown, E., Mascha, E., and Welch, K.M. (1993). Factors that predict antiphospholipid immunoreactivity in young people with transient focal neurological events. *Arch. Neurol. 50,* 833–836.

Tincani, A., Allegri, F., Sanmarco, M., Cinquini, M., Taglietti, M., Balestrieri, G., Koike, T., Ichikawa, K., Meroni, P., and Boffa, M.C. (2001). Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine determinations—a cooperative project of the European Antiphospholipid Forum. *Thromb. Haemost. 86*(2), 575–583.

Tincani, A., Gilburd, B., Abu-Shakra, M., Blank, M., Allegri, F., Ottaviani, R., Riboni, M., Meroni, P.L., Balestrieri, G., and Shoenfeld, Y. (2002). Immunization of naive BALB/c mice with human beta2-glycoprotein I breaks tolerance to the murine molecule. *Arthritis Rheum. 46*(5), 1399–1404.

Tincani, A., Spatola, L., Prati, E., Allegri, F., Ferremi, P., Cattaneo, R., Meroni, P., and Balestrieri, G. (1996). The anti-2-glycoprotein I activity in human anti-phospholipid syndrome sera is due to monoreactive low-affinity autoantibodies directed to epitopes located on native 2-glycoprotein I and preserved during species evolution. *J. Immunol. 157,* 5732–5738.

Tourbah, A., Clapin, A., Gout, O., Fontaine, B., Liblau, R., Batteux, F., Stievenart, J.L., Weill, B., Lubetzki, C., and Lyon-Caen, O. (1998). Systemic autoimmune features and multiple sclerosis: a 5-year follow-up study. *Arch. Neurol. 55*(4), 517–521.

Triolo, G., Ferrante, A., Ciccia, F., Accardo-Palumbo, A., Perino, A., Castelli, A., Giarratano, A., and Licata, G. (2003). Randomized study of subcutaneous low molecular weight heparin plus aspirin versus intravenous immunoglobulin in the treatment of recurrent fetal loss associated with antiphospholipid antibodies. *Arthritis Rheum. 48*(3), 728–731.

Vianna, J.L., Khamashta, M.A., Ordi-Ros, J., Font, J., Cervera, R., Lopez-Soto, A., Tolosa, C., Franz, J., Selva, A., Ingelmo, M., Vilardell, M., and Hughes, G.R.V. (1994). Comparison of the primary and secondary antiphospholipid syndrome: a European multicenter study of 114 patients. *Am. J. Med. 96,* 3–9.

Visvanathan, S. and McNeil, H.P. (1999). Cellular immunity to β_2-glycoprotein I in patients with the antiphospholipid syndrome. *J. Immunol. 162*(11), 6919–6925.

Wang, X., Campos, B., Kaetzel, M.A., and Dedman, J.R. (1999). Annexin V is critical in the maintenance of murine placental integrity. *Am. J. Obstet. Gynecol. 180*(4), 1008–1016.

Wiener, M.H., Burke, M., Fried, M., and Yust, I. (2001). Thromboagglutination by anticardiolipin antibody complex in the antiphospholipid syndrome: a possible mechanism of immune-mediated thrombosis. *Thromb. Res. 103*(3), 193–199.

Willems, G.M., Janssen, M.P., Comfurius, P., Galli, M., Zwaal, R.F., and Bevers, E.M. (2000). Competition of annexin V and anticardiolipin antibodies for binding to phosphatidylserine containing membranes. *Biochemistry 39*(8), 1982–1989.

Willems, G.M., Janssen, M.P., Pelsers, M.M.A.L., Comfurius, P., Galli, M., Zwaal, R.F.A., and Bevers, E.M. (1996). Role of divalency in the high-affinity binding of anticardiolipin antibody-β_2-glycoprotein I complexes to lipid membranes. *Biochemistry 35,* 13833–13842.

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*(7), 1309–1311.

Wurm, H. (1984). β_2-glycoprotein I (apolipoprotein H) interactions with phospholipid vesicles. *Int. J. Biochem. 16,* 511–515.

Yasuda, S., Atsumi, T., Ieko, M., and Koike, T. (2004). Beta2-glycoprotein I, anti-beta2-glycoprotein I, and fibrinolysis. *Thromb. Res. 114*(5–6), 461–465.

Yasuda, S., Tsutsumi, A., Chiba, H., Yanai, H., Miyoshi, Y., Takeuchi, R., Horita, T., Atsumi, T., Ichikawa, K., Matsuura, E., and Koike, T. (2000). β_2-glycoprotein I deficiency: prevalence, genetic background and effects on plasma lipoprotein metabolism and hemostasis. *Atherosclerosis 152*(2), 337–346.

Yetman, D.L., and Kutteh, W.H. (1996). Antiphospholipid antibody panels and recurrent pregnancy loss: prevalence of anticardiolipin antibodies

compared with other antiphospholipid antibodies. *Fert. Steril. 66*(4), 540–546.

Yin, E.T., and Gaston, L.W. (1965). Purification and kinetic studies on a circulating anticoagulant in a suspected case of lupus erythematosus. *Thromb. Diath. Haemorrh. 14,* 89–115.

Yoshida, K., Arai, T., Kaburaki, J., Ikeda, Y., Kawakami, Y., and Kuwana, M. (2002). Restricted T-cell receptor beta-chain usage by T cells autoreactive to beta(2)-glycoprotein I in patients with antiphospholipid syndrome. *Blood 99*(7), 2499–2504.

Zhou, H., Wolberg, A.S., and Roubey, R.A.S. (2004). Characterization of monocyte tissue factor activity induced by IgG antiphospholipid antibodies and inhibition by dilazep. *Blood 104*(8), 2353–2358.

CHAPTER

31

Sjögren Syndrome

MENELAOS N. MANOUSSAKIS AND HARALAMPOS M. MOUTSOPOULOS

Department of Pathophysiology, School of Medicine, National University of Athens, Greece.

Sjögren syndrome (SS) is an autoimmune disorder characterized by exocrine gland dysfunction and destruction, mainly the salivary and lachrymal glands, leading to dryness of mouth and eyes. Exocrinopathy is associated with dense lymphocytic infiltrates of glandular tissues and B-cell hyper-reactivity. Sjögren syndrome appears unique for two reasons. First, it has a broad clinical presentation, extending from local exocrinopathy to involvement of multiple organs; it may be found alone (primary SS) or in association with other autoimmune diseases (secondary SS). Although clinical, serologic and genetic features may distinguish primary from secondary SS, it remains unclear whether these entities are etiologically and pathogenically different. Second, it is a model disorder where a benign process can evolve into a lymphoid malignancy. Thus, the study of SS may provide insights into the pathogenesis of autoimmune disorders and lymphoid malignancy. Sjögren syndrome has been long regarded as an autoimmune disease, due to the presence of lymphoid infiltrates in the exocrine glands, the plethora of serum autoantibodies, the various hyperimmune clinical complications, as well as its frequent association with other autoimmune disorders.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Most SS patients have an indolent course. The initial manifestations can be nonspecific and usually antedate the full-blown development of the syndrome by a decade. In approximately one-third of primary SS patients mild extraglandular manifestations are present, whereas a small but significant number of patients develop lymphoid neoplasia.

Glandular Manifestations

Dryness of mouth (xerostomia) and eyes are the major glandular manifestations of SS. Patients variably complain of difficulty in chewing and swallowing, sore mouth, and/or a sandy feeling or itchiness in the eyes. The oral mucosa is dry, sticky, and erythematous, often with fungal overgrowth. The tongue appears with fissures and atrophy of the filiform papillae. Teeth show increased plaque formation, hypocalcification, and caries at the gingival margins. Diminished secretion of tears (aqueous layer) leads to chronic irritation and destruction of the corneal and bulbar conjunctival epithelium [keratoconjunctivitis sicca (KCS)]. Major sali-

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

vary gland enlargement, particularly of the parotid glands (Figure 31.1), occurs episodically or chronically in 25–65% of primary SS patients, but is uncommon among patients with secondary SS (Moutsopoulos et al., 1979).

Salivary flow rates measured for whole saliva or for separate secretions from each major salivary gland (with or without stimulation) reveal reduced salivary flow rates. Sialography reveals various degrees of sialectasis in the majority of patients. Scintigraphy, a functional assessment of salivary glands, appears unable to differentiate between SS patients and controls (Adams et al., 2003). Ultrasonography, conventional magnetic resonance imaging and magnetic resonance sialography may provide noninvasive diagnostic alternatives (Niemela et al., 2004).

Decreased tear secretion rate as measured by the Schirmer's test (wetting of the paper strip of less than 5 mm per 5 minutes), is an indication of diminished secretion but is not diagnostic for KCS (reviewed by Kincaid, 1987). Slit-lamp examination after Rose-Bengal staining reveals punctuate or filamentary keratitis lesions that are characteristic for KCS (Figure 31.2) (Kincaid, 1987). The tear breakup time (overly rapid breakup time) is used to evaluate tear film integrity labeled by a drop of fluorescein.

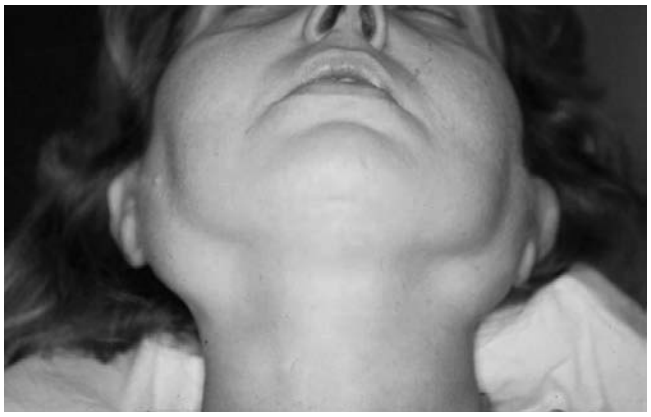


FIGURE 31.1 Firm, painless, nodular parotid gland enlargement in a 54-year old patient with primary Sjögren syndrome.

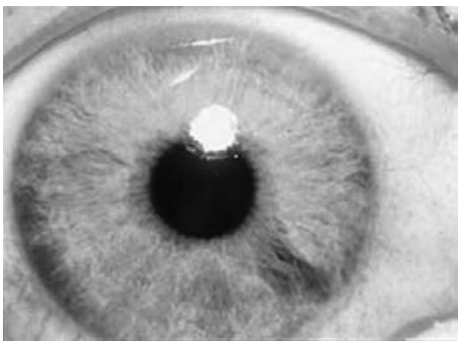


FIGURE 31.2 Staining of damaged corneal and conjunctival epithelia by Rose Bengal dye in a patient with primary Sjögren syndrome and keratoconjunctivitis sicca (KCS). See color plate section.

Extraglandular Manifestations

Patients with clinically extraglandular manifestations may complain of easy fatigue, general malaise, low-grade fever, and of myalgias and arthralgias.

Musculoskeletal Manifestations

Arthralgias, myalgias, morning stiffness, intermittent synovitis, and, infrequently, chronic symmetric polyarthritis may be encountered in primary SS patients. Polymyositis is rare.

Respiratory Tract Involvement

Various respiratory tract manifestations have been described in SS patients affecting the respiratory tract, the mediastinum, and the pleura. These are frequent but usually mild and of low clinical importance. Patients can present with dry cough secondary to dryness of the tracheobronchial mucosa (xerotrachea), dyspnea from small airway obstruction, and, very rarely, interstitial disease.

Gastrointestinal and Hepatobiliary Manifestations

Dysphagia, nausea, dyspepsia, and epigastric pain are seen. Biopsies of gastric mucosa show chronic atrophic gastritis and lymphocytic infiltrates. Liver involvement in primary SS patients is rare (5%) and subclinical, presenting with elevated liver enzymes and/or with antimitochondrial antibodies associated with stage I primary biliary cirrhosis (PBC). Sicca manifestations have been described in half of PBC patients (Trevino et al., 1987).

Urinary Tract Involvement

Less than 10% of SS patients manifest renal disease. Abnormal urine acidification test is found in one-fourth of the patients. Interstitial disease is found early in the disease and manifests with renal colici, hyposthenuria, and hypokalemic hyperchloremic renal tubular acidosis (type I). Immune complex glomerulonephritis (membranoproliferative or mesangial proliferative) is a late-disease manifestation and is associated with cryoglobulinemia and hypocomplementemia (Goules et al., 2000). Interstitial cystitis has been also described (Van de Merwe et al., 1993).

Vascular involvement

Raynaud phenomenon is seen in one-third of the patients with primary SS and precedes sicca manifestations (Skopouli et al., 1990). Small vessel hypersensitivity vas-

culitis and, rarely, polyarteritis nodosa-like vasculitis have been observed in primary SS patients (Alexander, 1987). Episodic palpable purpuric or petechial lesions of variable intensity in the lower extremities (dependent purpura) are common. Recurrent urticaria-like lesions, various maculopapular erythematous lesions, subcutaneous nodules, and skin ulcerations may be also seen. Cases of systemic vasculitis with visceral involvement affecting kidney, lung, and gastrointestinal tract have been described.

Neuropsychiatric involvement

Neurologic involvement occurs in approximately 10–20% of primary SS patients manifesting with peripheral sensory or sensory-motor polyneuropathy and/or mononeuritis multiplex. Carpal tunnel and other entrapment syndromes as well as sensory gangliopathy have also been described. Trigeminal and/or optic neuropathy has been observed. Sensorineural hearing loss is usually subclinical (Tumiati et al., 1997).

Hemiparesis, sensory deficits, seizures, movement disorders, cerebellar defects, neurogenic bladder, transverse myelopathy, and a multiple sclerosis-like picture have been described in SS patients (Alexander et al., 1994). However, other investigators using strict criteria for the classification of primary SS have not substantiated these observations (Soliotis et al., 2004). Sjögren syndrome patients often present various neuropsychiatric features, which may at least partly be attributed to dysregulation of the stress response (Johnson and Moutsopoulos, 1992). Anxiety, depressed mood, and personality structure disorders are frequently manifest and may necessitate therapeutic intervention.

Lymphoproliferative disease

In primary SS, malignant non-Hodgkin lymphomas (most often low-grade marginal-zone B-cell lymphoma) have been estimated to occur in approximately 4–8% of patients followed for approximately 8–10 years (Kassan et al., 1978; Kauppi et al., 1997; Voulgarelis et al., 1999) (Figure 31.3). This usually is seen in patients with low C4, mixed monoclonal cryoglobulinemia, and purpura (Skopouli et al., 2000). In most cases, the patients are in fairly good clinical performance status, at an early clinical stage, whereas extranodal localization is frequent (Table 31.1) (Voulgarelis et al., 1999). The survival is closely associated with the histologic grade of lymphoma.

Secondary Sjögren Syndrome and Overlapping Entities

Sicca manifestations can be found alone or in conjunction with several other autoimmune rheumatic diseases, such as RA, systemic lupus erythematosus (SLE) and systemic sclerosis. In addition, manifestations of SS have been described in patients with polymyositis, polyarteritis nodosa, PBC, mixed connective tissue disease, and myasthenia gravis (Moutsopoulos et al., 1980).

Approximately 5% of patients with rheumatoid arthritis (RA) present a clinically overt SS, while subclinical sicca complaints can be affirmed by as many as 20% of such patients. The diagnosis of RA usually precedes that of SS by

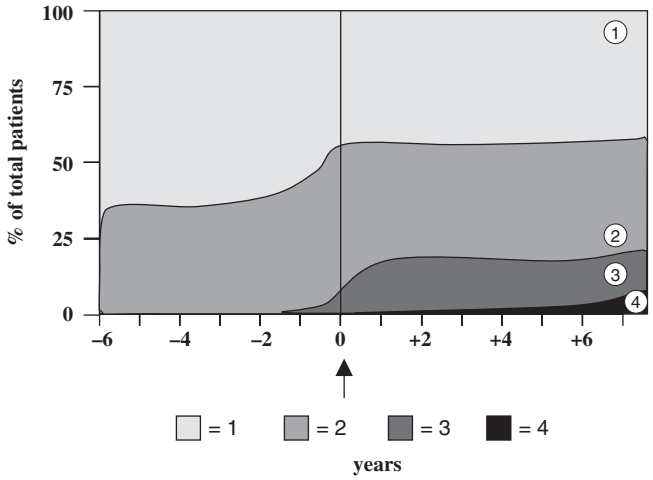


FIGURE 31.3 The clinical spectrum of primary Sjögren syndrome (SS) and disease evolution. Schematic representation of the proportion of the various subgroups of primary SS patients in different time points relatively to the time of diagnosis, as derived from a synthesis of published information. 1: patients with disease confined in the exocrine glands; 2: patients with extraglandular involvement without adverse prognostic signs (palpable purpura, low C4 complement levels and/or mixed monoclonal cryoglobulins); 3: patients with extraglandular involvement with adverse prognostic signs; 4: patients with extraglandular involvement and lymphoma development.

TABLE 31.1 Clinical characteristics of malignant non-Hodgkin's lymphoma in primary Sjögren syndrome patients

Features of lymphoma*	% positive
Clinical performance status:	
0–1	82
2–4	18
B symptoms	24
Localization:	
Nodal	63
Extranodal	81
Clinical stage:	
I/II	58
III/IV	42

*Performance status was graded (0–4 scale) at the time of lymphoma diagnosis using the Eastern Cooperative Oncology Group scale, whereas the Clinical Staging (I–V scale) was based on the Ann Arbor classification system (Voulgarellis et al., 1999). B symptoms: fever, night sweats, and weight loss.

many years (Moutsopoulos et al., 1979). Keratoconjunctivitis sicca is the predominant sicca manifestation of patients with RA and SS, whereas xerostomia, salivary gland enlargement, lymphadenopathy, renal involvement, and Raynaud phenomenon are uncommon.

Based on distinct clinical, serologic, and immunogenetic profiles of patients with SS alone and of those with SS associated with RA (Moutsopoulos et al., 1979; 1980), the terms "primary SS" and "secondary SS" were proposed for the former and the latter groups of patients, respectively (Moutsopoulos et al., 1979). During recent decades, the term "secondary SS" has generally been applied to connote sicca disorder that occurs together with any autoimmune disorder; however, this disregards the possibility for true overlapping entities. In this context, a recent study has revealed that patients with coexisting SLE and SS (SLE SS) appear to constitute a subgroup (9%) of SLE patients with distinct clinical, serologic, pathologic, and immunogenetic features, in whom SS is expressed as an overlapping entity, is largely similar to primary SS and usually precedes SLE (Manoussakis et al., 2004).

Oral and/or ocular dryness are also observed in approximately 20% of patients with systemic sclerosis (Medsger, 1987), often associated with fibrosis of the exocrine glands. In contrast, sicca manifestations associated with frank lymphocytic infiltrative lesions are extremely common (60%) in patients with limited systemic sclerosis (Drosos et al., 1991).

Pathologic Features

The histopathologic assessment of SS patients usually reveals focal or diffuse mononuclear infiltrations in one or more organs that are potentially progressive and likely contribute to the dysfunction of the affected organs. Destructive and degenerative lesions, such as diffuse acinar atrophy, duct dilation and fibrosis may be also present; however, the loss of secretory function of the exocrine glands is often disproportionate to the destruction encountered.

Salivary gland involvement is central in primary SS and, therefore, the pathologic study of the salivary gland tissues has been long applied for the diagnostic confirmation of SS (Daniels et al., 1987) and the focus of an extensive research of its pathogenesis. The pathologic assessment of labial salivary gland biopsy reveals chronic focal sialadenitis that typically consists of focal mononuclear aggregates (at least 50 lymphocytes and plasma cells), which are adjacent to and replace the normal acini (Daniels et al., 1987). Larger foci forming germinal centers may be found. Among several proposed histologic classifications, a focus score of more than 1 focus/4 mm^2 (with focus defined as an aggregate of 50 or more mononuclear cells) has been proposed as a specific measure for SS (Chisholm and Mason, 1968) and the best single classification criterion (Vitali et al., 1993). In a modification of this method, Greenspan and Daniels had considered scores from 1 to 12 foci/4 mm^2 and found a significant positive correlation between a higher score and larger foci (Greenspan et al., 1974, Daniels et al., 1987).

Clinical Course and Evolution of the Disease

The initial presentation of primary SS at diagnosis essentially determines its outcome (Figure 31.3) (Skopouli et al., 2000). In the vast majority of patients, the glandular sicca features and serologic profile remain unchanged during the disease course (Gannot et al., 2000; Skopouli et al., 2000). Ordinarily, lymphoma and glomerulonephritis are late events (Skopouli et al., 2000). Lymphoproliferative disease increases with patient follow-up (2.6% at 5 years and 3.9% at 10 years) and is predicted by the presence of palpable purpura and low complement levels at the first visit (Ioannidis et al., 2002). These manifestations appear to distinguish high-risk patients (type I) from patients with low risk (type II) (Ioannidis et al., 2002; Theander et al., 2004). The overall mortality of primary SS is not different from that of the general population, whereas mortality increases in high-risk patients (Martens et al., 1999; Skopouli et al., 2000; Pertovaara et al., 2001; Ioannidis et al., 2002; Theander et al., 2004).

Diagnosis and Differential Diagnosis

Combined clinical, pathologic, and serologic assessment of patients can easily lead to diagnosis. Differential diagnosis should consider various medical conditions that may cause dryness of the mucosae, with or without salivary gland enlargement, including adverse effects of drugs, infections, tumors, metabolic disorders, and irradiation. In particular, sarcoidosis (Drosos et al., 1989), lipoproteinemias (types II, IV, and V), chronic graft-versus-host disease, lymphomas, amyloidosis and infection by human immunodeficiency virus (HIV) (Itescu, 1991) and hepatitis C virus (HCV) (Haddad et al., 1992, Ramos-Casals et al., 2002) might be misinterpreted as SS. Clinical, pathologic, and serologic features can be helpful for the differentiation of these processes from genuine SS. In particular, the identification of serum autoantibodies to Ro(SSA) or La(SSB) proteins is a strong indication for primary SS (Table 31.2). In elderly individuals, mucosal dryness is frequently an age-related atrophic process (Drosos et al., 1988).

A prospective concerted action from 12 European countries resulted in the validated definition of the European criteria for SS (Vitali et al., 1993; 1996). Recently, the American-European Consensus Group has modified the criteria. Among others, the presence of histopathologic evidence of sialadenitis and of serum anti-Ro(SSA)/La(SSB) autoantibodies were introduced as obligatory classification criteria (Box 31.1) (Vitali et al., 2002). These criteria can be

TABLE 31.2 Differential diagnosis of primary Sjögren syndrome (SS) from sicca syndrome associated with infection by HIV or HCV or with sarcoidosis

	Primary SS	Sarcoidosis	HCV infection with sicca syndrome	HIV infection with sicca syndrome
Sex predilection	Female	No difference	No difference	Male
Age	Middle-age	Middle-age	All ages	Young
Serum autoantibodies to Ro(SSA) and La(SSB)	Frequently present	Absent	Absent	Absent
Predominant T-cell subset in lymphoid infiltrates	$CD4^+$	Non-caseating granuloma	$CD4^+$	$CD8^+$
HLA-association	DR3, DRw53, DQA1*0501	Unknown	Unknown	HLA-DR5
Diagnostic viral serologic test	None	None	HCV tests	HIV tests

HCV, hepatitis B virus; HIV, human immunodeficiency virus.

Box 31.1
The American-European Consensus Group classification criteria for Sjögren syndrome

I. Ocular symptoms: a positive response to at least one of the three following questions:
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than three times a day?

II. Oral symptoms: a positive response to at least one of the three following questions:
1. Have you had a daily feeling of dry mouth for more than 3 months?
2. Have you had recurrently or persistently swollen salivary gland as an adult?
3. Do you frequently drink liquids to aid in swallowing dry food?

III. Ocular signs: objective evidence of ocular involvement defined as a positive result in at least one of the following two tests:
1. Schirmer's I test, performed without anesthesia (≤5 mm in 5 minutes) ¶
2. Rose-Bengal score or another ocular dye score (≥4 according to van Bijsterveld's scoring system)

IV. Histopathology

Presence of focal lymphocytic sialadenitis in minor salivary glands (obtained through normal-appearing mucosa), evaluated by an expert histopathologist with a focus score ≥1, defined as a number of lymphocytic foci which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes per 4 mm^2 of glandular tissue.

V. Salivary gland involvement: objective evidence of salivary gland involvement defined as a positive result in at least one of the following three diagnostic tests:
1. Unstimulated salivary flow (≤1.5 mL in 15 minutes)
2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts.
3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer.

VI. Autoantibodies: presence in the serum of the following autoantibodies:

Antibodies to Ro(SSA) or La(SSB) or both

Rules for classification: In patients without any potentially associated disease the presence of any four of the six items is indicative of definitive primary SS. In patients with a potentially associated disease (for instance another connective tissue disease) item-1 or item-2 plus any two from items 3, 4, 5 is indicative of secondary SS.

Exclusion criteria: prior head and neck irradiation, pre-existing lymphoma, acquired immunodeficiency disease (AIDS), hepatitis C infection, sarcoidosis, graft-versus-host disease, sialoadenosis, use of neuroleptic, anti-depressant, anti-hypertensive or parasympatholytic drugs.

¶Test should be excluded from the criteria or not considered indicative for a diagnosis of SS in elderly subjects (older than 60 years).

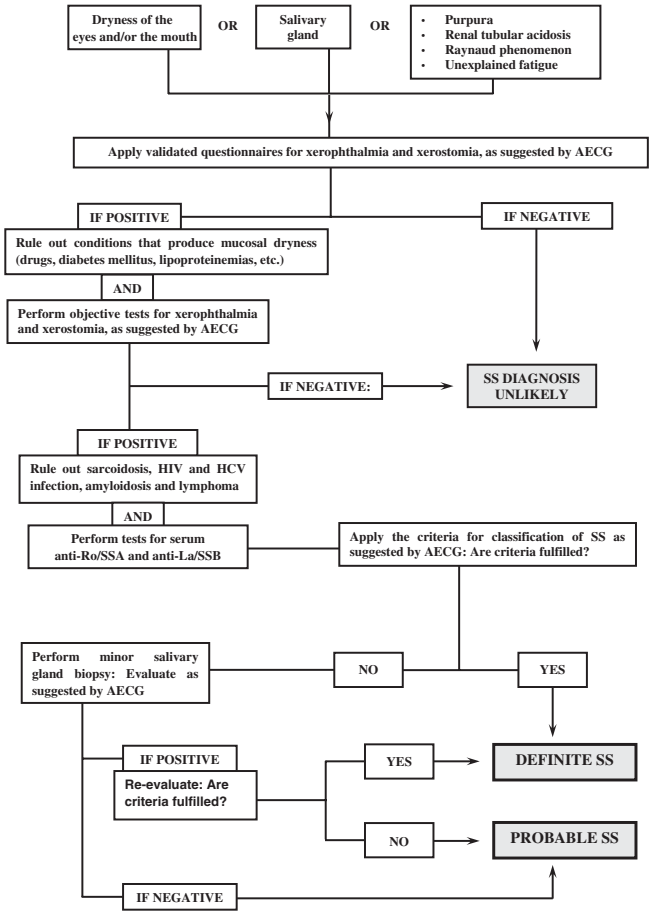


FIGURE 31.4 A practical algorithm for the assessment and diagnosis of Sjögren syndrome (SS) based on the sequential application of validated questionnaires and objective tests, as suggested by the American-European Consensus Group (AECG) criteria for the classification of SS (see Box 31.1). Adapted from Vitali et al. (2002).

also utilized to derive a practical algorithm for diagnosis (Figure 31.4).

Epidemiology

SS affects primarily women (women to men, 9:1), mainly in the fourth and fifth decades of life. However, reported cases include individuals of all ages, from children to elderly persons (Stiller et al., 2000). In different population studies, the overall prevalence of primary SS has been estimated to be approximately 3% (Jacobsson et al., 1989; Dafni et al., 1997).

AUTOIMMUNE FEATURES AND PATHOGENIC MECHANISMS

Humoral Autoimmune Features

Primary SS is one extraordinary example of disorders associated with polyclonal B-cell hyper-reactivity, as attested by profound hypergammaglobulinemia, multiple autoantibodies, and cryoglobulins (Harley, 1987). Organ-specific autoantibodies include those to antigens of salivary ducts, thyroid, gastric mucosa, erythrocytes, pancreas, prostate, and nerve cells. Non-organ-specific autoantibodies include antibodies to cellular antigens (Ro/SSA and La/SSB, histones, single-stranded DNA, α-fodrin) and rheumatoid factors.

Ro/SSA and La/SSB represent heterogeneous ribonucleoprotein complexes consisting of antigenic proteins (two main proteins of 52 kDa [Ro52] and 60 kDa [Ro60] for Ro/SSA and one protein of 48 kDa for La/SSB) associated with small cytoplasmic RNAs (hYRNAs) (Slobbe et al., 1991). The function of Ro/SSA hYRNA complexes remains largely unknown. The La/SSB protein has been suggested to participate in the transcription termination of RNA polymerase III and in the initiation of the translation of at least the poliovirus mRNA, whereas it is capable of melting DNA/RNA hybrids by virtue of its ATPase activity (Bachmann et al., 1990). Antibodies to Ro/SSA and La/SSB antigens frequently coexist in the serum of primary SS patients, and their detection has been recently included as a classification criterion for SS (St Clair, 1992; Vitali et al., 2002). Several mechanisms, including molecular mimicry with viral antigens or antigen-driven responses, have been proposed to explain the genesis of these autoantibodies in SS. Furthermore, these particular autoantibodies appear to represent a meaningful link between sicca syndrome that occurs in primary SS and in SS that coexists with SLE (Manoussakis et al., 2004). These findings are especially important not only for classification purposes but also because they strongly suggest a relationship between the biologic processes that generate these specific immunologic responses and the autoimmune sicca disorder itself.

By gel-precipitation assays, anti-Ro/SSA and anti-La/SSB antibodies are extremely uncommon in healthy individuals, whereas they are detected in approximately 38–60% and 25–40%, respectively, of primary SS patients. More sensitive techniques, such as enzyme-linked immunosorbent assay (ELISA), using purified or recombinant antigens, may reveal anti-Ro/SSA and anti-La/SSB in up to 95% and 87% of patients, respectively, but also in 10–15% of healthy controls. Of note, these autoantibody specificities are not specific for the primary syndrome and may be found also in 3–20% of patients with several other autoimmune diseases, usually in the presence of associated SS. Except for certain anti-La/SSB responses (Tzioufas et al., 2002), attempts to demonstrate linear immunodominant epitopes using small synthetic peptides have been inconclusive. In a recent study, autoimmune sera from patients with primary SS and SLE have been found to contain anti-idiotypic antibodies targeting a common anti-La/SSB idiotype that mask the anti-La/SSB response (Routsias et al., 2002). Interestingly, these anti-idiotypic antibodies can be detected using complementary peptides of La/SSB epitopes and hidden anti-La/SSB

antibodies can be released and detected using complementary epitope analogs (Routsias et al., 2002).

Viral infections or the induction of cellular stress by heat, UV light, serum starvation, and chemicals on cultured epithelial cells and keratinocytes has been shown to induce the translocation of Ro/SSA and La/SSB ribonucleoproteins to the cell surface (Rosen et al., 1995). Interestingly, abnormal translocation and localization to the outer membranes of the nuclear autoantigen La/SSB have also been observed in the conjunctival and salivary epithelial cells of SS patients (Yannopoulos et al., 1992; Price et al., 1994). Also, various autoantigenic ribonucleoproteins have been detected in the exosomal bodies normally released by cultured salivary gland epithelial cells (Kapsogeorgou et al., 2003).

Multiple other non-organ-specific autoantibodies have been described in primary SS patients, including anticentromere antibodies, antimitochondrial antibodies, antibodies to the U1nRNP ribonucleoprotein, antiphospholipid, anticytokeratin and antineutrophil antibodies. IgG present in the sera of SS patients has been found to bind to and activate cholinergic receptors present on rat parotid gland that were identified as M3-muscarinic receptors (Bachman et al., 1998) with possible inhibitory effect on parasympathetic neurotransmission (Waterman et al., 2000) (see Chapter 17). The detection of autoantibodies to the cytoskeletal protein α-fodrin was initially considered a specific diagnostic marker for SS (Haneji et al., 1997), but subsequent studies by ELISA gave a sensitivity of only 48% and a specificity less than that of anti-La (Zandbelt et al., 2004).

Cellular Autoimmune Features

Peripheral Blood Lymphocytes

The analysis of the absolute number of the total peripheral blood lymphocytes as well as T and B cells does not generally reveal significant differences compared with normal individuals. Recent comparative studies of peripheral blood and parotid glands from primary SS patients have indicated the depletion of CD27-expressing memory B cells from the peripheral blood and accumulation and/or retention of these antigen-experienced B cells in the inflamed salivary gland tissues (Dorner et al., 2002).

Tissue-Infiltrating Lymphocytes

The majority of infiltrating lymphocytes in the immunopathologic lesions of the exocrine glands are T cells, while B lymphocytes constitute approximately 20–25% of infiltrating cells. Most of the T lymphocytes (60–70%) bear the $CD4^+$ phenotype, the αβ T-cell receptor (TCR), and the CD45 Ro memory inducer marker. A relative predominance of certain TCR-Vβ (e.g., Vβ2 and Vβ13) and TCR-Vα (e.g., Vα2, Vα11.1, and Vα17.1) genes and a relatively limited junctional (Jβ-genes) usage has been reported, which possibly support an antigen-driven proliferation of T cells in the inflammatory lesions (Matsumoto et al., 1996).

Oligomonoclonal B-cell Expansion

Monoclonal light chains or immunoglobulins have been demonstrated in the serum and/or the urine of approximately 80–100% of patients with extraglandular involvement, compared with approximately 25–40% of patients with disease limited to the exocrine glands. In addition, approximately one-third of primary SS patients exhibit high serum levels of mixed monoclonal cryoglobulins (type II) that contain an IgMκ monoclonal rheumatoid factor. It appears that SS patients with polyclonal B-cell activation generate monoclonal B-cell processes very early in their disease course, and this monoclonality is most frequent in patients with extraglandular involvement, which, in fact, is associated with an increased risk for developing lymphoid malignancy (Kassan et al., 1978).

Monoclonal immunoglobulin gene rearrangements are also detected in the salivary glands and may antedate the development of non-Hodgkin lymphoma (Freimark et al., 1989). Recent studies have indicated that in primary SS patients there is no apparent major molecular abnormality in the generation of the IgV chain repertoire; however, there is biased usage of V(L) chain genes caused by selection and clonal expansion of B cells expressing particular V(L) genes, as well as accumulation of B cells bearing mutated V(L) gene rearrangements within the inflamed parotid glands (Dorner et al., 2002). In addition to the less aggressive oligoclonal B-cell expansion, other tumor-inciting events, such as Bcl-2 translocation appear essential for the development of SS-associated lymphoma (Pisa et al., 1991).

Cell Trafficking, Adhesion, and Activation of Infiltrating Cells

Glandular endothelium of SS patients likely represents the entry site for inflammatory cells. Vessels in the inflamed salivary glands of patients have been shown to express vascular cell adhesion molecule 1 (VCAM1, CD106), intercellular adhesion molecule 1 (ICAM1, CD54), as well as P- and E-selectins, whereas infiltrating mononuclear cells surrounding blood vessels express the counter-receptors lymphocyte function-associated antigen-1 (LFA1), α4- and α5-integrins, and CD44 (Aziz et al., 1996). In addition, high levels of the chemokine CXCL-13/BCA1 have been found on endothelial cells of patients, whereas its counter-receptor CXCR-5 is observed on B cells, a fact that possibly indicates the contribution of this chemokine to the establishment of germinal center-like structures of patients (Amft et al., 2001; Xanthou et al., 2001; Salomonsson et al., 2003).

Immunohistochemical and *in situ* hybridization studies have demonstrated the presence of the proinflammatory cytokines tumor necrosis factor (TNF) and interleukins 6

and 1β (IL-6I, L-1β) in the salivary gland biopsies of SS patients that localize to mononuclear cell infiltrates and epithelium (Oxholm et al., 1992; Fox et al., 1994; Boumba et al., 1995; Cauli et al., 1995; Ohyama et al., 1996). Both TNF and TNF-receptor are present in the inflammatory infiltrates, vascular endothelium, and ductal epithelium (Koski et al., 2001). Salivary gland $CD4^+$ T cells produce abundant IL-2, IL-2R, interferon-γ, IL-1a, and IL-10 mRNA, but minimal or no IL-4, IL-5, and IL-13, suggesting the relative predominance of a Th1 response in SS (Fox et al., 1994; Boumba et al., 1995; Ajjan et al., 1998). Recently, the infiltrating $CD68^+$ macrophages in the minor salivary gland biopsies of SS patients were shown to express high amounts of the proinflammatory cytokine IL-18 (Boiu-Zahiu et al., 2004).

The majority of T cells expresses activation molecules such as major histocompatibility complex (MHC) class II, LFA1, as well as other cell adhesion molecules (CD2/LFA2, CD58/LFA3, CD54/ICAM1, CD154/CD40L) (Skopouli et al., 1991; St. Clair et al., 1992, Kapsogeorgou et al., 2001a; Dimitriou et al., 2002a). Interestingly, infiltrating T lymphocytes have been shown to express large quantities of the serine protease granzyme-A and perforin, possibly involved in the salivary tissue destruction (Alpert et al., 1994; Polihronis et al., 1998; Xanthou et al., 1999).

The Role of Apoptosis (Programmed Cell Death)

Abnormalities in apoptotic pathways may be critically involved in the deterioration of immunologic tolerance, in altered homeostasis of tissue infiltrative processes, as well as in the presentation of autoantigens. Studies in murine models indicated that genetic abnormalities in programmed cell-death processes (mutations in the apoptosis-related proteins Fas(CD95) or Fas-ligand(CD95L) lead to the persistence of autoimmune T-cell clones. Importantly, the infection of exocrine glands of such mutant mice by epitheliotropic viruses may result in chronic inflammatory glandular lesions (Fleck et al., 2001). In SS patients, peripheral blood $CD4^+$ T lymphocytes have been shown to display accelerated *in vitro* apoptosis, enhanced expression of Fas and decreased expression of Bcl-2 (apoptosis inhibitor) proteins (Ichikawa et al., 1995; Ogawa et al., 1996). On the other hand, the salivary gland-infiltrating mononuclear cells express elevated levels of Fas(CD95), Fas-ligand and Bax (pro-apoptotic proteins) and Bcl-2 (apoptosis inhibitor) and rarely undergo apoptosis: a phenomenon referred to as "blocked apoptosis" (Kong et al., 1997; Polihronis et al., 1998; Ohlsson et al., 2001). In direct contrast, apoptosis appears to play a role in the destruction of glandular epithelial cells, since these cells display DNA-strand breaks; widespread and high expression of the apoptotic proteins Fas, Fas-ligand, and Bax; and downregulation of Bcl-2 (Kong et al., 1997; Polihronis et al., 1998), associated with caspase-3 activation (Jimenez et al., 2002). The expression of the negative regulator molecules CTLA-4 and PD-1 is also increased (Bolstad et al., 2003). Furthermore, using cultured non-neoplastic cell lines, salivary gland epithelial cells have been shown to be susceptible to Fas-mediated and Fas-unrelated apoptotic death after stimulation by interferon-γ (but not TNF or IL-1) (Abu-Helu et al., 2001). These findings indicate that epithelial cell apoptosis contributes to the glandular lesions of SS, either by the action of local infiltrating cytotoxic T cells or via intrinsic mechanisms, possibly prior to lymphocytic infiltration.

The apoptotic epithelial cell death possibly results in the release of autoantigens that are captured by antigen-presenting cells and presented to the immune cells, thus resulting in the perpetuation and the expansion of immune responses. Apoptosis may also represent a specialized mechanism whereby nuclear antigens may gain access to the immune system, leading to the induction of humoral responses to Ro/SSA and La/SSB ribonucleoproteins (Rosen et al., 1995). In fact, during early apoptosis, La/SSB autoantigen has been shown to redistribute diffusely to the cytoplasm, whereas both Ro/SSA and La/SSB autoantigens lead to surface apoptotic blebs and bodies (Ohlsson et al., 2002).

Implication of Glandular Epithelial Cells in Immunopathology

During the last decade, a new immunopathogenetic concept has emerged, placing the glandular or acinar epithelial cells at the center of the immunopathologic processes. Several lines of evidence indicate that the epithelia of SS display features of activation and participate in the induction and maintenance of the autoaggressive lymphocytic infiltrations that characterize the syndrome. In fact, the early lymphoepithelial lesions in primary SS have been demonstrated to begin around the ducts.

In salivary gland biopsies of SS patients, ductal and/or acinar SGEC display high levels of several immunoactive proteins, such as adhesion, presentation molecules that are known to mediate the lymphoid cell homing and the amplification of interactions between epithelial and immune cells. Epithelial cells adjacent to sites of intense inflammation display high levels of MHC class I and class II (DR) molecules (Lindahl et al., 1985), CD54/ICAM1, CD106/VCAM, and E-selectin adhesion molecules (St. Clair et al., 1992; Kapsogeorgou et al., 2001a; Tsunawaki et al., 2002), as well as CD80/B7.1 costimulatory molecules (Manoussakis et al, 1999; Tsunawaki et al., 2002; Fujihara et al., 1999). Also, high expression of CD54/ICAM1 has been detected in the periacinar myoepithelial cells of SS patients (Kapsogeorgou et al., 2001a).

Salivary epithelia have been also found to express increased levels of the proinflammatory cytokines IL-1,

IL-6, and TNF (Fox et al., 1994; Boumba et al., 1995) and of several T-cell-attracting and germinal-center-forming chemokines, including CCL3/MIP-1α, CCL4/MIP-1β, IL-8 and CCL5/RANTES (Cuello et al., 1998), STCP-1/MDC, CXCL-9/Mig, CXCL-10/IP-10, CXCL12/SDF-1, CCL17/TARC, CXCL13/BCA-1, and CCL19/ELC (Amft et al., 2001; Xanthou et al., 2001), whereas CXCR-4 (the counter-receptor for CXCL-12) and CXCR-3 (the receptor for CXCL-9 and CXCL-10) have been shown on periductally infiltrating T cells (Amft et al., 2001; Ogawa et al., 2002). Thus, the epithelial cells in the glandular lesions of SS, by virtue of aberrant expression of various immunoactive factors, are potentially capable of attracting lymphocytes and of promoting chronic inflammatory reactions. This fact is in agreement with the widespread involvement of epithelial tissues in primary SS patients that has been described as autoimmune epithelitis (Moutsopoulos, 1994).

The activated cell phenotype of glandular epithelial cells that is encountered *in situ* in SS patients may be solely due to the influence of factors produced locally by the infiltrating cells. On the other hand, their expression could also be a result of intrinsic cellular activation processes that may operate in patients' epithelia. Interestingly, the careful analysis of CD54/ICAM1 expression in salivary tissues of SS patients has revealed that the epithelial expression is observed throughout glandular tissues and is not confined in areas proximal to lymphoid infiltrates (Kapsogeorgou et al., 2001a). Such peculiar activation of epithelial cells was also previously recognized for the proto-oncogene c-myc expression (Skopouli et al., 1992).

The establishment and analysis of long-term cultured non-neoplastic SGEC obtained from minor salivary gland tissues (Dimitriou et al., 2002b) indicated that SGEC were capable of expressing various immunoactive molecules, including MHC class I and class II molecules, adhesion molecules, and functional CD80 (B7) and CD40 costimulatory molecules (Manoussakis et al, 1999; Kapsogeorgou et al., 2001a; 2001b; Dimitriou et al, 2002a; 2002b), and thus, are suitably equipped for the presentation of antigenic peptides and the transmittance of activation signals to T cells. The expression of functional CD80 (B7) costimulatory proteins deserves particular attention (Manoussakis et al, 1999; Kapsogeorgou et al., 2001b; Tsunawaki et al., 2002), since these proteins are typically expressed by classical antigen-presenting cells and are critical for the regulation of naïve T-cell activation. Notably, the (CD86) B7.2 molecules that are expressed by SGEC have been shown to display unique binding properties denoted by the functional interaction with CD28-receptor and reduced binding to the negative-regulator of immune responses CTLA-4 (Kapsogeorgou et al., 2001b).

Furthermore, parallel analyses of non-neoplastic SGEC cultures derived from SS patients and controls revealed the constitutive and stably higher expression of various immunoactive molecules in cells derived from SS patients, including MHC class I, costimulatory, adhesion, and apoptosis molecules (Manoussakis et al, 1999; Abu-Helu et al., 2001; Kapsogeorgou et al., 2001a; Dimitriou et al, 2002a; 2002b). This fact most likely indicates an intrinsic activation process taking place in the epithelia of patients. The nature of such intrinsic activation is presently unclear; however, the implication of an exogenous agent, such as an epitheliotropic virus remains a reasonable possibility (Figure 31.5).

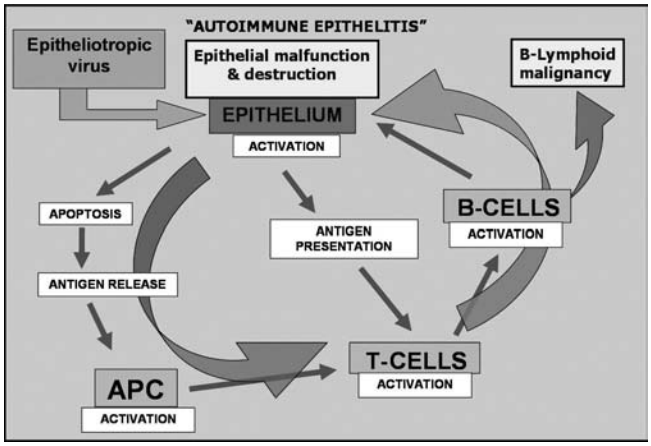


FIGURE 31.5 A schematic model for the pathogenesis of autoimmune epithelitis lesions in the salivary glands of Sjögren syndrome patients. An epitheliotropic virus and/or other intrinsic aberration(s) may initiate a constant degree of activation of epithelia and/or of apoptosis. During the course of recurring activation of salivary gland epithelial cells, persistent presentation of antigen(s) to T cells and salivary gland epithelial cell apoptosis, focal lymphocytic infiltrates, and the destruction of epithelia likely ensue. Salivary-gland epithelial cells, by virtue of aberrant expression of various immunoactive molecules, such as class I and class II MHC proteins, adhesion molecules, costimulatory proteins, proinflammatory cytokines, and chemokines, are likely able to attract lymphocytes and to induce the activation of T cells by typical antigen-presentation mechanisms. The cytokines (such as interferon-γ) that are produced by activated T cells may further trigger the antigen-presenting capacities of salivary-gland epithelial cells. In addition, epithelial apoptosis by direct cytotoxic and/or Fas-mediated mechanisms are also further induced. This leads to the release of epithelial constituents that are subsequently endocytosed and presented to T cells by professional antigen-presenting cells (APC), such as dendritic cells, that reside in the gland, thus further potentiating antigen-induced responses and chronic proliferation of T- and B-cells. The polyclonal activation of B lymphocytes is associated with the production of autoantibodies to epithelial cell constituents that may induce the dysfunction and/or the destruction of the glandular epithelia. Chronic B-lymphocyte stimulation may give rise to monoclonal B-cell processes and occasionally to frank malignant non-Hodgkin lymphoma.

GENETIC FEATURES

Multiple immunogenetic studies, familial clustering, and the increased frequency of certain MHC class II alleles strongly indicate the genetic predisposition of SS. Studies

from different countries and ethnic groups have revealed an increased frequency of several MHC HLA-DR alleles (DRB1 genes), including HLA-DR3, HLA-DR5, HLA-DRw11, HLA-DR8, and HLA-DRw53. Particular HLA class II alleles may have an important role in the regulation of the immune responses against the Ro/SSA and LA/SSB ribonucleoproteins. The generation of these autoantibodies has been correlated with heterozygosity for HLA-DQwl/DQw2 and particularly with the alleles DRB1*03, DQB1*02, DQA1*0501, DQB1*0201, as well as alleles comprising DQw6 (Harley et al., 1986; Reveille et al., 1991; Bolstad et al., 2001; Gottenberg et al., 2003). In addition, human recombinant Ro60 was recently shown to induce strong immunologic responses in transgenic mice carrying DR2, DR3, or DQ8 HLA genes, but not control HLA-DQ6 gene (Paisansinsup et al., 2002).

During recent years, the role of single-base-exchange polymorphisms of gene promoters or microsatellite regions of various immunologic molecules has been addressed in primary SS patients. Polymorphisms of transforming growth factor-β (TGF-β) and TNF have been shown to associate with the production of anti-La/SSB antibodies (Gottenberg et al., 2004). In addition, the genomic absence of the T-cell receptor Vβ7.2 gene in SS patients has been shown to associate with the presence of autoantibodies (Manavalan et al., 2004).

ENVIRONMENTAL INFLUENCES

A viral infection has been long suspected for the induction of SS, since transient or persistent infection of epithelial cells by a putative virus may be an initiating event for autoimmune reactions. Research has been focused on various types of viruses, including the herpesviruses (cytomegalovirus, Epstein–Barr virus, and human herpesvirus type-6), retroviruses, and the HCV virus; however, results have been inconclusive. Chronic lymphocytic sialadenitis is found in 14–50% of patients infected by HCV (Haddad et al., 1992); however, SS patients seldom show evidence of HCV infection. Type A retroviral particles have been identified in labial salivary gland extracts from patients with SS (Garry et al., 1990). Recently, based on the exclusive detection of viral genome sequences in minor salivary gland lesions of primary SS, the enterovirus coxsackie B virus arises as a possible candidate etiologic agent (Triantafylopoulou et al., 2004).

ANIMAL MODELS

Clinicopathologic features resembling human SS have been observed to develop in several animal species, either spontaneously or following experimental induction (Hoffman and Walker, 1987), including immunization with salivary gland extracts or tissues, graft-versus-host reactions, viral infections, as well as in TGF-β-knockout mice, HTLV1-transactivating factor-transgenic mice, and BAFF- (Groom et al., 2002).

The development of spontaneous sialodacryoadenitis-transgenic mice resembling primary SS has been reported in several inbred mouse strains, including the nonobese diabetic (NOD) mice, several murine lupus models (NZB, NZB/NZW, and MLR), the IQI/Jcl (Saegusa et al., 1994) and the NFS/sld mutant mice (Haneji et al., 1994). NOD mice, an inbred animal model for insulin-dependent diabetes, develop lymphocytic infiltrates of the salivary glands and antisalivary autoantibodies. In this strain, over 15 genetic loci have been associated with diabetes, insulitis and/or sialadenitis. Idd5 and Idd3 loci appear primarily to relate to the development of sialadenitis, whereas Idd1 appears protective (Brayer et al., 2000; Boulard et al., 2002; Cha et al., 2002). In addition, aberrant histomorphologic differentiation of submandibular glands has been observed (Cha et al., 2001). In these mice, the disruption of the ICA69 locus (a self-antigen expressed in brain, pancreas, salivary, and lacrimal glands) or immunotherapy against ICA69-specific T cells has been shown to ameliorate glandular disease (Winer et al., 2002).

TREATMENT

Treatment of SS patients is directed to provide symptomatic relief, to lessen mucosal dryness-induced damages, and to timely recognize and treat disease complications (Vlachoyiannopoulos and Moutsopoulos, 1997). In particular, patients with adverse prognostic factors should be regularly followed for disease complications.

For KCS, the application of eye lubricants as often as necessary is the mainstay of treatment. Diuretics, antidepressants, and antihistamines may worsen lachrymal and salivary hypofunction and should be used with caution. Patients with residual glandular function may benefit significantly from the oral administration of the muscarinic M3-receptor agonists pilocarpine (Vivino et al., 1999) and cevimeline (Petrone et al., 2002). Ordinary or closed glasses and soft contact lenses offer additional corneal protection. Corticosteroid-containing ophthalmic solutions should be avoided as they may induce corneal lesions or promote infections. Dental treatment with fluoride retards damage of teeth surfaces. For tender salivary gland enlargement, local application of moist heat and nonsteroidal anti-inflammatory drugs may be helpful. Bacterial infection as well as lymphoma should be always considered. Vaginal lubricants may be used to treat vaginal dryness and dyspareunia.

The decision for systemic therapeutic intervention remains largely empirical and is primarily based on the severity of extraglandular manifestations (Table 31.3).

TABLE 31.3 Classification of clinical manifestations and management of Sjögren syndrome according to the severity of systemic involvement

	Type I Debilitating but not life-threatening manifestations	Type II Potentially severe manifestations	Type III Severe or life-threatening manifestations
Chronic	Xerostomia Xerophthalmia Xerotrachea Dyspareunia Constitutive symptoms Raynaud phenomenon Thyroiditis	Distal tubular acidosis Tubulo-interstitial nephritis Bronchiolitis Diffuse interstitial lung disease High risk for lymphomatous transformation*	Progressive interstitial lung disease Lymphoma
Acute	Recurrent salivary gland enlargement Arthritis Purpura	Local infections	Visceral vasculitis Membranoproliferative glomerulonephritis Peripheral neuropathy Myositis CNS involvement Systemic infections
Clinical	Periodic follow-up	Regular follow-up	Frequent follow-up
Management	Conservative measures	Cautious medical intervention	Aggressive medical intervention

*Disease manifestations associated with high risk for lymphoma development (low C4 complement levels, and possibly purpura, persistent salivary gland enlargement, splenomegaly, lymphadenopathy and type II mixed monoclonal cryoglobulinemia).

Nonsteroidal anti-inflammatory drugs or hydroxychloroquine may be administered for arthralgias and myalgias and methotrexate for persistent arthritis. Despite encouraging initial results, the oral administration of natural human interferon-α has shown questionable efficacy (Cummins et al, 2003). Also, a recent study with the anti-TNF agent infliximab did not show any efficacy in primary SS (Mariette et al., 2004).

Patients with overt distal renal tubular acidosis may be orally treated with sodium bicarbonate to avoid nephrocalcinosis. For Raynaud phenomenon, avoidance of cold exposure, emotional stress, and smoking should be recommended and oral nifedipine may be tried. Leukocytoclastic vasculitis does not require specific therapy. Corticosteroids are mainly employed for severe extraglandular manifestations. Aggressive treatment with cytotoxic drugs carries an increased risk for lymphoma development and should be reserved for threatening systemic manifestations. Finally, the treatment of malignant lymphoma in SS patients is based on histology and extent, as for lymphoid neoplasias in general.

PERSPECTIVES ON IMMUNOPATHOGENESIS

Chronic immune system stimulation has a central role in the pathogenesis of SS, as illustrated by the extraordinary systemic immunologic hyper-reactivity and the lymphoplasmacytic infiltrates in affected organs. The further delineation of immune system functions such as memory, tolerance and suppression, the production and function of immunologic mediators, antigen presentation, apoptosis, and the effect of sexual, hormonal and neuroendocrine factors may further elucidate the pathogenesis of autoimmune disorders. Future immunologic studies of SS should address two major issues that may provide insight not only into the pathogenesis but also into specific treatment strategies. The first concerns the nature of processes that initiate and perpetuate the cellular and humoral autoreactive processes. The second involves how and to what extent these processes take part in the actual pathogenesis of the disorder. Although lymphocytic infiltrates characterize the lesions, it is still unclear to what extent these represent the primary cause of tissue destruction. Also, it is unknown whether epithelial tissues in SS patients are more vulnerable to autoaggressive attacks by the immune system, compared with healthy individuals. Glandular infiltrative lesions of SS may be actually perceived as idiomorphous chronic inflammatory reactions to antigens persistently expressed by epithelial cells. Such persistence may be due to repetitive exposure to antigenic substances and/or to the ineffective clearance of damaged cells by the immune system.

The anatomic proximity of salivary and lachrymal glands to mucosal surfaces and ports of antigen entry renders these tissues potentially accessible to various infectious agents and environmental antigens. In fact, salivary glands are sites that frequently harbor pathogenic viruses, and chronic infection of glandular tissues (e.g., by a slow pathogenic virus) has long been evoked to explain the pathogenesis of SS.

Such a process could induce various changes in infected epithelial cells, including aberrant function and differentiation, degeneration and altered survival, altered expression of various proteins and antigens, and enhanced antigen presentation capacities. Indeed, several lines of evidence indicate that the epithelial cells in the salivary and lacrimal glands, and probably elsewhere, are not simply the targets of activated immune cells but they actively participate in the genesis of lesions. These include the expression of various immunoactive substances and the widespread involvement of epithelial tissues.

Such "disseminated" epithelitis apparently arises as a direct response of immune cells to nonspecific inflammatory mediators secreted by local epithelial tissues and/or antigen-specific stimulation. Although not considered to be professional antigen-presenting cells, epithelial cells of certain tissues, such as those of the gastrointestinal tract, are capable of stimulating T cells in an antigen-specific manner, through the presentation of MHC-antigenic peptide complexes and expression of costimulatory molecules. In this context, glandular epithelial cells, initially affected and intrinsically activated by an environmental agent, may trigger a cascade of immune-mediated reactions, which in turn may incite chronic inflammatory and potentially injurious autoimmune reactions. The delineation of the antigenic specificity of tissue-infiltrating lymphocytes is of paramount importance for the understanding of SS. The precise appraisal of involvement of these cells in tissue injury, whether foreign-specific, cross-reactive, or truly autoreactive clones (e.g., Ro/SSA- or La/SSB-specific), may direct specific therapeutic interventions. Innate characteristics of SS patients, by and large directed by their genetic background, may also be determining factors for the induction and perpetuation of the disease. For instance, MHC class II molecules, by virtue of preferential binding and shaping of distinct peptides, influence specific T-cell activation and thus can be directly implicated in the generation of harmful immune responses. In addition, neuroendocrine factors, stress mediators, and sex hormones have been also recognized as active participants in immunological reactions. The elucidation of the role of such factors in the pathogenesis of autoimmune diseases is expected to improve our understanding. In this context, it should not escape attention that the exocrine glands are organs with one of the richest autonomic innervations, evolved to sense and to express emotional stresses.

References

Abu-Helu, R.F., Dimitriou, I.D., Kapsogeorgou, E.K., Moutsopoulos, H.M., and Manoussakis, M.N. (2001). Induction of salivary gland epithelial cell injury in Sjögren's syndrome: *in vitro* assessment of T cell-derived cytokines and Fas protein expression. *J. Autoimmun. 17,* 141–153.

Adams B.K., Al Attia H.M., Parkar S. (2003). Salivary gland scintigraphy in Sjögren's syndrome: are quantitative indices the answer? *Nucl. Med. Commun. 24,* 1011–1016.

Ajjan, R.A., McIntosh, R.S., Waterman, E.A., Watson, P.F., Franklin, C.D., Yeoman, C.M., and Weetman, A.P. (1998). Analysis of the T-cell receptor Valpha repertoire and cytokine gene expression in Sjögren's syndrome. *Br. J. Rheumatol. 37,* 179–185.

Alexander, E.I. (1987). Inflammatory vascular disease in Sjögren's syndrome. In N. Talal. H.M. Moutsopoulos, and S.S. Kassan. (eds.), *Sjögren's Syndrome: Clinical and Immunological Aspects* (pp. 102–125). Berlin: Springer-Verlag.

Alexander, E.L., Ranzenbach, M.R., Kumar, A.J., Kozachuk, W.E., Rosenhaum, A.E., Patronas, N., Harley, J.B., and Reichlin, M. (1994). Anti-Ro(SSA) autoantibodies in central nervous system disease associated with Sjögren's syndrome (CNS SS): Clinical, neuroimaging, and angiographic correlates. *Neurology 44,* 899–908.

Alpert, S., Kang, H.I., Weissman, I., and Fox, R.I. (1994). Expression of granzyme A in salivary gland biopsies from patients with primary Sjögren's syndrome. *Arthritis Rheum. 37,* 1046–1054.

Amft, N., Curnow, S.J., Scheel-Toellner, D., Devadas, A., Oates, J., Crocker, J., Hamburger, J., Ainsworth, J., Mathews, J., Salmon, M., Bowman, S.J., and Buckley, C.D. (2001). Ectopic expression of the B cell-attracting chemokine BCA-1 (CXCL13) on endothelial cells and within lymphoid follicles contributes to the establishment of germinal center-like structures in Sjögren's syndrome. *Arthritis Rheum. 44,* 2633–2641.

Aziz, K.E., McCluskey, P.J., and Wakefield, D. (1996). Expression of selectins (CD62 E,L,P) and cellular adhesion molecules in primary Sjögren's syndrome. *Clin. Immunol. Immunopathol. 80,* 55–66.

Bachmann, M., Pfeifer, K., Schroder, H.C., and Muller, W.E.G. (1990). Characterization of the autoantigen La as a nucleic acid-dependent ATPase/ATPase with melting properties. *Cell 60,* 85–93.

Bacman, S., Perez Leiros, C., Sterin-Borda, L., Hubscher, O., Arana, R., and Borda, E. (1998). Autoantibodies against lacrimal gland M3 muscarinic acetylcholine receptors in patients with primary Sjögren's syndrome. *Invest Ophthalmol Vis Sci. 39,* 151–156.

Boiu-Zahiu, S., Kapsogeorgou, E.K., Dimitriou, I.D., Spahidou, M.P., Moutsopoulos, H.M., and Manoussakis, M.N. (2004). Elevated expression of IL-18 in Sjögren's syndrome: distinct expression patterns of the active and proactive forms. *Arthritis Res. Ther. 6*(Suppl 1), S8.

Bolstad, A.I., Wassmuth, R., Haga, H.J., and Jonsson, R. (2001). HLA markers and clinical characteristics in Caucasians with primary Sjögren's syndrome. *J. Rheumatol. 28,* 1554–1562.

Bolstad, A.I., Eiken, H.G., Rosenlund, B., Alarcon-Riquelme, M.E., and Jonsson, R. (2003). Increased salivary gland tissue expression of Fas, Fas ligand, cytotoxic T lymphocyte-associated antigen 4, and programmed cell death 1 in primary Sjögren's syndrome. *Arthritis Rheum. 48,* 174–185.

Boulard, O., Fluteau, G., Eloy, L., Damotte, D., Bedossa, P., and Garchon, H.J. (2002) Genetic analysis of autoimmune sieladenitis in nonobese diabetic mice: a major susceptibility region on chromosome 1. *J Immunol 168,* 4192–4199.

Boumba, D., Skopouli, F.N., and Moutsopoulos, H.M. (1995). Cytokine mRNA expression in the labial salivary gland tissues from patients with primary Sjögren's syndrome. *Br. J. Rheumatol. 34,* 326–333.

Brayer, J., Lowry, J., Cha, S., Robinson, C.P., Yamachika, S., Peck, A.B., and Humphreys-Beher, M.G. (2000) Alleles from chromosomes 1 and 3 of NOD mice combine to influence Sjögren's syndrome-like autoimmune exocrinopathy. *J. Rheumatol. 27,* 1896–1901.

Cauli, A., Yanni, G., Pitzalis, C., Challacombe, S., and Panayi, G.S. (1995). Cytokine and adhesion molecule expression in the minor salivary glands of patients with Sjögren's syndrome and chronic sialoadenitis. *Ann. Rheum. Dis. 54,* 209–215.

Cha, S., van Blockland, S.C., Versnel, M.A., Homo-Delarche, F., Nagashima, H., Brayer, J., Peck, A.B., and Humphreys-Beher, M.G. (2001) Abnormal organogenesis in salivary gland development may initiate adult onset of autoimmune exocrinopathy. *Exp. Clin. Immunogenet. 18,* 143–146.

Cha, S., Nagashima, H., Brown, V.B., Peck, A.B., and Humphreys-Beher M.G. (2002). Two NOD Idd-associated intervals contribute synergistically to the development of autoimmune exocrinopathy (Sjögren's syndrome) on a healthy murine background. *Arthritis Rheum. 46,* 1390–1398.

Chisholm, D.M. and Mason, D.K. (1968). Labial salivary gland biopsy in Sjögren's syndrome. *J. Clin. Pathol. 21,* 656–660.

Cuello, C., Palladinetti, P., Tedla, N., Di Girolamo, N., Lloyd, A.R., McCluskey, P.J., and Wakefield, D. (1998). Chemokine expression and leucocyte infiltration in Sjögren's syndrome. *Br. J. Rheumatol. 37,* 779–783.

Cummins, M.J., Papas, A., Kammer, G.M., and Fox, P.C. (2003). Treatment of primary Sjögren's syndrome with low-dose human interferon alfa administered by the oromucosal route: combined phase III results. *Arthritis Rheum. 49,* 585–93.

Dafni, U.G., Tzioufas, A.G., Staikos, P., Skopouli, F.N., and Moutsopoulos, H.M. (1997). Prevalence of Sjögren's syndrome in a closed rural community. *Ann. Rheum. Dis. 56,* 521–525.

Daniels, T.E., Aufdemorte, T.B., and Greenspan, J.S. (1987). Histopathology of Sjögren's syndrome. In N. Talal, H.M. Moutsopoulos, and S.S. Kassan, (eds.), *Sjögren's Syndrome: Clinical and Immunological Aspects.* (pp. 41–52). Berlin: Springer-Verlag.

Dimitriou, I.D., Kapsogeorgou, E.K., Moutsopoulos, H.M., and Manoussakis, M.N. (2002a). CD40 on salivary gland epithelial cells: High constitutive expression by cultured cells from Sjögren's syndrome patients indicating their intrinsic activation. *Clin. Exp. Immunol. 127,* 386–392.

Dimitriou, I.D., Kapsogeorgou, E.K., Abu-Helu, R.F., Moutsopoulos, H.M., and Manoussakis, M.N. (2002b). Establishment of a convenient system for the long-term culture and study of non-neoplastic human salivary gland epithelial cells. *Eur. J. Oral Sci. 110,* 21–30.

Dorner T., Hansen A., Jacobi A., and Lipsky P.E. (2002). Immunglobulin repertoire analysis provides new insights into the immunopathogenesis of Sjögren's syndrome. *Autoimmun. Rev. 1,* 119–124.

Drosos, A.A., Antonopoulos, A.P., Costopoulos, J.S., Papadimitriou, C., and Moutsopoulos, H.M. (1988). Prevalence of primary Sjögren's syndrome in an elderly population. *Br. J. Rheumatol. 27*, 123–127.

Drosos, A.A., Constantopoulos, S.H., Phsychos, D.. Stefanou. D., Papadimitriou, C.S., and Moutsopoulos, H.M. (1989). The forgotten cause of sicca complex; sarcoidosis. *J. Rheumatol. 16,* 1548–1551.

Drosos, A.A., Pennec, Y.L., Elisaf, M., Acritidis, N.C., Jouquan, J., Moutsopoulos, H.M., and Youinou, P. (1991). Sjögren's syndrome in patients with the CREST variant of progressive systemic scleroderma. *J. Rheumatol. 18,* 1685–1688.

Fleck M., Zhang H.G., Kern E.R., Hsu, H.C., Muller-Ladner, U., and Mountz, J.D. (2001). Treatment of chronic sialadenitis in murine model of Sjögren's syndrome by local FasL gene transfer. *Arthritis Rheum. 44,* 964–973.

Fox, R.I.. Kang H.I., Ando, D., Abrams, J., and Pisa, E. (1994). Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. *J. Immunol. 152,* 5532–5539.

Freimark, B., Fantozzi, R., Bone, R., Bordin, G., and Fox, R. (1989). Detection of clonally expanded salivary gland lymphocytes in Sjögren's syndrome. *Arthritis Rheum. 32,* 859–869.

Fujihara, T., Fujita, H., Tsubota, K., Saito, K., Tsuzaka, K., Abe, T., and Takeuchi, T. (1999). Preferential localization of CD8+alpha E beta 7+ T cells around acinar epithelial cells with apoptosis in patients with Sjögren's syndrome. *J Immunol. 163*, 2226–2235.

Gannot G., Lancaster H.E., and Fox P.C. (2000). Clinical course of primary Sjögren's syndrome: salivary, oral, and serologic aspects. *J. Rheumatol. 27,* 1905–1909.

Garry, R.F., Fermin, C.D., Hart, D.J., Alexander, S.S., Donehower, L.A., and Luo-Zhang, H. (1990). Detection of a human intracisternal A type retroviral particle antigenically related to HIV. *Science 250,* 1127–1130.

Gottenberg, J.E., Busson, M., Loiseau, P., Cohen-Solal, J., Lepage, V., Charron, D., Sibilia, J., and Mariette X. (2003) In primary Sjögren's syndrome, HLA class II is associated exclusively with autoantibody production and spreading of the autoimmune response. *Arthritis Rheum. 48,* 2240–2245.

Gottenberg, J.E., Busson, M., Loiseau, P., Dourche, M., Cohen-Solal, J., Lepage, V., Charron, D., Miceli, C., Sibilia, J., and Mariette, X. (2004). Association of transforming growth factor beta1 and tumor necrosis factor alpha polymorphisms with anti SSB/La antibody secretion in patients with primary Sjögren's syndrome. *Arthritis Rheum. 50,* 570–580.

Goules, A., Masouridi, S., Tzioufas, A.G., et al. (2000). Clinically significant and biopsy-documented renal involvement in primary Sjögren's syndrome. *Medicine (Baltimore) 79,* 241–249.

Greenspan, J.S., Daniels, T.E., Talal, N. and Sylvester, R.A. (1974). The histopathology of Sjögren's syndrome in labial salivary gland biopsies. *Oral Surg. Oral Med. Oral Pathol. 37,* 217–229.

Groom, J., Kalled, S.L., Cutler, A.H., Olson, C., Woodcock, S.A., Schneider, P., Tschopp, J., Cachero, T.G., Batten, M., Wheway, J., Mauri, D., Cavill, D., Gordon, T.P., Mackay, C.R., and Mackay, F. (2002). Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J. Clin. Invest. 109,* 59–68.

Haddad, J., Deny, P., MunzGothiel, C., Amhrosini, J.C., Trinchet, J.C.. Pateron, D., Mal, F., Gallard, P., and Beaugrad, M. (1992). Lymphocytic sialadenitis of Sjögren's syndrome associated with chronic hepatitis C virus liver disease. *Lancet 339,* 321–323.

Haneji, N., Hamano, H., Yanagi, K., and Hayashi, Y. (1994). A new animal model for primary Sjögren's syndrome in NFS/sld mutant mice. *J. Immunol. 153,* 2769–2777.

Haneji, N., Nakamura T., Takio K., Yanagi K., Higashiyama H., Saito I., Noji S., Sugino H., Nayashi, Y., Identification of alpha-fodrin as a candidate antoantigen in primary Sjögren's syndrome. *Science 1997, 276*, 604–607.

Harley, J.B. (1987). Autoantibodies in Sjögren's syndrome. In N. Talal, H.M. Moutsopoulos, and S.S. Kassan, (eds.), *Sjögren's Syndrome: Clinical and Immunological Aspects.* (pp. 218–234). Berlin: Springer-Verlag.

Harley, J.B., Reichlin, M., Arnett, F.C., Alexander, E.L., Bias, W.B., and Provost, T.T. (1986). Gene interaction at HLA-DQ enhances autoantibody production in primary Sjögren's syndrome. *Science 232,* 1145–1147.

Hoffman, R.W. and Walker, S.E. (1987). Animal models of Sjögren's syndrome. In N. Talal. H.M. Moutsopoulos, and S.S. Kassan (eds.), *Sjögren's Syndrome: Clinical and Immunological Aspects.* (pp. 266–288). Berlin: Springer-Verlag.

Ichikawa, Y., Arimori, K., Yoshida, M., et al. (1995). Abnormal expression of apoptosis-related antigens, Fas and bcl–2, on circulating T-lymphocyte subsets in primary Sjögren's syndrome. *Clin. Exp. Rheumatol. 13,* 307–313.

Itescu, S. (1991). Diffuse infiltrative lymphocytosis syndrome in human immunodeficiency virus infection—a Sjögren's-like disease. *Rheum. Dis. Clin. N. Am. 17,* 99–115.

Ioannidis, J.P., Vassiliou, V.A., Moutsopoulos, H.M. (2002). Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjögren's syndrome. *Arthritis Rheum., 46,* 741–747.

Jacobsson, L.T.H., Axell, T.E., Hansen, B.U., Henriesson, V.J., Larsson., A., Lieberkind, K., Lilja, B., and Manthrope, R. (1989). Dry eyes or

mouth. An epidemiological study in Swedish adults with special reference to primary Sjögren's syndrome. *J. Autoimmun. 2,* 521–527.

Jimenez, F., Aiba-Masago, S., Al Hashimi, I., Vela-Roch, N., Fernandes, G., Yeh, C.K., Talal, N., and Dang, H. (2002). Activated caspase 3 and cleaved poly(ADP-ribose)polymerase in salivary epithelium suggest a pathogenetic mechanism for Sjögren's syndrome. *Rheumatology 41,* 338–342.

Johnson, E.O. and Moutsopoulos, H.M. (1992). Neuroimmunological axis and rheumatic diseases, (editorial). *Eur. J. Clin. Invest. 22,* 25.

Kapsogeorgou, E.K., Dimitriou, I.D., Abu-Helu, R.F., Moutsopoulos, H.M., and Manoussakis, M.N. (2001a). Activation of epithelial and myoepithelial cells in the salivary glands of patients with Sjögren's syndrome: high expression of intercellular adhesion molecule-1 (ICAM.1) in biopsy specimens and cultured cells. *Clin. Exp. Immunol. 124,* 126–133.

Kapsogeorgou, E.K., Moutsopoulos, H.M., and Manoussakis, M.N. (2001b). Functional expression of a costimulatory B7.2 (CD86) protein on human salivary gland epithelial cells that interacts with the CD28 receptor, but has reduced binding to CTLA4. *J. Immunol. 166,* 3107–3113.

Kapsogeorgou, E.K., Dimitriou, I.D., Abu-Helu, R.F., Moutsopoulos, H.M., and Manoussakis, M.N. (2003). Cultured salivary gland epithelial cells release exosomes that contain the Sjögren's syndrome-associated autoantigenic ribonucleoproteins Ro/SSA and La/SSB. *Arthritis Res. Ther. 5*(Suppl 1), S4.

Kassan, S.S., Thomas, T., 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.

Kauppi, M., Pukkala, E., and Isomaki, H. (1997). Elevated incidence of hematologic malignancies in patients with Sjögren's syndrome compared with patients with rheumatoid arthritis (Finland). *Cancer Causes Control 8,* 201–204.

Kincaid, M.C. (1987). The eye in Sjögren's syndrome. In N. Talal, H.M. Moutsopoulos, S.S. Kassan, (eds.), *Sjögren's syndrome: Clinical and Immunological Aspects*. (pp. 25–34). Berlin: Springer-Verlag,

Kong, L., Ogawa, N., Nakabayashi, T., Liu, G.T., D'Souza, E., McGuff, H.S., Guerrero, D., Talal, N., and Dang, H. (1997). Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum. 40,* 87–97.

Koski, H., Janin, A., Humphreys-Beher, M.G., Sorsa, T., Malmstrom, M., and Konttinen, Y.T. (2001). Tumor necrosis factor-alpha and receptors for it in labial salivary glands in Sjögren's syndrome. *Clin. Exp. Rheumatol. 19,* 131–137.

Lindahl, G., Hedfors, E., Klareskog, L., and Forsum, U. (1985). Epithelial HLA-DR expression and T lymphocyte subsets in salivary glands in Sjögren's syndrome. *Clin. Exp. Immunol. 61,* 475–482.

Manavalan, S.J., Valiando, J.R., Reeves, W.H., Arnett, F.C., Necker, A., Simantov, R., Lyons, R., Satoh, M., and Posnett, D.N. (2004). Genomic absence of the gene encoding T cell receptor Vbeta7.2 is linked to the presence of autoantibodies in Sjögren's syndrome. *Arthritis Rheum. 50,* 187–198.

Manoussakis, M.N., Dimitriou, I.D., Kapsogeorgou, E.K., Xanthou, G., Paikos, S., Polihronis, M., and Moutsopoulos, H.M. (1999). Expression of B7 costimulatory molecules by salivary gland epithelial cells in patients with Sjögren's syndrome. *Arthritis Rheum. 42,* 229–239.

Manoussakis, M.N., Georgopoulou, C., Zintzaras, E., Spyropoulou, M., Stavropoulou, A., Skopouli, F.N., and Moutsopoulos, H.M. (2004). Sjögren's syndrome associated with systemic lupus erythematosus: clinical and laboratory profiles and comparison with primary Sjögren's syndrome. *Arthritis Rheum. 50,* 882–891.

Mariette, X., Ravaud, P., Steinfeld, P., Baron, G., Goetz, J., Hachulla, E., Combe, B., Puechal, X., Pennec, Y., Sauvezie, B., Perdriger, A., Hayem, G., Janin, A., and Sibilia, J. (2004). Inefficacy of Infliximab in primary Sjögren's syndrome. results of the Randomized, Controlled Trial of Remicade in Primary Sjögren's Syndrome (TRIPSS). *Arthritis Rheum. 50,* 1270–1276.

Martens, P.B., Pillemer, S.R., Jacobsson, L.T., O'Fallon, W.M., and Matteson, E.L. (1999). Survivorship in a population based cohort of patients with Sjögren's syndrome, 1976–1992. *J. Rheumatol. 26,* 1296–1300.

Matsumoto, I., Tsubota, K., Satake, Y., Kita, Y., Matsumura, R., Murata, H., Namekawa, T., Nishioka, K., Iwamoto, I., Saitoh,Y., Sumida, T., and Dyck, P.J. (1996). Common T cell receptor clonotype in lacrimal glands and labial salivary glands from patients with Sjögren's syndrome. *J. Clin. Invest. 97,* 1969–1977.

Medsger, T.A., Jr. (1987). Sjögren's syndrome and systemic sclerosis (scleroderma). In N. Talal, H.M. Moutsopoulos, and S.S. Kassan (eds.), *Sjögren's Syndrome: Clinical and Immunological Aspects.* (pp. 182–187). Berlin: Springer-Verlag.

Moutsopoulos, H.M. (1994). Sjögren's syndrome: Autoimmune epithelitis. *Clin. Immunol. Immunopathol. 72,* 162–165.

Moutsopoulos, H.M., Webber, Vlagopoulos, T.P., Chused, T.M., and Decker, J.L. (1979). Differences in the clinical presentation of sicca syndrome in the presence and in the absence of rheumatoid arthritis. *Am. J. Med. 66,* 733–736.

Moutsopoulos, H.M., Chused, T.M., Mann, D.L., Klippel, J.H., Fauci, A.S., Frank, M.M., Lawley, T.J., and Hamburger, M.I.(1980). Sjögren's syndrome (sicca syndrome): Current issues. *Am. Intern. Med. 92,* 212–226.

Niemela, R.K., Takalo, R., Paakko, E., Suramo, I., Paivansalo, M., Salo, T., and Hakala, M. (2004). Ultrasonography of salivary glands in primary Sjögren's syndrome. A comparison with magnetic resonance imaging and magnetic resonance sialography of parotid glands. *Rheumatology 41*, 338–342.

Ogawa, N., Dang, H., Kong, L., Anaya, J.M., Liu, G.T., and Talal, N. (1996). Lymphocyte apoptosis and apoptosis-associated gene expression in Sjögren's syndrome. *Arthritis Rheum. 39,* 1875–1885.

Ogawa, N., Ping, L., Zhenjun, L., Takada, Y., and Sugai, S.. (2002). Involvement of the interferon-gamma-induced T cell-attracting chemokines, interferon-gamma-inducible 10-kd protein (CXCL10) and monokine induced by interferon-gamma (CXCL9), in the salivary gland lesions of patients with Sjögren's syndrome. *Arthritis Rheum. 46,* 2730–2741.

Ohlsson, M., Skarstein, K., Bolstad, A.I., Johannessen, A.C., and Jonsson, R. (2001). Fas-induced apoptosis is a rare event in Sjögren's syndrome. *Lab. Invest. 81,* 95–105.

Ohlsson, M., Jonsson, R., and Brokstad, K.A.(2002). Subcellular redistribution and surface exposure of the Ro52, Ro60 and La48 autoantigens during apoptosis in human ductal epithelial cells: a possible mechanism in the pathogenesis of Sjögren's syndrome. *Scand. J. Immunol. 56,* 456–469.

Ohyama, Y., Nakamura, S., Matsuzaki, G., Shinohara, M., Hiroki, A., Fujimura, T., Yamada, A., Itoh, K., and Nomoto, K. (1996). Cytokine messenger RNA expression in the labial salivary glands of patients with Sjögren's syndrome. *Arthritis Rheum. 39,* 1376–1384.

Oxholm, P., Daniels, T.E., and Bendtzen, K. (1992). Cytokine expression in labial salivary glands from patients with primary Sjögren's syndrome. *Autoimmunity 12,* 185–191.

Paisansinsup, T., Deshmukh, U.S., Chowdhary, V.R., Luthra, H.S., Fu, S.M., and David, C.S. (2002). HLA class II influences the immune response and antibody diversification to Ro60/Sjögren's syndrome-A: heightened antibody responses and epitope spreading in mice expressing HLA-DR molecules. *J. Immunol. 168,* 5876–5884.

Petrone, D., Condemi, J.J., Fife, R., Gluck, O., Cohen, S., and Dalgin, P. (2002). A double-blind, randomized, placebo-controlled study of cevimeline in Sjögren's syndrome patients with xerostomia and keratoconjunctivitis sicca. *Arthritis Rheum., 46,* 748–754.

Pertovaara, M., Pukkala, E., Laippala, P., Miettinen, A., and Pasternack, A. (2001). A longitudinal cohort study of Finnish patients with primary Sjögren's syndrome: clinical, immunological, and epidemiological aspects. *Ann. Rheum. Dis. 60,* 467–472

Pisa, E.H., Pisa, P., Hang, H., 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,* 1245–1250.

Polihronis, M., Tapinos, N.I., Theocharis, S.E., Economou, A., Kittas, C., and Moutsopoulos, H.M. (1998). Modes of epithelial cell death and repair in Sjögren's syndrome (SS). *Clin. Exp. Immunol. 114,* 485–490.

Price, E.J., Brooks, S.M., Shattles, W.S., and Venables, P.J.W. (1994). Antibodies to Ro and La in the salivary pathology of Sjögren's syndrome. In M. Homma, S. Sugai, T. Tojo, N. Miyakaka, and M. Akizuki (eds.), *Sjögren's Syndrome. State of the Art.* (pp 157–160). Amsterdam: Kugler.

Ramos-Casals, M., Garcia-Carrasco, M., Brito Zeron, M.P., Cervera, R., and Font, J. (2002). Viral etiopathogenesis of Sjögren's syndrome: role of the hepatitis C virus. *Autoimmun. Rev. 1,* 238–243.

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(SSA)/La(SSB) autoantibody responses. *J. Immunol. 146,* 3871–3875.

Rosen, A., Casciola-Rosen, L., and Ahearn, J. (1995). Novel packages of viral and selfantigens are generated during apoptosis. *J. Exp. Meet. 181,* 1557–1561.

Routsias, J.G., Touloupi, E., Dotsika, E., Moulia, A., Tsikaris, V., Sakarellos, C., Sakarellos-Daitsiotis, M., Moutsopoulos, H.M., and Tzioufas, A.G. (2002). Unmasking the anti-La/SSB response in sera from patients with Sjögren's syndrome by specific blocking of anti-idiotypic antibodies to La/SSB antigenic determinants. *Mol. Med. 8,* 293–305.

Saegusa, J., Yasuda, A., Kubota, H., and Ito, T. (1994). Spontaneous sialoadenitis in IQI/JCL mice. A new animal model of primary Sjögren's syndrome. In M. Homma, S. Sugai, T. Tojo, N. Miyakaka, and M. Akizuki. (eds.), *Sjögren's Syndrome. State of the Art.* (pp. 559–561). Amsterdam: Kugler.

Salomonsson, S., Jonsson, M.V., Skarstein, K., Brokstad, K.A., Hjelmstrom, P., Wahren-Herlenius, M., and Jonsson, R. (2003). Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjögren's syndrome. *Arthritis Rheum. 48,* 3187–3201.

Skopouli, F.N., Fox, P.C, Galanopoulou. V., Atkinson, J.C, Jaffe, E.C., and Moutsopoulos, H.M. (1991). T cell subpopulations in the labial minor salivary gland histopathologic lesion of Sjögren's syndrome. *J. Rheumatol. 18,* 210–214.

Skopouli, F.N., Kousvelari, E., Mertz, P., Jaffe, E.S., Fox, P.C., and Moutsopoulos, H.M. (1992). C-myc mRNA expression in minor salivary glands of patients with Sjögren's syndrome. *J. Rheumatol.19,* 693–699.

Skopouli, F.N., Dafni, U., Ioannidis, J.P., et al. (2000). Clinical evolution, and morbidity and mortality of primary Sjögren's syndrome. *Semin. Arthritis Rheum. 29,* 296–304.

Skopouli, F.N., Talal, N., Galanopoulou, V., Tsampoulas, C.G., Drosos, A.A., and Moutsopoulos H.M. (1990) Raynaud's phenomenon in primary Sjögren's syndrome. *J. Rheumatol. 17,* 618–620.

Slobbe, R.L., Pruijn, G.J.M., and Van Venrooij, W.J. (1991). Ro(SSA) and La(SSB) ribonucleoprotein complexes: Structure, function and antigenicity. *Ann. Med. Interne 142,* 592–600.

Soliotis, F.C., Mavragani, C.P., and Moutsopoulos, H.M. (2004). Central nervous system involvement in Sjögren's syndrome. *Ann. Rheum. Dis. 63,* 616–620.

St. Clair, E.W. (1992). Anti-La antibodies. *Rheum. Dis. Din. N. Am. 18,* 359–377.

St. Clair, E.W., Angellilo, J.C., and Signer, K.H. (1992). Expression of cell adhesion molecules in the salivary gland microenviroment of Sjögren's syndrome. *Arthritis Rheum. 35,* 62–66.

Stiller, M., Golder, W., and Doring, E. (2000). Primary and secondary Sjögren's syndrome in children—a comparative study. *Clin. Oral Invest. 4,* 176–182.

Theander, E., Manthorpe, R., Jacobsson, L.T.H. (2004). Mortality and causes of death in primary Sjögren's syndrome. A prospective cohort study. *Arthritis Rheum. 50*, 1262–1269.

Trevino, H., Tsaianos, E.B., and Schenker, S. (1987) In N. Talal, H.M., Moutsopoulos, and S.S. Kassan (eds.), *Sjögren's syndrome. Clinical and Immunolotical Aspects.* Gastrointestinal and hepatobiliary features in Sjögren's syndrome. Berlin: Springer-Verlag. Pp. 8895.

Triantafyllopoulou, A., Tapinos, N., and Moutsopoulos, H.M. (2004). Evidence for coxsackievirus infection in primary Sjögren's syndrome. *Arthritis Rheum. 50*, 2897–2902.

Tsunawaki, S., Nakamura, S., Ohyama, Y., Sasaki, M., Ikebe-Hiroki, A., Hiraki, A., Kadena, T., Kawamura, E., Kumamaru, W., Shinohara, M., and Shirasuna, K. (2002). Possible function of salivary gland epithelial cells as non-professional antigen-presenting cells in the development of Sjögren's syndrome. *J. Rheumatol. 29,* 1884–1896.

Tumiati, B., Casoli, P., and Parmeggiani, A. (1997). Hearing loss in Sjögren's syndrome. *Ann. Intern. Med. 126,* 450–453.

Tzioufas, A.G., Wassmuth, R., Dafni, U.G., Guialis, A., Haga, H.J., Isenberg, D.A., Jonsson, R., Kalden, J.R., Kiener, H., Sakarellos, C., Smolen., J.S., Sutcliffe, N., Vitali, C., Yiannaki, E., and Moutsopoulos, H.M. (2002). Clinical, immunological, and immunogenetic aspects of autoantibody production against Ro/SSA, La/SSB and their linear epitopes in primary Sjögren's syndrome (pSS): a European multicentre study. *Ann. Rheum. Dis. 61,* 398–404.

Van de Merwe, J.P., Kamerling, R., Arendsen, H.S., Mulder, A.H., and Hooijkaas, H. (1993). Sjögren's syndrome in patients with interstitial cystitis. *J. Rheumatol. 20,* 962–966.

Vitali, C., Bombardieri, S., Moutsopoulos. H.M., Balestrieri, G., Bencivelli, W., Bernstein, R.M., Bjerrum, K.B., Braga, S., Coll, J., and de Vita, S. (1993). Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum. 36,* 340–347.

Vitali, C., Bombardieri, S., Moutsopoulos. H.M., and the European Study Group on Diagnostic Criteria for Sjögren's syndrome (1996). Assessment of the European classification criteria for Sjögren's syndrome in a series of clinically defined cases: Results of a prospective multicentre study. *Ann. Rheum. Dis. 55,* 116–121.

Vitali, C., Bombardieri, S., Jonsson, R., Moutsopoulos, H.M., Alexander, E.L., Carsons, S.E., Daniels, T.E., Fox, P.C., Fox, R.I., Kassan, S.S., Pillemer, S.R., Talal, N., Weisman, M.H., and the European Study Group on Classification Criteria for Sjögren's Syndrome (2002). Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann. Rheum. Dis. 61,* 554–558.

Vivino, F.B., Al-Hashimi, I., Khan, Z., LeVeque, F.G., Salisbury, P.L., 3rd, Tran-Johnson, T.K., Muscoplat, C.C., Trivedi, M., Goldlust, B., and Gallagher, S.C. (1999). Pilocarpine tablets for the treatment of dry mouth and dry eye symptoms in patients with Sjögren's syndrome. *Arch. Intern. Med. 159,* 174–181.

Vlachoyiannopoulos, P.G. and Moutsopoulos, H.M. (1997). Therapy in Sjögren's syndrome. In L.B.A. Van de Putte, D.E. Furst, H.J. Williams, P.L.C.M. Van Riel (eds), *Therapy of Systemic Rheumatic Disorders*. (pp. 615–628). New York: Marcel Dekker.

Voulgarelis, M., Dafni, U.G., Isenberg, D.A., and Moutsopoulos, H.M. (1999). Malignant lymphoma in primary Sjögren's syndrome: a multicenter, retrospective, clinical study by the European Concerted Action on Sjögren's Syndrome. *Arthritis Rheum. 42,* 1765–1772.

Waterman, S.A., Gordon, T.P., and Rischmueller, M. (2000). Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjögren's syndrome. *Arthritis Rheum. 4,* 1647–1654.

Winer, S., Astsaturov, I., Cheung, R., Tsui, H., Song, A., Gaedigk, R., Winer, D., Sampson, A., McKerlie, C., Bookman, A., and Dosch, H.M. (2002). Primary Sjögren's syndrome and deficiency of ICA69. *Lancet 360,* 1063–1069.

Xanthou, G., Tapinos, N.I., Polihronis, M., Nezis, I.P., Margaritis, L.H., and Moutsopoulos, H.M. (1999). CD4 cytotoxic and dendritic cells in the immunopathologic lesion of Sjögren's syndrome. *Clin. Exp. Immunol.118,* 154–163.

Xanthou, G., Polihronis, M., Tzioufas, A.G., Paikos, S., Sideras, P., and Moutsopoulos, H.M. (2001). "Lymphoid" chemokine messenger RNA expression by epithelial cells in the chronic inflammatory lesion of the salivary glands of Sjögren's syndrome patients. *Arthritis Rheum. 44,* 408–418.

Yannopoulos, D.I., Roncin, S., Lamour, A., Pennec, Y.L., Moutsopoulos, H.M., and Youinou, P. (1992). Conjunctival epithelial cells from patients with Sjögren's syndrome inappropriately express major histocompatibility complex molecules, La/SSB antigen and heat shock proteins. *J. Clin. Immunol. 12,* 259–265.

Zandbelt, M.M., Vogelzangs, J., Van De Putte, L.B., Van Venrooij, W.J., and Van Den Hoogen, F.H. (2004). Anti-alpha-fodrin antibodies do not add much to the diagnosis of Sjögren's syndrome. *Arthritis Res. Ther. 6,* R33–R38.

CHAPTER

32

Rheumatoid Arthritis

SARAH MACKIE, MARK QUINN, AND PAUL EMERY

Academic Unit of Musculoskeletal Medicine, University of Leeds, Great George Street, Leeds, United Kingdom

Rheumatoid arthritis (RA) is a systemic inflammatory disorder characterized by symmetrical synovitis of peripheral joints and tendon sheaths. Without treatment there is progressive damage to soft tissue, cartilage, and bone, resulting in deformity and disability. Rheumatoid factor, an autoantibody that binds the constant region (Fc) of immunoglobulin, is usually present. Subcutaneous nodules and involvement of other organs may also occur.

Until the nineteenth century, there was no concept of RA as a disease entity. Descriptions of inflammatory arthritis date back to antiquity, but these contain insufficient detail to clearly differentiate RA from gout, except arguably in a few writings from Eastern cultures (Sturrock et al., 1977). Rheumatoid arthritis was first described in the medical literature by Landré-Beauvais in 1800, and was first named by Garrod in 1859. Paleopathologic evidence suggests that RA may have existed at least since the seventh century in Europe and for several millennia in North America (Aceves-Avila et al., 2001).

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Clinical studies utilize the classification of RA developed by the American College of Rheumatology (ACR), which is shown in Box 32.1 (Arnett et al., 1988). Because these tend to favor active RA, they may be modified for use in population studies (MacGregor et al., 1994). However, the ACR criteria are less useful in diagnosing RA of less than 12 weeks duration (Green et al., 1999). Assessments of disease severity include measures of joint inflammation (disease activity) and the rate of joint destruction. Predictors of future severe disease include the presence of rheumatoid factor (RF), the rapid appearance of erosions, shared-epitope positivity (discussed below), active disease at onset, extra-articular features, and poor functional status at disease onset.

Epidemiologic Features

The prevalence of RA is approximately 1% in the United States and Western Europe (Lawrence et al., 1998; Symmons et al., 2002). In some other populations, such as China, Japan and some black populations in rural South Africa, estimates of the prevalence are as low as 0.2–0.3%

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Box 32.1
The 1987 American Rheumatology Association criteria: at least four criteria must be fulfilled for classification as rheumatoid arthritis (RA)

1. Morning stiffness of joints—lasting at least 1 hour before maximal improvement for at least 6 weeks
2. Arthritis in 3 or more joint areas—soft tissue swelling or effusion in 3 or more joint areas simultaneously for at least 6 weeks
3. Arthritis of hand joints—swelling of wrist, metacarpophalangeal, or proximal interphalangeal joints for at least 6 weeks
4. Symmetric arthritis—simultaneous involvement of the same joint areas bilaterally for at least 6 weeks
5. Rheumatoid nodules—subcutaneous nodules over bony prominences, extensor surfaces, or in juxta-articular regions
6. Rheumatoid factor—detected by a method that is positive in <5% of normal controls
7. Radiographic changes—typical RA changes on hand and wrist radiographs, e.g., erosions, peri-articular osteopenia

Adapted from Arnett et al. (1988) ©Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley and Sons, Inc.

(Adejabo, 1995); while in certain Native American tribes, such as the Pima Yakima and Chippewa Indians, the prevalence is over 5% (Jacobsson and Pillemer, 1994). In most populations, RA is 2 to 3 times as common in women as in men, particularly in the premenopausal years.

Estimates of the incidence of RA in Westernized countries have shown a peak at 45–55 years of age for women and a continued increase up to 75 years for men (Wiles et al., 1999). However, the incidence of RA may be in decline over recent decades (Doran et al., 2002).

Clinical Features

Articular

The classical presentation of RA is that of a symmetrical inflammatory polyarthritis. The metacarpophalangeal joints, wrists, and metatarsophalangeal joints are most commonly involved at the onset, but any synovial joint may be affected. The inflammation primarily affects the synovial membrane—the lining of the joint; this is termed synovitis. The tendon sheath lining may also become inflamed. The inflammation causes the joints to become painful, swollen, and stiff after rest. As disease advances, progressive damage to bone and soft-tissues occurs, with consequent joint subluxation or deformity. Ulnar deviation and volar subluxation at the wrists and metacarpophalangeal joints are common. The fingers classically exhibit "swan neck" or "boutonniere" deformities. The cervical spine may also become involved.

Early radiographic signs of RA include soft-tissue swelling, peri-articular osteopenia, joint-space loss, and bony erosions. These erosions tend to appear at characteristic sites, such as the ulnar styloid and fifth metatarsophalangeal joints, and contribute to deformity. Serial radiographs are often used to monitor progression of RA, but ultrasound and magnetic resonance imaging are more sensitive in the detection of subclinical synovitis and early erosions. Such studies suggest that bone damage occurs in proportion to the level of synovitis but not in its absence (Conaghan et al., 2003).

TABLE 32.1 Extra-articular features of rheumatoid arthritis

System	Extra-articular features
Skin	Subcutaneous rheumatoid nodules
Lung	Fibrosing alveolitis Obliterative bronchiolitis Pleuritis Pleural nodules
Cardiac	Pericarditis/pericardial effusion Myocardial fibrosis
Eye	Scleritis Episcleritis Scleromalacia perforans Keratoconjunctivitis sicca
Bone	Osteoporosis
Hematologic	Felty syndrome
Neurologic	Peripheral nerve compression Peripheral sensory neuropathy Mononeuritis multiplex Cord compression Transverse myelitis

Extra-articular

Active RA may produce fatigue, low-grade fever, or weight loss. Extra-articular features, systemic vasculitis or amyloidosis, may be observed, particularly in late or severe RA (Table 32.1, Figures 32.1 and 32.2).

Prognosis

Most studies show that RA is associated with higher mortality, often due to respiratory disease, cardiovascular disease, and malignancy, including non-Hodgkin lymphoma and lung cancer (Mellemkjaer et al., 1996; Symmons et al., 1998). Early diagnosis and successful treatment of

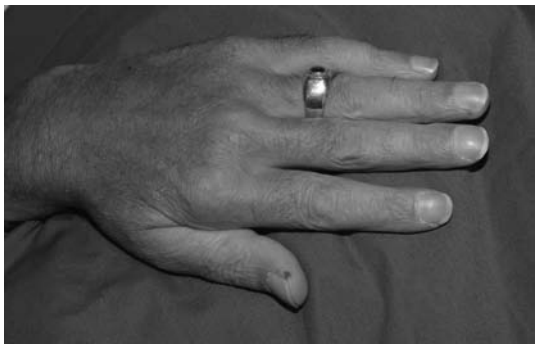


FIGURE 32.1 Rheumatoid arthritis with nailfold vasculitis (see color plate section).

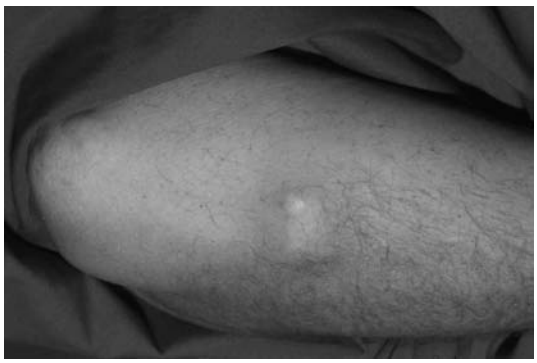


FIGURE 32.2 Rheumatoid nodule on extensor aspect of forearm (see color plate section).

inflammation with methotrexate are associated with better mortality rates (Symmons et al., 1998; Krause et al., 2000). Rheumatoid arthritis also carries an increased risk of infections and of osteoporosis.

Pathologic features

Normal Synovium

The synovial membrane lines the joint capsule. The lining layer (intima) is made up of cells called synoviocytes; lacking a basement membrane, it is supported directly by the subintima layer. The subintima contains loose connective tissue, blood vessels, lymphatics, and nerve endings. The synovial fluid lubricates and nourishes the adjacent cartilage.

Most synoviocytes are "fibroblast-like." These, also called type B synoviocytes, express adhesion molecules and synthesize constituents of the synovial fluid and the extracellular matrix. Others (type A synoviocytes) express macrophage markers and originate from bone marrow-derived monocytes. In this chapter, the terms "fibroblast" and "macrophage" are used as convenient terms for these two synoviocyte populations, but in RA these cells may take on altered properties such that exact correspondence with other fibroblasts and macrophages cannot be assumed.

Synovium in Rheumatoid Arthritis

In RA, synoviocytes of both types proliferate. The inflamed synovium also contains many new blood vessels, including specialized high endothelial venules that are formed early in RA, allowing leukocytes to move from the bloodstream into the tissues. Subintimal blood vessels are surrounded by $CD4^+$ T cells. The synovial membrane contains T and B lymphocytes and macrophages, sometimes in structures resembling lymphoid follicles or even germinal centers (Duke et al., 1982); synovial plasma cells produce autoantibodies. Neutrophils accumulate in synovial fluid, which becomes a fibrinous inflammatory exudate. Local release of matrix-degrading enzymes and inflammatory mediators promotes damage to adjacent cartilage (Zvaifler and Firestein, 1994).

In end-stage RA, fibrous scar tissue replaces most of the synovial cells and blood vessels. Ultimately the cartilage too is destroyed and the joint may fuse.

AUTOIMMUNE FEATURES

Evidence for the role of autoimmunity in RA is substantial, to the extent that it is sometimes regarded as a prototype autoimmune disease. The presence of RF and the association with other autoimmune diseases such as autoimmune hypothyroidism provided early support for this view. Strong evidence in support of an autoimmune basis for RA is provided by the observation that it may be ameliorated by immunomodulation by many different modalities, whether physiological (such as pregnancy), pathologic [RA tends to improve as $CD4^+$ counts decline with human immunodeficiency virus (HIV) infection], or pharmacologic (a wide variety of immunosuppressant drugs are used in treatments).

The role of humoral autoimmunity is supported by the presence of RF and other autoantibodies, discussed more fully below. Rheumatoid factor is produced in the synovium of affected joints, and its presence in the serum is related to adverse outcomes. Lately there has been much interest in antibodies against citrullinated epitopes, which are present in the joints of patients with RA. Commercial availability of serologic tests for these "anti-cyclic citrullinated peptide" (anti-CCP) antibodies has led to interesting data. Like RF, anti-CCP antibodies may antedate the development of clinical RA by several years (Nielen et al., 2004), and their presence predicts more aggressive disease. However, the corollary to this is that the presence of RF or anti-CCP does not appear necessary for the development of RA, nor is the presence of either or both sufficient to produce

disease. Yet the effectiveness of depletion of B-cell precursors in the treatment of RA, discussed below, is intriguing, since symptomatic response may correlate more closely with levels of RA-related autoantibody than with total immunoglobulin.

The role of T-cell-based autoimmunity is supported most strongly by the association with certain major histocompatibility complex type II (MHC II) haplotypes; although no single T-cell reactant appears unique to RA, certain self-antigens, such as BiP, are interesting (see section on B cells, below). Therapies directed against T cells, such as total lymphoid irradiation, cyclosporine, anti-CD4 therapy (Isaacs et al., 1997), and CTLA4-Ig (see below) tend to ameliorate RA. Research into new T-cell-directed therapies has already led to the introduction of drugs such as leflunomide into the clinical armamentarium.

Finally, diseases resembling RA may be produced experimentally in animals by various immunomodulatory strategies, as discussed below. These serve to illustrate the wide variety of immunologic mechanisms by which a similar clinical picture may be produced. By definition, this clinical picture must feature an inflammatory arthritis, and thus strategies directed primarily at inflammation, ranging in potency from nonsteroidal anti-inflammatory drugs (NSAIDs) to anti-tumor necrosis factor (TNF) therapies, are still among the most reliable strategies to relieve the symptoms of RA.

The concept of a self/non-self dichotomy in immunology has given way to more complex models in which innate and adaptive immunity interact with each other and with immunoregulatory mechanisms. It is difficult, therefore, to entirely separate autoimmune from inflammatory features, since innate immunity is so closely interwoven with both. Examples of such interactions will be given throughout the section dealing with pathogenic mechanisms.

GENETIC FEATURES

Twin studies in Finland and the UK suggest a genetic influence on the etiology of RA. Compared with a prevalence of 1% in the general population, a sibling or a dizygotic twin of a patient with RA has a 4% risk of developing RA, and monozygotic twin concordance is 15–20% (Aho et al., 1986). Epidemiologic data suggests that RA has a heritability of 50–60%, of which half may be attributed to the HLA genes (MacGregor et al., 2000).

HLA-DR4 is present in about 70% of North American and European white patients with RA, compared with a background prevalence of 28% in the non-RA population. In other populations, such as Israeli Jews, RA is associated with HLA-DR1.

The different RA-associated HLA-DR subtypes have in common a "shared epitope," a particular sequence of amino acids (residues 67–74: LLEQKRAA in HLA-DRB1*0401; LLEQRRAA in *0404, *0405, and *0101; and LLERRRAA in *1001) on the third hypervariable region of the HLA-DR β chain (Gregersen et al., 1987). Approximately 90% of Western Europeans with RA possess at least one HLA-DR carrying the shared epitope, and these patients have a higher risk of severe (seropositive, erosive, and extra-articular) disease (McDonagh et al., 1997). The prevalence of the shared epitope in RA is affected by the ethnicity of the background population, but the risk of severe disease given shared-epitope-positive RA seems similar in many populations (del Rincon et al., 2003). Other amino acid patterns in the 67–74 region of HLA may conversely be protective against RA (de Vries et al., 2002). A possible association between shared-epitope-negative RA and presence of the shared epitope in the mother of the RA patient has been explored in some studies (Harney et al., 2003).

No individual gene provides such a strong association with RA as the HLA region, necessitating larger population cohorts. Candidate gene approaches have yielded various associations in selected cohorts, but interesting results from high-throughput techniques in genomics are generating new hypotheses. The results of whole-genome linkage screens using DNA microsatellite data from sibling pairs affected by RA are also not always replicated in other cohorts (Eyre et al., 2004). Linkage analysis using single-nucleotide polymorphisms (SNPs) has yielded an association with the (*PADI4*) gene in Japanese RA (Suzuki et al., 2003), but this result has not been replicated in a UK population (Barton et al., 2004). An association with a missense polymorphism of a protein tyrosine phosphatase has also been identified in RA as in several other autoimmune diseases (Begovich et al., 2004).

ENVIRONMENTAL INFLUENCES

Disentangling genetic and environmental features is difficult. One approach taken is to compare the prevalence of RA exposed to different environmental conditions, but associations with factors such as urbanization and breastfeeding have not always been replicated in other studies. Smokers appear to have an increased risk of developing seropositive RA (Stolt et al., 2003), particularly if they are shared-epitope-positive (Padyukov et al., 2004). Evidence for the role of infection and the related "hygiene hypothesis" remains controversial (Carty et al., 2003).

ANIMAL MODELS

Autoimmune arthritis may be induced in animals by various methods: by immunization with cartilage components, by non-specific immune stimulation, by infectious

agents, or by genetic manipulation. None of these models exactly resembles human RA, but nevertheless may provide useful information relating to pathogenesis and treatment of autoimmune arthritis.

Most traditional models of autoimmune arthritis featurea stimulus to overcome normal immunologic tolerance to self-antigens, often by introducing immunostimulatory foreign material in close proximity to joint-related self-antigens. For example, collagen-induced arthritis (CIA) is induced by immunizing mice with rat type II collagen. This produces T cells directed against autologous cartilage antigens, causing a destructive synovitis. The disease can be transferred to an unaffected animal by T-cell transfer. A particular epitope of the rat collagen (peptide 256–270) was shown to be immunodominant in CIA (Malmstrom et al., 1996); glycosylation of this peptide was important (Corthay et al., 1998). Mice transgenic for RA-associated HLA molecules, which bind to the 261–273 peptide of type II collagen, are particularly susceptible to CIA (Andersson et al., 1998).

Transfer of monoclonal antibodies specific to native type II collagen into naïve mice, however, induces an acute form of arthritis that could be potentiated by lipopolysaccharide stimulation and displayed massive neutrophil infiltration (Nandakumar et al., 2003).

Streptococcal cell wall (SCW) arthritis is induced by intraperitoneal injection of SCW fragments in the Lewis rat. This produces a relapsing-remitting arthritis, the severity of which correlates with the level and persistence of SCW antigen within synovium. The arthritis is initially T-cell independent; it requires intact Toll-like receptor 2 (TLR-2) signaling (Joosten et al., 2003) and involves the alternative complement pathway. Later, T-cell-dependent mechanisms supervene (van den Broek, 1990).

Adjuvant-induced arthritis (AIA) in rats is produced by immunization with *Mycobacterium tuberculosis* in oil. The immunodominant epitope involved is the mycobacterial heat-shock protein (hsp) hsp65, which has a homolog in rats. AIA can also be induced in certain rat strains (presumably against self epitopes) by injecting adjuvant or pristane alone into the joint. In both AIA and pristane-induced arthritis, the oil is taken up by macrophages but is non-degradable, so that the macrophages are persistently activated and "primed" towards development of an autoreactive adaptive immune response. Interestingly, preimmunization with mycobacterial hsp65 or DNA coding a particular human hsp60 epitope (Quintana et al., 2003) protects rats not only against AIA but against various other experimental arthritides (including CIA and SCW arthritis); this protection appears to mediated by Th2 cytokine-secreting regulatory T cells (van Eden and Waksman, 2003), which normally compensate for the incomplete tolerance to self-hsp65 in Lewis rats (Durai et al., 2004).

Some animal models feature high levels of RF secretion, but the clinical manifestations often more closely resemble systemic lupus erythematosus (SLE) than RA. For example, the MRL/lpr mouse has a reduced expression of the Fas protein, involved in apoptosis, and produces high levels of RF in addition to SLE-type autoantibodies. Another example is the BAFF-overexpressing mouse; this cytokine influences peripheral B-cell numbers and may perhaps "rescue" autoreactive B cells from deletion (Lesley et al., 2004). The BAFF transgene produces an SLE-like syndrome featuring RF- and SLE-related autoantibodies such as antibodies against double-stranded DNA.

The K/BxN mouse, a non-derived mouse with a transgene T-cell receptor, develops a spontaneous destructive arthritis mediated by antibodies against glucose-6-phosphate isomerase (G6PI) (Kouskoff et al., 1996). Glucose-6-phosphate isomerase is a ubiquitous intracellular protein, but may also be found extracellularly over the cartilage surface of normal joints (Matsumoto et al., 2002). Production of these antibodies requires T cells and occurs only in lymph nodes that drain the affected joints (Mandik-Nayak et al., 2002). Transfer of anti-G6PI antibodies to healthy mice produces a transient arthritis that does not require the presence of B or T cells (Korganow et al., 1999). The antibodies cause disease via Fc receptors and complement (Ji et al., 2002), but localization of antibodies to the joint depends on mast cells, neutrophils, and Fc receptors (Wipke et al., 2004).

A different spontaneous T-cell dependent model of arthritis is produced by a mutation in ZAP-70, a T-cell signaling protein (Sakaguchi et al., 2003). Abnormal T-cell signaling produces alteration in T-cell selection in the thymus resulting in autoreactive T cells. Mice with the disease show elevated IgG-RF and antibodies to type II collagen but not to double-stranded DNA. The disease may be transferred by $CD4^+$ T cells into nude mice, but not by transfer of serum. ZAP-70 signaling is also suppressed in patients with RA, but this seems to be a consequence of the systemic inflammation rather than a gene mutation; it improves with successful treatment of RA.

Animal models demonstrate the wide variety of mechanisms that may produce a similar phenotype of autoimmune arthritis. For example, mechanisms of innate immunity may be the initiating trigger or the final effector mechanism; some autoantibodies are sufficient to cause arthritis, whereas others are not; and a pathogenic T-cell repertoire can be produced by means of a single transgenic T-cell receptor or by affecting T-cell signaling more globally. However, none of these animal models exactly resemble RA (many resembling psoriatic arthritis more closely), and treatments that work in animal models do not always translate well into clinical practice. Nevertheless, these approaches will continue to produce fascinating insights into autoimmunity and to generate hypotheses relevant to human disease.

PATHOGENIC MECHANISMS

Different mechanisms are likely to be responsible for initiation, perpetuation, and chronic inflammation (Thomas and Lipsky, 1996). The presence of autoantibodies and the HLA association suggest that the adaptive immune system is involved in RA. An exogenous or endogenous "danger" signal from the innate immune system may be necessary for an adaptive autoimmune response to develop (Matzinger, 2002). A failure of self tolerance may also implicate dysfunctional regulatory T cells or natural killer T (NKT) cells.

Cytokines are present in complex networks that may be self-reinforcing (Firestein and Zvaifler, 1990). Inflammation may be also perpetuated by the continued ingress of im-mune cells, due to signals from adhesion molecules and chemokines, and by the antigen-specific amplification inherent in adaptive immunity. Defective apoptosis of immune cells may also perpetuate inflammation, and the stromal environment may play a role in this (Salmon et al., 1997); the fibroblasts themselves also tend to proliferate rather than undergo apoptosis. Potential mechanisms by which cells in RA resist apoptosis include the apoptosis inhibitor, Fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein (FLIP), in RA macrophages (Perlman et al., 2001), activation of NFκB, and overexpression of the signaling molecules Bcl-2 and Stat-3 (Liu and Pope, 2003), and of heat shock proteins such as hsp70 (Mosser et al., 1997).

Role of Shared Epitope

Given the location of the shared epitope on the class II HLA gene, its effect on susceptibility and severity of RA (see above) is likely to operate via an interaction with T cells. However, the exact mechanism of this remains unclear (Buckner and Nepom, 2002); broadly speaking, it may permit autoreactive T cells to escape peripheral or central tolerance. Some theoretical possibilities follow and are laid out in Table 32.2.

1. The MHC has a vital role in generating normal self-tolerance, and is involved in both central and peripheral mechanisms of this. One of the mechanisms of peripheral tolerance involves regulatory T cells. The shared epitope might be involved in a failure of regulatory T cells to keep autoreactivity in check.
2. The shared epitope may predispose to immunologic tolerance to an important pathogen-related peptide, causing an inadequate immune response and persistence of the pathogen, theoretically triggering the onset of RA. Epstein–Barr virus, for example, expresses a protein with amino-acid sequence homology to the shared epitope.
3. The shared epitope may predispose to presentation of a particular self peptide, triggering autoimmunity. However, the identity of such an "arthritogenic" self peptide is unclear, partly because of epitope spreading, whereby autoimmunity comes to involve progressively greater numbers of epitopes from the target organ. Some novel autoantibodies that are relatively specific for RA have lately been discovered, but the role of the shared epitope in producing T-cell help for the production of such autoantibodies remains undefined. One suggestion is that the shared epitope may cause imperfect intracellular antigen processing—for example, compared with non-RA associated HLA DR*04 alleles, the rheumatoid arthritis-associated HLA class II alleles DR*0401, *0404 and 0405 form relatively unstable DR-CLIP (class II associated Ii peptide) complexes, which may result in a tendency to present non-CLIP self peptides (Patil et al., 2001).
4. The shared epitope may predispose to presentation of a particular foreign peptide, which then cross-reacts with a self peptide. For example, the shared epitope may tend to present a peptide from dnaK (the 70 kDa heat shock protein of *Escherichia coli*), which has a natural ligand that has a QKRAA sequence; other sequences on dnaK have homology with sequences of type II collagen (Auger and Roudier, 1997).

TABLE 32.2 Possible mechanisms by which the shared epitope might confer susceptibility to rheumatoid arthritis

	Predisposition towards decreased immune reactivity	**Predisposition towards increased immune reactivity**
Immune response to endogenous (self) antigen	1. Impaired response of regulatory T cells that are necessary for maintaining peripheral tolerance to autoantigen	3. Shared epitope predisposes to presentation of self peptide, e.g., intracellular self peptide; *or* shared-epitope peptide sequence itself is immunogenic
Immune response to exogenous (foreign) antigen	2. Shared epitope predisposes to impaired defense against pathogen; pathogen persists and itself affects the immune system	4. Defense against pathogens is overexuberant, leading to cross-reactivity to self antigens by "molecular mimicry"

T Cells

There is substantial evidence for the role of T cells in RA. The strong association with HLA-DR and the shared epitope, the Th1 dominance in RA synovium, the evidence for both $CD4^+$ and $CD8^+$ T-cell activation in RA, and the involvement of T cells in many animal models are all supportive; the efficacy of T cell-directed therapies such as total lymphoid irradiation, thoracic duct drainage, cyclosporine, leflunomide, and costimulatory blockade provides further evidence.

The expression of early activation markers such as CD69 by synovial, but not peripheral blood, T cells suggests that T-cell activation occurs within the inflamed joint (Iannone et al., 1996). Activated RA T cells, via expression of CD154 (CD40), are capable of activating B cells and dendritic cells (MacDonald et al., 1997). Polyclonal T cells enter the joint in established RA, so that T-cell receptor repertoire studies have failed to consistently identify a particular pathogenic T-cell clone. The pathogenic autoantigen could be related to any of the targets of the RA-associated autoantibodies described below, and may not be the same in every individual. Mechanisms potentially involved in breaking tolerance have been discussed in the section above, and include molecular mimicry (perhaps by the effects of infection) and the appearance of new epitopes, or modification of existing epitopes, with ongoing inflammation or joint damage—citrullination of joint-related antigens, discussed below, provides a potential example of this.

The cytokine profile in RA is broadly Th1-like, but is rather atypical in that there are high levels of TNF-α and moderate levels of interferon-γ (IFN-γ), but minimal interleukin 2 (IL-2). T cells in RA exist in a state of partial activation, as shown by their markers (expression of IL-2 receptor is limited [Emery et al., 1988]), by functional tests *in vitro* (the autologous mixed lymphocyte response is impaired [Bergroth et al., 1989], as is the response to mitogens or recall antigens), and by anergy to tuberculin testing *in vivo* (Emery et al., 1984). $CD4^+$ T cells in RA are also atypical in that they possess some cytotoxic functions and receptors more usually associated with natural killer (NK) cells (Weyand et al., 2003). They have impaired ability to generate a clonal burst or to undergo activation-induced cell death or apoptosis (Salmon et al., 1997; Szodoray et al., 2003).

T-cell function seems to improve when RA is treated. Cytokines such as TNF-α (Cope, 2003) or IFN-β (Pilling et al., 1999), and oxidative stress can all alter T-cell function *in vitro*; defective thymic output of T cells has also been proposed as a primary cause (Weyand et al., 2003).

Regulatory T cells have a role in maintaining peripheral tolerance of autoantigens, and evidence from animal models suggests that they have the potential to suppress autoimmune disease (see above). Regulatory T cells operate by various mechanisms, including direct cell–cell contact and by secretion of cytokines such as TGF-β, causing "bystander suppression" of immune cells in the locality of the antigen recognized by the regulatory T cell. This is a rapidly-developing field of research. For example, RA synovium contains $CD25^+CD4^+$ T cells with the capability to inhibit responses of other T cells (Cao et al., 2003); it is as yet unclear what role these play in either promoting or limiting the autoimmunity of RA.

B Cells

The presence of RF was originally thought to be pathognomic of RA, and thus RA was originally conceptualized as an autoantibody-driven disease. Localization of immune complexes to the joint, and the presence of polyclonal B-cell activation in RA provide further evidence for a role for B cells. However, early studies showed that infusion of RF-containing serum into healthy volunteers did not elicit arthritis. Rheumatoid arthritis may occur in the absence of RF, although presence of RF correlates strongly with severity and extra-articular manifestations. Some autoantibodies, such as anti-G6PI, even appear to be correlated with severity and extra-articular manifestations but not with susceptibility to RA (van Gaalen et al., 2004a). Rheumatoid factor and anti-CCP antibodies may be detected in asymptomatic patients years before the development of clinical RA; 49% of RA patients were positive for either IgM-RF or anti-CCP on at least one occasion before the development of RA, compared with 1% and 0.6% respectively for patients who did not later develop RA (Nielen et al., 2004). These results suggest that these autoantibodies might have a role in pathogenesis of early RA, but demonstrate that their presence alone is not sufficient to elicit clinical disease. Evidence that would support a pathogenic role for an autoantibody in RA would include the demonstration of T cells reacting to the same antigen in RA, class switching, and affinity maturation. These have been described for various candidate autoantibodies, as will be seen below.

Beyond production of autoantibodies, B cells can also act as antigen presenting cells with or without costimulatory molecules to induce T-cell activation and expansion. B cells also produce a variety of cytokines, including lymphotoxin, IL-6, and IL-10 (Dorner and Burmester, 2003). They appear to play a role in formation of lymphoid organs and germinal centers and influence the activation and regulation of dendritic cells and effector T cells. It has been suggested that a similarity between the molecules expressed by synoviocytes to those expressed by follicular dendritic cells may promote B-cell persistence in RA synovium (Edwards and Cambridge, 1995).

Rheumatoid factor is produced by normal individuals, usually transiently, in response to events such as infection or vaccination; it preferentially binds IgG that is aggregated,

such as IgG bound in immune complexes; therefore, it may have a physiological role in modulating the effects of small immune complexes, or in stimulating RF B cells to present antigens contained in those immune complexes. The RF produced due to such stimuli is usually germline-encoded and of IgM isotype, but in RA there is evidence of class switching and affinity maturation, suggesting the involvement of RF-specific T-helper cells. A requirement for activation of TLRs of RF B cells (Leadbetter et al., 2002) may help explain why more immunoglobulin is produced in affected joints and draining lymph nodes. RF B cells can be triggered by T cells specific for a variety of foreign antigens (Roosnek and Lanzavecchia, 1991).

Various novel autoantibodies have recently been described in RA; many appear to be directed against "cryptic" antigens, those exposed in the context of cell stress or joint damage (Table 32.3).

Many of these autoantibodies recognize citrullinated autoantigens. For example, anti-filaggrin antibodies, antiperinuclear factor, antikeratin, and anti-Sa antibodies all recognize citrullinated epitopes. Citrullination tends to occur when inflammatory cells undergo apoptosis, activating the enzyme peptidyl arginine deiminase. Sera from different patients with RA react with different combinations of citrullinated antigens, such that no particular peptide is dominantly antigenic. An enzyme-linked immunosorbent assay (ELISA) detecting antibodies reacting to CCP is available for clinical use; epidemiologic studies have revealed an association with the shared epitope (van Gaalen et al., 2004b). This is interesting, since citrullinated peptides can be shown to fit into the P4 pocket of the shared epitope, but the homologous arginine peptides do not (Hill et al., 2003). B cells from the synovial fluid of anti-CCP-positive RA patients spontaneously produce anti-CCP antibodies, but B cells from the peripheral blood of these patients do not. This suggests antigen-driven maturation of CCP-specific B cells at the site of inflammation in RA (Reparon-Schuijt et al., 2001). Anti-Sa antibodies fall into at least two populations: one, recognizing 50 and 46 kDa antigenic determinants, is highly specific for RA and tends to be elevated during flares of disease; the other, recognizing the 68 kDa Sa antigen, is also observed in patients with SLE and tends to be seen in RA patients during remissions (Escalona et al., 2002).

The RA33 antigen, a shuttling protein involved in mRNA transport, is overexpressed in RA synovium; Th1-polarized T cells directed against RA33 have also been identified in RA (Fritsch et al., 2002). While anti-RA33 antibodies are described in RA, they are not specific for the disease, being also associated with multisystem autoimmune diseases.

BiP, a chaperone protein, migrates onto the cell surface under conditions of cell stress, and is overexpressed in RA synovium. Many patients with RA have T cells reactive to BiP (Blass et al., 2001).

Neutrophils

Neutrophils within RA synovium are involved in generating reactive oxygen species, and proinflammatory and chemoattractant molecules. They also produce vascular endothelial growth factor (VEGF) (Kasama et al., 2000). They express class II MHC, but not the costimulatory molecules CD80 and CD86 (Cross et al., 2003). Synovial fluid has an antiapoptotic effect on neutrophils (Ottonello et al., 2002).

Synoviocytes

As discussed above, macrophage-like synoviocytes are capable of antigen presentation whereas fibroblast-like synoviocytes in RA express high levels of proteases that degrade cartilage, and may exhibit properties similar to those of follicular dendritic cells.

Fibroblast-like synoviocytes in RA appear "transformed" in some respects (Zvaifler and Firestein, 1994). They produce high levels of TGF-β and platelet-derived growth factor (PDGF), perhaps stimulating their own growth in autocrine fashion, and evidence of monoclonal expansion in the pannus area may be observed (Imamura et al., 1998). Synoviocytes from subjects with RA co-implanted into

TABLE 32.3 Some novel autoantibodies in rheumatoid arthritis

Antibody	Antigen	Sensitivity (%)	Specificity (%)
Antikeratin	Filaggrin (citrullinated portion)	40	92–99
Anti-cyclic citrullinated peptides	Deiminated arginine in molecules such as filaggrin, fibrin	53	96
Anti-RA33	Heterogenous nuclear ribonucleoprotein A2	32	90–96 in absence of connective tissue disease
Anti-Sa	Unknown	42	98
Anti-p68	BiP (formerly known as grp78)	40	96

Adapted from Steiner and Smolen (2002) with permission from the publishers, BioMed Central.

SCID mice adjacent to articular cartilage are capable of cartilage destruction that is independent of inflammatory cell infiltration; unlike conventionally "transformed" cells, however, their proliferative capacity is limited (Seemayer et al., 2003). The pathogenic significance of this is controversial, but RA synoviocytes can be shown to exhibit reduced apoptosis *in vivo* and are resistant to apoptotic signals mediated via CD95 (Fas) and TNF-α *in vitro*. Embryonic genes are expressed by synovial fibroblasts in RA (Sen et al., 2000).

Cytokines and Soluble Mediators

Many proinflammatory cytokines are produced in RA (Feldmann et al., 1996). They may form self-perpetuating networks; however, disease reactivates once treatment with IL-1 or TNF-α blockers is stopped, suggesting that cytokine networks are not the sole mechanism of disease persistence in RA. The following is limited to a brief overview of a complex field.

Most of the cytokines in RA synovium (Table 32.4) appear to derive from macrophages rather than from T cells; levels of T-cell products such as IFN-γ and IL-2 are relatively scanty (Firestein et al., 1990), although there is debate regarding whether the observed levels of IFN-γ are biologically significant. The cytokine profile displays a bias towards a Th1 type (Simon et al., 1994; Steiner et al., 1999), although this may vary according to the histologic features (Klimiuk et al., 1997).

In RA, IL-1 and TNF-α are principally produced by macrophages, and in smaller quantities by B cells, T cells, and (in the case of TNF-α) fibroblasts (Firestein et al., 1990). Injection of these cytokines into animal joints synergistically produces synovitis (Henderson and Pettipher, 1989). They have many actions, including stimulation of T cells, B cells, macrophages, and fibroblasts. IL-1 and TNF-α induce expression of chemokines and vascular adhesion molecules (ICAM1, E-selectin and VCAM), which enable recruitment of leucocytes including polyclonal memory T cells (Sedgwick et al., 2000). Both TNF-α and IL-1 induce expression of further IL-1, of many other cytokines, and of matrix- and tissue-degrading enzymes. Bone metabolism is also affected: Il-1 regulates production of osteoprotegerin (OPG); IL-1 and TNF-α cause osteo-

TABLE 32.4 Expression of some cytokines and chemokines in RA synovial tissue

	Cells			
mRNA and protein	**Monocytes**	**Fibroblasts**	**T cells**	**Dendritic cells**
IL-1 α and β	•	•		
IL-1Ra	•	•		
IL-2			○	
IL-6	•	•		
Il-8 (chemokine)	•	•		
IL-10	•		•	
IL-12	•			•
IL-15	•	•		
IL-17			•	
IL-18	•	•		
TNF-α	•	•		
IFN-γ			○	
GMCSF	•	•		
PDGF	•			
VEGF	•			
FGF	•	•		
TGF-β	•	•		
LIF	•	•		
MCP-1	•	•		
MIP-1α	•	•		
RANTES		•		

•, expressed; ○, expressed at low levels; FGF, fibroblast growth factor; GMCSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; LIF, leukocyte inhibitory factor; MCP-1, monocyte chemotactic protein 1; MIP-1α, monocyte inflammatory protein 1α; PDGF, platelet-derived growth factor; RANTES, regulated on activation, normal T expressed and secreted; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Adapted from Arend (2001)

blasts to increase production of receptor activator of NFκB ligand (RANKL). Effects of RANKL include maturation of osteoclast precursors and bone resorption by mature osteoclasts.

Interleukin 6 is produced by a wide range of cells in RA, including fibroblasts under the influence of IL-1 and TNF-α. In combination with its soluble receptor, sIL-6R, IL-6 induces both B- and T-cell proliferation, and promotes differentiation of macrophages, osteoclasts and megakaryocytes. Interleukin 6 also stimulates production of acute phase proteins by the liver.

Interleukin 12, produced by dendritic cells and activated macrophages, increases production of IFN-γ by $CD4^+$ T cells and NK cells, biasing the cytokine response towards a Th1 pattern.

Interleukin 15 is a cytokine, produced primarily by macrophages, with structural similarities to IL-2. It is chemotactic for activated T cells and promotes the production of IL-1, TNF-α and IL-6 (McInnes et al., 1997).

Interleukin 17 is produced by a small number of T cells in RA synovium, but synergizes with IL-1 or TNF-α and is proinflammatory in its action on fibroblasts (Miossec, 2003).

Interleukin 18 shares a signal transduction pathway with IL-1, and is produced by macrophages and fibroblasts. In synergy with IL-15 and IL-12, it increases the production of TNF-α and IFN-γ (Liew et al., 2003).

Macrophage migration inhibitory factor (MIF) is a cytokine of unique structure with many proinflammatory effects, including upregulation of IL-1, TNF-α, prostaglandins, and matrix metalloproteinases (Morand et al., 2003). It induces proliferation of fibroblast-like synoviocytes via extracellular signal regulated protein kinase (ERK) mitogen-activated protein (MAP) kinase rather than NfκB (Lacey et al., 2003). Levels of synovial MIF are elevated in RA and correlate with disease activity.

In RA, there is inflammation despite compensatory upregulation of immunoregulatory cytokines and cytokine antagonists, such as IL-10, IL-13, TGF-β and IL-1Ra and the soluble TNF-α receptors. Interleukin 10, from synovial fibroblasts, inhibits production of proinflammatory cytokines by macrophages and may contribute to the low level of production of IL-2 and IFN-γ by synovial T cells. Transforming growth factor-β is profibrotic and chemotactic for monocytes. Interleukin 1Ra, produced by mature macrophages and by stimulated intrasynovial neutrophils, is a competitive antagonist of IL-1 at its receptor binding site.

Growth Factors

Growth factors upregulated in RA include PDGF and fibroblast growth factor (FGF), which may have autocrine effects, and VEGF, a major angiogenic factor.

Chemokines

Chemokines comprise four supergene families according to their cysteine residue structure: C-X-C, C-C, C, and C-X_3-C (Szekanecz et al., 2003).

Most chemokine ligands act on more than one chemokine receptor within each supergene family. The receptors are differentially expressed on various inflammatory cells (Katschke et al., 2001; Shadidi et al., 2003). Integrins and chemokine receptors may direct organ-specific lymphocyte homing; in RA, some of this specificity seems to be lost (Buckley, 2003).

The C-X-C family contains ligands such as IL-8 (CXCL8), which is produced in large quantities by macrophages and fibroblasts in RA synovium and has proinflammatory and proangiogenic effects; it influences leukocyte chemotaxis, adhesion molecule expression, cell–matrix interactions, and the respiratory burst of neutrophils. CXCL9 and 10 are also produced by RA synovium (Patel et al., 2001), and their receptor CXCR3 is expressed on almost all T cells infiltrating the joint in RA, but only a minority of circulating T cells in these patients (Qin et al., 1998). Another member of this family, SFD-1 (CXCL12) has its own receptor, CXCR4, is also proangiogenic and may play a role in retention of T cells within the RA synovium.

C-C chemokines such as MCP-1 (CCL2), MIP-1α (CCL3) and RANTES (CCL5) are all produced in large amounts by RA synoviocytes and have roles in chemotaxis and activation of macrophages and in promotion of angiogenesis. T cells infiltrating high endothelial venules in RA synovium bear the receptor CCR5 in addition to CXCR3 (Patel et al., 2001).

The C family includes lymphotactin (XCL1), which seems to play a role in T-cell chemotaxis in RA (Wang et al., 2004).

The C-X_3-C family has a sole member, fractalkine (CX3CL1), which has its own receptor, CX3CR1. Effects in RA may include monocyte chemotaxis and angiogenesis.

Complement

C4 depletion in synovial fluid is evidence of complement activation in RA (Ruddy and Austen, 1975; Konttinen et al., 1996). Complement's many roles may be relevant in RA pathogenesis, including neutrophil chemotaxis, opsonization, and local inflammation. Low levels of complement may be associated with serious extra-articular manifestations of RA, although the association is not sufficient to be clinically useful (Saraux et al., 2001).

Pro- and Anti-Angiogenic Factors

In RA, angiogenesis (neovascularization) occurs and may play a significant role in joint damage. In early RA, high

synovial vascularity on magnetic resonance imaging (MRI) or Doppler ultrasound correlates with increased joint erosions (Taylor, 2002). Angiogenesis is promoted by many factors, including VEGF, VCAM1, and E-selectin. Other stimulators of angiogenesis include angiogenin, the angiopoietins (Ang-1 and Ang-2), G-CSF, IL-8, TNF-α, FGF, epidermal growth factor (EGF), insulin-like growth factor (IGF) and TGF-β; inhibitors include IL-12, IFN-γ and tissue inhibitor of metalloproteinases (TIMP). The joint in RA is hypoxic compared with normal joints and this induces expression of HIF-1α; in addition to optimizing intracellular metabolism for hypoxic conditions, hypoxia inducible factor-1α (HIF-1α) regulates transcription of mediators such as VEGF. Production of VEGF is also induced in RA synovial T cells by cytokines TNF-α and IL-1. The same appears true of many other proangiogenic factors including αVβ3 integrin.

Adhesion Molecules

The intercellular adhesion molecule 1 (ICAM1), expressed widely in RA synovium and induced by TNF-α, IL-1 or IFN-γ, helps recruit lymphocytes, monocytes, and neutrophils.

The vascular cell adhesion molecule 1 (VCAM1) is expressed by the RA synovial intima and by activated endothelium. It is upregulated by IL-1, TNF-α, IFN-γ, and IL-4. It binds α4β1 integrin (found on lymphocytes and macrophages) and also α4β7 integrin, expressed by many synovial lymphocytes as well as those homing to gut lymphoid tissue.

Recognition of Pathogen-Associated Molecular Patterns

Pathogen-associated molecular patterns (PAMPs) are recognized by pattern-recognition receptors (PRRs), which play a key role in innate immunity in the recognition of pathogens or of cellular injury. Macrophage mannose receptors and scavenger receptors help mediate phagocytosis. Non-phagocytic immune cells may be directly activated by TLRs. TLR4 may be activated not only by bacterial lipopolysaccharide but also by hsp60, hsp70, and fibronectin, all present within the inflamed joint. In a serum transfer model, the intracellular effects of TLR4 and Il-1 activation were similar (Choe et al., 2003). TLR2 expression by fibroblasts is upregulated by inflammatory cytokines (Siebl et al., 2003) and its effects are mediated by nuclear factor-κB (NFκB).

Small amounts of bacterial mRNA may be detected in inflamed joints, mainly relating to commensal skin and gut organisms (Kempsell et al., 2000). These commensal organisms are unculturable and do not cause clinical evidence of joint sepsis.

Intracellular Signaling Pathways

The NFκB pathway is activated by a wide range of inflammatory and immune stimuli such as cytokines, TLR engagement, and cellular stress. NFκB is a transcription factor that, when released from inhibition by cytoplasmic IκB, translocates to the nucleus and induces transcription of many proinflammatory cytokines, such as TNF, IL-6 and IL-1β; enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2); and adhesion molecules. NFκB also inhibits apoptotic cell death. The NFκB pathway thus appears to summate diverse immune signals and to be an important local regulator of inflammation. Expression of both NFκB, particularly in macrophage-like synoviocytes, and members of the activator protein 1 (AP-1) transcription factor family can be demonstrated in RA (Handel et al., 1995); they are synergistically activated by cytokines such as TNF-α and IL-1 (Granet et al., 2004). It is notable that steroids, which are among the most reliably effective drugs for RA, inhibit the NFκB and AP-1 pathways; various other drugs used for RA also inhibit NFκB. Specific inhibitors of NFκB dramatically ameliorate disease manifestations in mouse models of erosive inflammatory arthritis (Clohisy et al., 2003).

Rheumatoid arthritis synovium shows activation of the mitogen- and stress-activated protein kinase pathways: p38 MAP kinase, ERK, and c-Jun amino-terminal kinase (JNK) (Schett et al., 2000). Each of these pathways is predominant in a particular region of the synovial membrane. For example, the p38 pathway predominates in synovial endothelium and mediates angiogenesis, chemoattraction and vasodilatation; the JNK pathway mediates Th1 differentiation of T cells, and cytokine and MMP expression by mononuclear cells.

Pathogenesis of Bony Erosions

Recently, data has emerged that osteoclast recruitment, maturation, activation and osteolysis are regulated by RANKL in synergy with IL-1 and TNF-α (Romas et al., 2002a). RANKL is produced in RA by activated T cells and synovial fibroblasts. In rat AIA, T-cell activation leads to a RANKL-mediated increase in osteoclasts and bone loss (Kong et al., 1999). Knockout of RANKL, or administration of analogs of OPG, the soluble decoy receptor to RANK, blocks bone destruction in animal models (Romas et al., 2002b) while having little effect on cartilage damage (Pettit et al., 2001).

Enzymes Involved in Tissue Damage

Zinc-dependent metalloproteinases include matrix metalloproteinases (MMPs), transmembrane ADAMs (e.g., the TNF-α converting enzyme [TACE]) and soluble ADAMTS

such as the aggrecanases ADAMTS-4 and -5. Tissue inhibitors of metalloproteinases (TIMPs) inhibit many of these. Matrix metalloproteinase levels are increased in RA. These enzymes cleave matrix proteins, such as collagen and aggrecan, and may activate or inactivate various inflammatory mediators. It has been proposed that products of MMP activity may be immunogenic in themselves (Descamps et al., 2003). The TNF-α converting enzyme causes shedding from the cell membrane of TNF-α, soluble TNF receptors, and fractalkine. Serine and cysteine proteases, such as cathepsins, also contribute to degradation of the cartilage matrix.

Many key inflammatory mediators participate in pathways of tissue damage. For example, the inducible COX-2 is overexpressed in RA synovium, causing increased production of proinflammatory prostaglandins, including PGE2. Nitric oxide, which is involved in both cartilage and bone damage, is produced by iNOS. Reactive oxygen intermediates are generated by the respiratory burst of neutrophils and contribute to local tissue damage.

Neurotransmitters and Mechanical Stress

RA spares the joints on the side of a pre-existing hemiparesis (Thompson and Bywaters, 1961). This may be partly due to a proinflammatory effect of mechanical stress: cultured synovial fibroblasts produce heat shock proteins in response to shear stress (Schett et al., 1998), and joint areas subject to greater mechanical stress exhibit greater inflammation in RA (Tan et al., 2003). However, AIA in rats is less severe in limbs previously treated with the sympathetic blocker guanethidine, suggesting that sympathetic neurotransmitters or cotransmitters may also play a role in inflammation. Neuropeptides such as substance P, released locally on stimulation of sensory nerves, have proinflammatory effects; substance P has been shown to stimulate RA synoviocytes to proliferate via the neurokinin-1 receptor (Lotz et al., 1987). Antagonists of this receptor have shown promise in animal models but remain untested in RA.

Summary: Interaction of Pathogenic Mechanisms

With such a large number of interdependent factors involved in pathogenesis of RA, many different models of pathogenesis might be constructed. Most of these would involve predisposing factors such as the genetic background (including shared epitope) and the existing T-cell repertoire, which controls tolerance to autoantigens. For example, shared-epitope positivity may predispose to the development of anti-CCP antibodies, which are specific for RA and may precede clinical symptoms by some years. The trigger for disease initiation seems most likely to be a stimulus, such as infection or injury, which causes activation of innate immunity in the joint. This would activate local dendritic cells, fibroblast-like synoviocytes and macrophages. Dendritic cells would travel to the local lymph nodes and there present auto-antigen to T cells. T cells allow B cells to produce high-affinity, class-switched autoantibodies, perhaps initially in the local lymph node and later within the joint itself. Chemokines and adhesion molecules expressed by the affected joint would stimulate the ingress of T cells and other inflammatory cells, such as neutrophils. There the T cells would interact with dendritic cells and with macrophage- and fibroblast-like synoviocytes, eliciting production of pro-inflammatory cytokines, tissue-degrading enzymes and partial transformation of the synoviocytes into an erosive, proliferating pannus. Once established, autoimmune joint inflammation would become self-perpetuating by means of a number of factors, and spread to a characteristic distribution of joints, perhaps due to mechanical and neurogenic factors. Extra-articular features usually develop in the context of severe disease in the presence of high titer RF and are associated with other autoantibodies such as anti-G6PI; whether these directly cause the extra-articular features is unclear.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

As yet no single immunologic marker is in itself diagnostic of RA, particularly in early disease when early treatment has the maximum potential to modify disease progression. However, in combination with careful clinical assessment and radiographic imaging studies, positive immunological tests may be extremely useful in increasing the certainty of the diagnosis of RA.

Rheumatoid factor is an autoantibody directed against the Fc region of IgG. An elevated RF is included in the ACR classification criteria for rheumatoid arthritis. Between 70% and 90% of patients with RA have RF, but levels may be lower in early RA. Rheumatoid factor may also be present in the normal elderly and in many other diseases, including Sjögren syndrome, SLE, and other rheumatological conditions, and in certain malignancies and chronic infections. Patients with RA who are positive for RF are classed as seropositive. A negative RF does not exclude RA and, in the presence of other defining criteria, the patients are classed as seronegative.

Rheumatoid factor can be any class of immunoglobulin but IgM-RF, IgA-RF, and IgG-RF are the most common. The RFs were traditionally detected by agglutination of sheep red cells, but more modern agglutination tests tend to use latex beads. Agglutination assays tend to favor the detection of IgM-RF. Quantitative analyzer-based nephelometric assays are now frequently used and results are standardized against a reference preparation; the results appear equiva-

lent to the latex test in diagnostic utility (Wolfe, 1998). These assays are reported to detect other classes of RF. Over 90% of patients with established RA have IgM-RF and a high titer increases the risk of severe, extra-articular, nodular disease particularly if IgG-RF and/or IgA-RF are also present. The combination of IgM-RF and IgA-RF appears highly specific for RA (Jonsson et al., 1998). A number of international external quality assurance schemes are available for total RF. Specific ELISAs for IgM-, IgG-, and IgA-RF are now widely available. These can be highly specific, but their performance depends on the quality of the detection system. There are no widely-used quality assurance schemes.

Several other autoantibodies have been described that appear to have greater specificity for RA. However, these assays were not convenient for widespread laboratory use. Antikeratin antibodies (AKA) can be detected using immunofluorescence to rat esophageal epithelium, antiperinuclear factor (APF) using immunofluorescence to the keratohyalin granules of human buccal mucosa epithelium, and antifilaggrin antibody (AFA) by immunoblot. Combinations of these assays increase sensitivity in diagnosis of RA (Ferraro-Peyret et al., 2002). It is now realized that these assays detect closely related autoantibodies. Both AKA and APF recognize filaggrin-related epitopes (Sebbag et al., 1995). The major target for AFA in RA synovium is deiminated (citrullinated) fibrin (Masson-Bessiere et al., 2001). Anti-Sa antibodies also recognize a similar epitope. Enzyme immunoassays were, therefore, developed for antibodies to a linear filaggrin peptide and subsequently to cyclic peptide variant of the deiminated filaggrin epitope (CCP). Second-generation ELISA-based anti-CCP kits are now available which appear to outperform the older assays in diagnosis of RA (Dubucquoi et al., 2004). Anti-CCP antibodies have specificity of 98% and sensitivity 68% in the diagnosis of established RA, and perform nearly as well in diagnosing early RA (Schellekens et al., 2000). In addition to their value in diagnosis, particularly in RF-negative patients, anti-CCP antibodies are also associated with aggressive disease and the development of radiologic erosions. Positive anti-CCP antibodies can predate clinical symptoms of RA by many years.

TREATMENT AND OUTCOME

Established Treatments

The treatment of patients with RA requires the collaboration of many healthcare professionals. First-line, symptomatic therapy is usually with NSAIDs, which relieve symptoms of inflammation but do not appear to alter the progression of disease or its prognosis. Some newer NSAIDs are more selective for the COX-2 isoenzyme and have been shown to have a lower incidence of gastrointestinal complications, but controversies remain over whether the COX-2 drugs may also increase the risk of thrombotic complications relative to standard NSAIDs.

The course of RA is characterized by episodic flares and remissions, superimposed on progressive damage to bones and joints. Most patients require long-term therapy to reduce the frequency and severity of flares: usually this requires immunomodulatory disease-modifying antirheumatic drugs (DMARDs), such as methotrexate, sulphasalazine or gold. In many cases, these are very effective (Pincus et al., 2002), but their onset of action is slow: they may take months to achieve their full effect. Leflunomide is the newest drug in this class and is more T-cell targeted. Much evidence suggests that early, aggressive therapy of RA matters more in the short and long term than which particular DMARD is chosen. The first DMARD used appears often to be the most effective (Aletaha and Smolen, 2002). However, although clinical parameters improve, radiographic progression may continue (Mulherin et al., 1996).

The modern treatment approach emphasizes early DMARD therapy to reduce long-term disability (Emery, 1994). However, a significant proportion of patients experience disease activity and progression of RA "resistant" to all traditional DMARDs. Systemic corticosteroids are effective in most patients, but serious adverse effects commonly occur if significant doses of steroid are continued long-term.

Biologic drugs are drugs designed to target particular steps in the pathogenesis of RA, either by mimicking endogenous molecules or administering monoclonal antibodies specific for an endogenous molecule. Currently, the principal class of biologics in clinical use is the anti-TNF class. The first of these was infliximab, a chimeric IgG1-type monoclonal antibody with murine variable domains. Infliximab binds soluble and membrane-bound TNF with high affinity, forming stable immune complexes and preventing TNF from binding to its receptor. These drugs may have dramatic efficacy, even in patients resistant to multiple conventional DMARDs, in rapidly reducing the inflammatory response and clinical disease activity, and retarding or even halting radiographic progression (Maini et al., 1999; Klareskog et al., 2004). Anti-TNF drugs have revolutionized clinical management of patients with RA, and have transformed expectations for the future from empirical immunosuppression to the hope of targeted, individualized therapies. Tumor necrosis factor-α has been conceptualized as a "dominant cytokine" in RA and it has been shown that blockade of the TNF-α pathway has benefit on many of the different characteristic aspects of RA, including synoviocyte proliferation, bony erosion, and angiogenesis (Table 32.5).

The anti-TNF drugs, however, have limitations. They are not universally efficacious, and an initial response may be lost after a period of treatment. Blockade of TNF-α is associated with an increased incidence of infections, including

TABLE 32.5 Anti-tumor necrosis factor (TNF) drugs currently licensed for treatment of rheumatoid arthritis

Anti-TNF drug	Structure	Administration	Action *in vitro*
Infliximab	Chimeric mouse/human IgG1, binding soluble and membrane-bound TNF-α	Intravenous	Binds soluble TNF-α, lyses cells carrying surface TNF-α
Etanercept	Fusion protein of Fc of IgG1 with p75 TNF-α receptor	Subcutaneous	Binds soluble TNF-α and lymphotoxin α
Adalimumab	As for infliximab but fully humanized	Subcutaneous	Similar to infliximab

encapsulated organisms. The drugs may cause a vasculitic syndrome, perhaps related to the commonly-observed induction of antinuclear antibodies or other autoantibodies. Problems with drug tolerability may range from non-specific infusion or injection site reactions to a frank anaphylacticoid reaction. Antichimeric antibodies develop in many patients receiving infliximab, but the clinical significance of this is unclear. There is not yet sufficient data to confirm or refute a theoretical association with malignancy. When anti-TNF drugs are given for established RA, efficacy is maintained only during the period of treatment; however, there is some emerging evidence to suggest that if given in early RA there may be a benefit lasting longer than the duration of treatment.

Anakinra, an IL-1Ra analog, is a biologic drug targeted at IL-1 mediated pathways. Although there is some evidence that it is moderately efficacious, perhaps particularly regarding bony erosions, of treatment benefit is relatively short-term and current clinical practice favors use of anti-TNF drugs in preference. Patients who do not respond to anti-TNF do not tend to respond to anakinra (Buch et al., 2004). Combination of anti-TNF drugs with anakinra does not seem to increase the benefit over treatment with anti-TNF alone, and is associated with an increased risk of infections (Genovese et al., 2004).

Other potential biologic targets will be discussed in the following section; their clinical utility currently remains largely unproven.

Experimental Drug Treatments

The success of anti-TNF therapy has stimulated much further interest in experimental therapies for RA. Some past, present and future directions of research are here briefly alluded to (see Chapter 76).

Intensive, Nonspecific Immunosuppression

Alemtuzumab (Campath-1H) is a monoclonal antibody directed against the lymphocytic antigen CD52, expressed on both B and T cells; it has some efficacy in RA, but this was only maintained in the short term (Schnitzer et al., 1997). High-dose chemotherapy and autologous bone marrow transplantation has been performed in some patients with severe RA resistant to conventional therapies; a temporary remission was achieved, but responsiveness to traditional DMARDs was often subsequently restored (Snowden et al., 2004).

T Cells

T-cell depletion is effective in RA but is too toxic to consider as a therapy. Anti-CD4 therapy produces some clinical response; this may relate to T-cell coating rather than to T-cell depletion (Mason et al., 2002).

A more subtle modulation of T-cell function is provided by costimulatory blockade. For an antigen-presenting cell to activate a T cell, rather than render it anergic or apoptotic, requires costimulation by CD80 or CD86. Once a T cell is fully activated, it expresses cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), which blocks CD80 or CD86 with very high avidity. Abatacept, a synthetic fusion protein of CTLA-4 with an immunoglobulin domain, is designed to mimic this signal and thereby block T-cell activation. This drug shows promising results in the treatment of RA in combination with methotrexate (Kremer et al., 2003).

Since regulatory T cells seem to be present in RA, another strategy might be to stimulate these cells, perhaps by means of molecules such as heat shock protein (Prakken et al., 2002).

Apoptosis

Induction of $CD4^+$ cell apoptosis using anti-CD95 (Fas) monoclonal antibody suppresses osteoclastogenesis in an animal model (Ogawa et al., 2003). Apoptosis of fibroblasts in RA synovium is mediated by the receptor TRAIL-R2, which represents a potential therapeutic target (Ichikawa et al., 2003).

Angiogenesis

Targeting angiogenesis in RA would be a novel approach since theoretically it would be less likely to predispose to infection or malignancy, although it could disrupt certain

other biologic processes such as fracture healing and fertility. Soluble VEGF receptor 1 is effective in CIA, and an anti-VEGF antibody has now been developed as a cancer treatment.

B Cells

Depletion of immature B cells using the anti-CD20 antibody rituximab appears efficacious in RA (Leandro et al., 2002); recent data demonstrate that response may be sustained up to 48 weeks (Edwards et al., 2004). Plasma cells and total levels of immunoglobulin are unaffected, but RA-specific autoantibodies are reduced. B-cell development may be targeted at a slightly later stage by targeting the B-cell survival factor BLyS, which is normally produced from monocytes. Antagonizing this, either by an anti-BLys monoclonal antibody or by giving TACI-Fc (a modified version of BLyS receptor), inhibits mature B-cell development and the ability of B cells to act as antigen presenting cells.

Cytokines, Chemokines, and Complement

With the success of anti-TNF therapies, attention has been directed to developing small-molecule TNF inhibitors, such as the PEGylated humanized anti-TNF fragment CDP870 (Choy et al., 2002a). Other strategies directed at TNF include inhibitors of TACE. It is hoped that a synthetic IL-1 binding molecule or IL-1 "trap," composed of human IgG1 Fc and the extracellular domain of the IL-1 receptor, will prove to be more potent than anakinra in RA.

Apart from anti-TNF and anti-IL-1 strategies, most targeted cytokine therapies, such as administration of recombinant human IL-4 or IL-10 (Smeets et al., 1999), have failed to demonstrate efficacy in RA. However, results of trials of anti-IL-6 receptor antibody are encouraging (Choy et al., 2002b; Nishimoto et al., 2004). Blockade of other cytokines such as IL-15 or IL-23 also shows promise.

It is hoped that blocking chemokine receptors may block leukocyte entry into affected joints. This has been demonstrated for CCR5 and CXCR2 in animal studies, and early clinical trials of CCR1 blockers show some promise (Haringman et al., 2003). A C5a antagonist has also been used with some success in animal models.

Other Targets

Many other therapeutic strategies might be described as more anti-inflammatory than immunologic in nature. Blockade of integrins or other adhesion molecules appears promising in animal models. Inhibitors of intracellular signaling molecules, such as NFκB and p38 MAP kinase, are theoretically attractive but must be made specific enough to avoid toxicity. Inhibiting osteoclasts reduces the rate of radiographic progression of RA, as shown by encouraging results with zolendronic acid. Strategies targeting the RANKL pathway also show promise. Matrix-degrading enzymes are another therapeutic target that has received some attention. However, the concern with therapeutic modalities that inhibit inflammation or other essential processes is that the treatment could cause toxicity by interfering with the physiological role of these processes. The hope of specific immunologic therapies is that, since RA is an autoimmune process, single or combination immunotherapies can act as a "master switch" that reverses the immunologic dysregulation that is presumed to drive the disease.

CONCLUDING REMARKS—FUTURE PROSPECTS

Rheumatoid arthritis is a rapidly-expanding field of knowledge. New animal models have challenged old conceptions of the disease. Developments in high-throughput techniques of genomics and proteomics promise further clues towards the pathogenesis of RA; further advances in our understanding of tolerance and immunoregulation will shed light on the key events leading to autoimmunity in RA. Attention will be directed also to the factors contributing to disease heterogeneity in RA. The advent of anti-TNF therapy has revolutionized the field and encouraged the development of further novel therapies. The characterization of specific autoantibodies and the efficacy of B-cell depletion has challenged paradigms of T-cell directed therapy in RA. Close cooperation between clinicians and scientists will continue to yield valuable insights as we move from an era of relentless disease progression in the face of nonspecific immunosuppression to a new era of early administration of individualized, probably combination therapies based on advances in our understanding of the pathogenesis of RA.

References

Aceves-Avila, F.J., Medina, F., and Fraga, A. (2001). The antiquity of rheumatoid arthritis: a reappraisal. *J. Rheumatol. 28,* 751–757.

Adebajo, A.O. (1995). Tropical rheumatology. Epidemiology and community studies: Africa. *Ballières Clin. Rheumatol. 9,* 21–30.

Aho, K., Roskenvuo, M., Tuominen, J., and Kaprio, J. (1986). Occurrence of rheumatoid arthritis in a nationwide series of twins. *J. Rheum. 13,* 899–902.

Aletaha, D. and Smolen, J.S. (2002). The rheumatoid arthritis patient in the clinic: comparing more than 1,300 consecutive DMARD courses. *Rheumatology 41,* 1367–1374.

Andersson, E.C., Hansen, B.E., Jacobsen, H., Madsen, L.S., Andersen, C.B., Engberg, J., Rothbard, J.B., McDevitt, G.S., Malmstrom, V., Holmdahl, R., Svejgaard, A., and Fugger, L. (1998). Definition of MHC and T cell receptor contacts in the HLA-DR4 restricted immunodominant epitope in type II collagen and characterization of collagen-induced arthritis in HLA-DR4 and human CD4 transgenic mice. *Proc. Natl. Acad. Sci. U.S.A. 23,* 7574–7579.

Arend, W.P. (2001). Physiology of cytokine pathways in rheumatoid arthritis. *Arthritis Rheum. 45,* 101–106.

Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H., Luthra, H.S., Mesdger, T.A., Mitchell, D.M., Neustadt, D.H., Pinalis, R.S., Schaller, J.G., Sharp, J.T., Wilder, R.L., and Hunder, G.G. (1988). The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum. 31,* 315–334.

Auger, I. and Roudier, J. (1997). A function for the QKRAA amino acid motif, mediating binding of DnaJ to DnaK. Implications for the association of rheumatoid arthritis with HLA-DR4. *J. Clin. Invest. 99,* 1818–1822.

Barton, A., Bowes, J., Eyre, S., Spreckley, K., Hinks, A., John, S., and Worthington, J. (2004). A functional haplotype of the PADI4 gene associated with rheumatoid arthritis in a Japanese population is not associated in a United Kingdom population. *Arthritis Rheum. 50,* 1117–1121.

Blass, S., Union, A., Raymackers, J., Schumann, F., Ungethum, U., Muller-Steinbach, S., De Keyser, F., Engel, J.M., and Burmester, G.R. (2001). The stress protein BiP is overexpressed and is a major B and T cell target in rheumatoid arthritis. *Arthritis Rheum*, *44*, 761–771.

Begovich, A.B., Carlton, V.E., Honigberg, L.A., Schrodi, S.J., Chokkalingam, A.P., Alexander, H.C., Ardlie, K.G., Huang, Q., Smith, A.M., Spoerke, J.M., Conn, M.T., Chang, M., Chang, S.Y., Saiki, R.K., Catanese, J.J., Leong, D.U., Garcia, V.E., McAllister, L.B., Jeffery, D.A., Lee, A.T., Batliwalla, F., Remmers, E., Criswell, L.A., Seldin, M.F., Kastner, D.L., Amos, C.I., Sninsky, J.J., and Gregersen, P.K. (2004). A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am. J. Hum. Genet. 75,* 330–337.

Bergroth, V., Tsai, V., and Zvaifler, N.J. (1989). Differences in responses of normal and rheumatoid arthritis peripheral blood T cells to synovial fluid and peripheral blood dendritic cells in allogeneic mixed leukocyte reactions. *Arthritis Rheum. 32,* 1381–1389.

Buch, M.H., Bingham, S.J., Seto, Y., McGonagle, D., Bejarano, V., White, J., and Emery, P. (2004). Lack of response to anakinra in rheumatoid arthritis following failure of tumor necrosis factor alpha blockade. *Arthritis Rheum. 50,* 725–728.

Buckner, J.H., and Nepom, G.T. (2002). Genetics of rheumatoid arthritis: is there a scientific explanation for the human leukocyte antigen association? *Curr. Opin. Rheumatol. 14,* 254–259.

Buckley, C. (2003). Why does chronic inflammatory joint disease persist? *Clin. Med. 3,* 361–366.

Cao, D., Malmstrom, V., Baecher-Allan, C., Hafler, D., Klareskog, L., and Trollmo, C. (2003). Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. *Eur. J. Immunol. 33,* 215–223.

Carty, S.M., Snowden, N., and Silman, A.J. (2003). Should infection still be considered as the most likely triggering factor for rheumatoid arthritis? *J. Rheum. 30,* 425–429.

Choe, J.Y., Crain, B., Wu, S.R., and Corr, M. (2003). Interleukin 1 receptor dependence of serum transferred arthritis can be circumvented by toll-like receptor 4 signaling. *J. Exp. Med. 197,* 537–542.

Choy, E.H., Hazleman, B., Smith, M., Moss, K., Lisi, L., Scott, D.G., Patel, J., Sopwith, M., and Isenberg, D.A. (2002a). Efficacy of a novel PEGylated humanized anti-TNF fragment (CDP870) in patients with rheumatoid arthritis: a phase II double-blinded, randomized, dose-escalating trial. *Rheumatology (Oxford) 41,* 1133–1137.

Choy, E.H., Isenberg, D.A., Garrood, T., Farrow, S., Ioannou, Y., Bird, H., Cheung, N., Williams, B., Hazleman, B., Price, R., Yoshizaki, K., Nishimoto, N., Kishimoto, T., and Panayi, G.S. (2002b). Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial. *Arthritis Rheum. 46,* 3143–3150.

Clohisy, J.C., Roy, B.C., Biondo, C., Frazier, E., Willis, D., Teitelbaum, S.L., and Abu-Amer, Y. (2003). Direct inhibition of NF-kappa B blocks bone erosion associated with inflammatory arthritis. *J. Immunol. 171,* 5547–5553.

Conaghan, P.G., OConnor, P., McGonagle, D., Astin, P., Wakefield, R.J., Gibbon, W.W., Quinn, M., Karim, Z., Green, M.J., Proudman, S., Isaacs, J., and Emery, P. (2003). Elucidation of the relationship between synovitis and bone damage: a randomized magnetic resonance imaging study of individual joints in patients with early rheumatoid arthritis. *Arthritis Rheum. 48,* 64–71.

Cope, A. (2003). Exploring the reciprocal relationship between immunity and inflammation in chronic inflammatory arthritis. *Rheumatology 42,* 716–731.

Corthay, A., Backlund, J., Broddefalk, J., Michaelsson, E., Goldschmidt, T.J., Kihlberg, J., and Holmdahl, R. (1998). Epitope glycosylation plays a critical role for T cell recognition of type II collagen in collagen-induced arthritis. *Eur. J. Immunol. 28,* 2580–2590.

Cross, A., Bucknall, R.C., Cassatella, M.A., Edwards, S.W., and Moots, R.J. (2003). Synovial fluid neutrophils transcribe and express class II major histocompatibility complex molecules in rheumatoid arthritis. *Arthritis Rheum. 48,* 2796–2806.

de Vries, N., Tijssen, H., van Riel, P.L., and van de Putte, L.B. (2002). Reshaping the shared epitope hypothesis: HLA-associated risk for rheumatoid arthritis is encoded by amino acid substitutions at positions 67–74 of the HLA-DRB1 molecule. *Arthritis Rheum. 46,* 921–928.

Del Rincon, I., Battafarano, D.F., Arroyo, R.A., Murphy, F.T., Fischbach, M., and Escalante, A. (2003). Ethnic variation in the clinical manifestations of rheumatoid arthritis: role of HLA-DRB1 alleles. *Arthritis Rheum. 49,* 200–208.

Descamps, F.J., Van den Steen, P.E., Nelissen, I., Van Damme, J., and Opdenakker, G. (2003). Remnant epitopes generate autoimmunity: from rheumatoid arthritis and multiple sclerosis to diabetes. *Adv. Exp. Mol. Biol. 535,* 69–77.

Doran, M.F., Pond, G.R., Crowson, C.S., O'Fallon, W.M., and Gabriel, S.E. (2002). Trends in incidence and mortality in rheumatoid arthritis in Rochester, Minnesota, over a forty-year period. *Arthritis Rheum. 46,* 579–581.

Dorner, T. and Burmester, G.R. (2003). The role of B-cells in rheumatoid arthritis: mechanisms and therapeutic targets. *Curr. Opin. Rheum. 15,* 246–252.

Dubucquoi, S., Solau-Gervais, E., Lefranc, D., Marguerie, L., Sibilia, J., Goetz, J., Dutoit, V., Fauchais, A.L., Hachulla, E., Flipo, R.M., and Prin, L. (2004). Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases. *Ann. Rheum. Dis. 63,* 415–419.

Duke, O., Panayi, G.S., Janossy, G., and Poulter, L.W. (1982). An immunohistological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies. *Clin. Exp. Immunol. 49,* 22–30.

Durai, M., Gupta, R.S., and Moudgil, K.D. (2004). The T cells specific for the carboxyl-terminal determinants of self (rat) heat-shock protein 65 escape tolerance induction and are involved in regulation of autoimmune arthritis. *J. Immunol. 172,* 2795–2802.

Edwards, J.C.W., and Cambridge, J. (1995). Is rheumatoid arthritis a failure of B cell death in synovium? *Ann. Rheum. Dis. 54,* 696–700.

Edwards, J.C., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Emery, P., Close, D.R., Stevens, R.M., and Shaw, T. (2004). Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N. Engl. J. Med. 350,* 2572–81.

Emery, P. (1994). The Roche Rheumatology Prize Lecture: The optimal management of early rheumatoid disease: the key to preventing disability. *Br. J. Rheumatol. 33,* 765–768.

Emery, P., Panayi, G., Symmons, D., and Brown, G. (1984). Mechanisms of depressed delayed-type hypersensitivity in rheumatoid arthritis: the role of protein energy malnutrition. *Ann Rheum Dis. 43,* 430–434.

Emery, P., Wood, N., Gentry, K., Stockman, A., Mackay, I.R., and Bernard, O. (1988). High-affinity interleukin-2 receptors on blood lymphocytes are decreased during active rheumatoid arthritis. *Arthritis Rheum. 31,* 1176–1181.

Escalona, M., Lopez-Longo, F.J., Gonzalez, C.M., Monteagudo, I., Rodriguez-Mahou, M., Grau, R., and Carreno, L. (2002). Anti-Sa sera from patients with rheumatoid arthritis contain at least 2 different subpopulations of anti-Sa antibodies. *J. Rheumatol. 29,* 2053–2060.

Eyre, S., Barton, A., Shephard, N., Hinks, A., Brintnell, W., MacKay, K., Silman, A., Ollier, W., Wordsworth, P., John, S., and Worthington, J. (2004). Investigation of susceptibility loci identified in the UK rheumatoid arthritis whole-genome scan in a further series of 217 UK affected sibling pairs. *Arthritis Rheum. 50,* 729–735.

Feldmann, M., Brennan, F.M., and Maini, R.N. (1996). Role of cytokines in rheumatoid arthritis. *Ann. Rev. Immunol. 14,* 397–440.

Ferraro-Peyret, C., Tebib, J., Desbos, A., Bihannic, R., Genestier, C., Letroublon, M.C., Veber, S., Benoit, E., Veysseyre-Balter, C., Monier, J.C., Fabien, N. (2002). Improvement in diagnosis of rheumatoid arthritis using dual indirect immunofluorescence and immunoblotting assays for antifilaggrin autoantibodies: a retrospective 3 year study. *J. Rheumatol. 29,* 276–281.

Firestein, G.S., Alvaro-Gracia, J.M., and Maki, R. (1990). Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J. Immunol. 144,* 3347–3353.

Firestein, G.S., and Zvaifler, N.J. (1990). How important are T cells in chronic rheumatoid synovitis? *Arthritis Rheum. 33,* 768–773.

Fritsch, R., Eselbock, D., Skriner, K., Jahn-Schmid, B., Scheinecker, C., Bohle, B., Tohidast-Akrad, M., Hayer, S., Neumuller, J., Pinol-Roma, S., Smolen, J.S., and Steiner, G. (2002). Characterization of autoreactive T cells to the autoantigens heterogeneous nuclear ribonucleoprotein A2 (RA33) and filaggrin in patients with rheumatoid arthritis. *J. Immunol. 169,* 1068–1076.

Genovese, M.C., Cohen, S., Moreland, L., Lium, D., Robbins, S., Newmark, R., and Bekker, P; 20000223 Study Group. (2004). Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. *Arthritis Rheum. 50,* 1412–9.

Granet, C., Maslinski, W., and Miossec, P. (2004). Increased AP-1 and NF-kappaB activation and recruitment with the combination of the pro-inflammatory cytokines IL-1beta, tumor necrosis factor alpha and IL-17 in rheumatoid synoviocytes. *Arthritis Res. Ther. 6,* R190–8.

Green, M., Marzo-Ortega, H., McGonagle, D., Wakefield, R., Proudman, S., Conaghan, P., Gooi, J., and Emery, P. (1999). Persistence of mild, early inflammatory arthritis: the importance of disease duration, rheumatoid factor, and the shared epitope. *Arthritis Rheum. 42,* 2184–2188.

Gregersen, P.K., Silver, J., and Winchester, R.J. (1987). The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum. 30,* 1205–1213.

Handel, M.L., McMorrow, L.B., and Gravallese, E.M. (1995). Nuclear factor-kappa B in rheumatoid synovium. Localization of p50 and p65. *Arthritis Rheum. 38,* 1762–1770.

Haringman, J.J., Kraan, M.C., Smeets, T.J., Zwinderman, K.H., and Tak, P.P. (2003). Chemokine blockade and chronic inflammatory disease: proof of concept in patients with rheumatoid arthritis. *Ann. Rheum. Dis. 62,* 715–721.

Harney, S., Newton, J., Milicic, A., Brown, M.A., and Wordsworth, B.P. (2003). Non-inherited maternal HLA alleles are associated with rheumatoid arthritis. *Rheumatology 42,* 171–174.

Henderson, B., and Pettipher, E.R. (1989). Arthritogenic actions of recombinant IL-1 and tumour necrosis factor alpha in the rabbit: evidence for synergistic interactions between cytokines *in vivo*. *Clin. Exp. Immunol. 75,* 306–310.

Hill, J.A., Southwood, S., Sette, A., Jevnikar, A.M., Bell, D.A., and Cairns, E. (2003). Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J. Immunol. 171,* 538–541.

Iannone, F., Corrigal, V.M., and Panayi, G.S. (1996). CD69 on synovial T cells in rheumatoid arthritis correlates with disease activity. *Br. J. Rheumatol. 35,* 397.

Ichikawa, K., Liu, W., Fleck, M., Zhang, H., Zhao, L., Ohtsuka, T., Wang, Z., Liu, D., Mountz, J.D., Ohtsuki, M., Koopman, W.J., Kimberly, R., and Zhou, T. (2003). TRAIL-R2 (DR5) mediates apoptosis of synovial fibroblasts in rheumatoid arthritis. *J. Immunol. 171,* 1061–1069.

Imamura, F., Aono, H., Hasunuma, T., Sumida, T., Tateishi, H., Maruo, S., and Nishioka, K. (1998). Monoclonal expansion of synoviocytes in rheumatoid arthritis. *Arthritis Rheum. 41,* 1979–1986.

Isaacs, J.D., Burrous, N., Wing, M., Keogan, M.T., Rebello, P.R., Watts, R.A., Pye, R.J., Norris, P., Hazelman, B.L., Hale, G., and Waldemann, H. (1997). Homanized anti-CD4 monoclonal antibody therapy of autoimmune and inflammatory disease. *Clin. Exp. Immunol. 110*, 158–166.

Jacobsson, L.T., and Pillemer, S.R. (1994). What can we learn about rheumatic diseases by studying Pima Indians? *J. Rheum. 21,* 1179–1182.

Ji, H., Ohmura, K., Mahmood, U., Lee, D.M., Hofhuis, F.M., Boackle, S.A., Takahashi, K., Holers, V.M., Walport, M., Gerard, C., Ezekowitz, A., Carroll, M.C., Brenner, M., Weissleder, R., Verbeek, J.S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (2002). Arthritis critically dependent on innate immune system players. *Immunity 16,* 157–168.

Jonsson, T., Steinsson, K., Jonsson, H., Geirsson, A.J., Thorsteinsson, J., and Valdimarsson, H. (1998). Combined elevation of IgM and IgA rheumatoid factor has high diagnostic specificity for rheumatoid arthritis. *Rheumatol. Int. 18,* 119–122.

Joosten, L.A., Koenders, M.I., Smeets, R.L., Heuvelmans-Jacobs, M., Helsen, M.M., Takeda, K., Akira, S., Lubberts, E., van de Loo, F.A., and van den Berg, W.B. (2003). Toll-like receptor 2 pathway drives streptococcal cell wall-induced joint inflammation: critical role of myeloid differentiation factor 88. *J. Immunol. 171,* 6145–6153.

Kasama, T., Kobayashi, K., Yajima, N., Shiozawa, F., Yoda, Y., Takeuchi, H.T., Mori, Y., Negishi, M., Ide, H., and Adachi, M. (2000). Expression of vascular endothelial growth factor by synovial fluid neutrophils in rheumatoid arthritis (RA). *Clin. Exp. Immunol. 121,* 533–538.

Katschke, K.J. Jr, Rottman, J.B., Ruth, J.H., Qin, S., Wu, L., LaRosa, G., Ponath, P., Park, C.C., Pope, R.M., and Koch, A.E. (2001). Differential expression of chemokine receptors on peripheral blood, synovial fluid, and synovial tissue monocytes/macrophages in rheumatoid arthritis. *Arthritis Rheum. 44,* 1022–1032.

Kempsell, K.E., Cox, C.J., Hurle, M., Wong, A., Wilkie, S., Zanders, E.D., Gaston, J.S., and Crowe, J.S. (2000). Reverse transcriptase-PCR analysis of bacterial rRNA for detection and characterization of bacterial species in arthritis synovial tissue. *Infect. Immun. 68,* 6012–6026.

Klareskog, L., van der Heijde, D., de Jager, J.P., Gough, A., Kalden, J., Malaise, M., Martin Mola, E., Pavelka, K., Sany, J., Settas, L., Wajdula, J., Pedersen, R., Fatenejad, S., and Sanda, M; TEMPO (Trial of Etanercept and Methotrexate with Radiographic Patient Outcomes) study investigators. (2004). Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet 363,* 675–81.

Klimiuk, P.A., Goronzy, J.J., Bjornsson, J., Beckenbaugh, R.D., and Weyand, C.M. (1997). Tissue cytokine patterns distinguish variants of rheumatoid synovitis. *Am. J. Pathol. 151,* 1311–1319.

Kong, Y.Y., Yoshida, H., Sarosi, I., Tan, H.L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A.J., Van, G., Itie, A., Khoo, W., Wakeham, A., Dunstan, C.R., Lacey, D.L., Mak, T.W., Boyle, W.J., and Penninger, J.M. (1999). OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature 397,* 315–323.

Konttinen, Y.T., Ceponis, A., Meri, S., Vuorkoski, A., Pirkko, K., Sorsa, T, Sukura, A., and Santavirta, S. (1996). Complement in acute and chronic arthritides: assessment of C3c, C9 and protectin (CD59) in synovial membrane. *Ann. Rheum. Dis. 55,* 888–894.

Korganow, A.S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J.L., Benoist, C., and Mathis, D. (1999). From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity 10,* 451–461.

Kouskoff, V., Korganow, A.S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (1996). Organ-specific disease provoked by systemic autoimmunity. *Cell 87,* 811–822.

Kraan, M.C., van Kuijk, A.W., Dinant, H.J., Goedkoop, A.Y., Sneets, T.J., de Rie M.A., Dijkmans, B.A., Vaishnaw, A.K., Bos, I.D., and Tak, P.P. (2002). Alefacept treatment in psoriatic arthritis: reduction of the effector T cell population in peripheral blood and synovial tissue is associated with improvement of clinical signs of arthritis. *Arthritis Rheum. 10,* 2776–2784.

Krause, D., Schleusser, B., Herborn, G., and Rau, R. (2000). Response to methotrexate treatment is associated with reduced mortality in patients with severe rheumatoid arthritis. *Arthritis Rheum. 43,* 14–21.

Kremer, J.M., Westhovens, R., Leon, M., Di Giorgio, E., Alten, R., Steinfeld, S., Russell, A., Dougados, M., Emery, P., Nuamah, I.F., Williams, G.R., Becker, J.C., Hagerty, D.T., and Moreland, L.W. (2003). Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N. Engl. J. Med. 349,* 1907–1915.

Lacey, D., Sampey, A., Mitchell, R., Bucala, R., Santos, L., Leech, M., and Morand, E. (2003). Control of fibroblast-like synoviocyte proliferation by macrophage migration inhibitory factor. *Arthritis Rheum. 48,* 103–109.

Lawrence, R.C., Helmick, C.G., Arnett, F.C., Deyo, R.A., Felson, D.T., Giannini, E.H., Heyse, S.P., Hirsch, R., Hochberg, M.C., Hunder, G.G., Liang, M.H., Pillemer, S.R., Steen, V.D. & Wolfe, F. (1998). Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum. 41,* 778–799.

Leadbetter, E.A., Rifkin, I.R., Hohlbaum, A.M., Beaudette, B.C., Shlomchik, M.J., and Marshak-Rothstein, A. (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature 416,* 603–607.

Leandro, M.J., Edwards, J.C., and Cambridge, G. (2002). Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann. Rheum. Dis. 61,* 883–888.

Lesley, R., Xu, Y., Kalled, S.L., Hess, D.M., Schwab, S.R., Shu, H.B., and Cyster, J.G. (2004). Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. *Immunity 20,* 441–453.

Liew, F.Y., Wei, X.Q., and McInnes, I.B. (2003). Role of interleukin 18 in rheumatoid arthritis. *Ann. Rheum. Dis. 62*(Suppl 2), 48–50.

Liu, H. and Pope, R.M. (2003). The role of apoptosis in rheumatoid arthritis. *Curr. Opin. Pharm. 3,* 323–328.

Lotz, M., Carson, D.A., and Vaughan, J.H. (1987). Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. *Science 235,* 893–895.

MacDonald, K.P., Nishioka, Y., Lipsky, P.E., and Thomas, R. (1997). Functional CD40 ligand is expressed by T cells in rheumatoid arthritis. *J. Clin. Invest. 100,* 2404–2414.

MacGregor, A.J., Bamber, S., and Silman, A.J. (1994). A comparison of the performance of different methods of disease classification for rheumatoid arthritis. Results of an analysis from a nationwide twin study. *J. Rheumatol. 21,* 1420–1426.

MacGregor, A.J., Streider, H., Rigby, A.S., Koskenvuo, M., Kaprio, J., Aho, K., and Silman, A.J. (2000). Characterising the quantitative genetic contribution to RA using data from twins. *Arthritis Rheum. 43,* 30–37.

Maini, R., St Clair, E.W., Breedveld, F., Furst, D., Kalden, J., Weisman, M., Smolen, J., Emery, P., Harriman, G., Feldmann, M., and Lipsky, P. (1999). Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet 354,* 1932–1939.

Malmstrom, V., Michaelsson, E., Burkhardt, H., Mattsson, R., Vuorio, E., and Holmdahl, R. (1996). Systemic versus cartilage-specific expression of a type II collagen-specific T-cell epitope determines the level of tolerance and susceptibility to arthritis. *Proc. Natl. Acad. Sci. U.S.A. 30,* 4480–4485.

Mandik-Nayak, L., Wipke, B.T., Shih, F.F., Unanue, E.R., and Allen, P.M. (2002). Despite ubiquitous autoantigen expression, arthritogenic autoantibody response initiates in the local lymph node. *Proc. Natl. Acad. Sci. U.S.A. 99,* 14368–14373.

Mason, U., Aldrich, J., Breedveld, F., Davis, C.B., Elliott, M., Jackson, M., Jorgensen, C., Keystone, E., Levy, R., Tesser, J., Totoritis, M., Truneh, A., Weisman, M., Wiesenhutter, C., Yocum, D., and Zhu, J. (2002). CD4 coating, but not CD4 depletion, is a predictor of efficacy with primatized monoclonal anti-CD4 treatment of active rheumatoid arthritis. *J. Rheumatol. 29,* 220–229.

Masson-Bessiere, C., Sebbag, M., Girbal-Neuhauser, E., Nogueira, L., Vincent, C., Senshu, T., and Serre, G. (2001). The major synovial targets of the rheumatoid arthritis-specific antifilaggrin autoantibodies are deiminated forms of the alpha- and beta-chains of fibrin. *J. Immunol. 166,* 4177–4784.

Matsumoto, I., Maccioni, M., Lee, D.M., Maurice, M., Simmons, B., Brenner, M., Mathis, D., and Benoist, C. (2002). How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat. Immunol. 3,* 360–365.

Matzinger, P. (2002). The danger model: a renewed sense of self. *Science 296,* 301–305.

McDonagh, J.E., Dunn, A., Ollier, W.E., and Walker, D.J. (1997). Compound heterozygosity for the shared epitope and the risk and severity of rheumatoid arthritis in extended pedigrees. *Br. J. Rheumatol. 36,* 322–327.

McInnes, I.B., Leung, B.P., Sturrock, R.D., Field, M.and Liew, F.Y. (1997). Interleukin–15 mediates T-cell dependent regulation of tumour necrosis factor-alpha production in rheumatoid arthritis. *Nat. Med. 3,* 189–195.

Mellemkjaer, L., Linet, M.S., Gridley, G., Frisch, M., Moller, H., and Olsen, J.H. (1996). Rheumatoid arthritis and cancer risk. *Eur. J. Cancer 32A,* 1753–1757.

Miossec P. (2003). Interleukin–17 in rheumatoid arthritis: if T cells were to contribute to inflammation and destruction through synergy. *Arthritis Rheum. 48,* 594–601.

Morand, E.F., Bucala, R., and Leech, M. (2003). Macrophage migration inhibitory factor: an emerging therapeutic target in rheumatoid arthritis. *Arthritis Rheum. 48,* 291–299.

Mosser, D.D., Caron, A.W., Bourget, L., Denis-Larose, C., and Massie, B. (1997). Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Mol. Cell. Biol. 17,* 5317–5322.

Mulherin, D., Fitzgerald, O., and Bresnihan, B. (1996) Clinical improvement and radiological deterioration in rheumatoid arthritis: evidence that the pathogenesis of synovial inflammation and articular erosion may differ. *Br. J. Rheumatol. 35,* 1263–1268.

Nandakumar, K.S., Svensson, L., and Holmdahl, R. (2003). Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. *Am. J. Pathol. 163,* 1827–1837.

Nielen, M.M.J., van Schaardenburg, D., Reesink, H.W., van de Stadt, R.J., van der Horst-Bruisma, I.E., de Koning, M.H., Habibuw, M.R., Vandenbroucke, J.P., and Dijkmans, B.A. (2004). Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum. 50,* 380–386.

Nishimoto, N., Yoshizaki, K., Miyasaka, N., Yamamoto, K., Kawai, S., Takeuchi, T., Hashimoto, J., Azuma, J., and Kishimoto, T. (2004). Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum. 50,* 1761–1769.

Ogawa, Y., Ohtsuki, M., Uzuki, M., Sawai, T., Onozawa, Y., Nakayama, J., Yonemura, A., Kimura, T., and Matsuno, H. (2003). Suppression of osteoclastogenesis in rheumatoid arthritis by induction of apoptosis in activated CD4+ T cells. *Arthritis Rheum. 48,* 3350–3358.

Ottonello, L., Cutolo, M., Frumento, G., Arduino, N., Bertolotto, M., Mancini, M., Sottofattori, E., and Dallegri, F. (2002). Synovial fluid from patients with rheumatoid arthritis inhibits neutrophil apoptosis: role of adenosine and proinflammatory cytokines. *Rheumatology 41,* 1249–1260.

Padyukov, L., Silva, C., Stolt, P., Alfredsson, L., and Klareskog, L. (2004). A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum. 50,* 3085–3092.

Patel, D.D., Zachariah, J.P., and Whichard, L.P. (2001). CXCR3 and CCR5 ligands in rheumatoid arthritis synovium. *Clin. Immunol. 98,* 39–45.

Patil, N.S., Pashine, A., Belmares, M.P., Liu, W., Kaneshiro, B., Rabinowitz, J., McConnell, H., and Mellins, E.D. (2001). Rheumatoid arthritis (RA)-associated HLA-DR alleles form less stable complexes with class II-associated invariant chain peptide than non-RA-associated HLA-DR alleles. *J. Immunol. 167,* 7157–7168.

Perlman, H., Pagliari, L.J., Liu, H., Koch, A.E., Haines, G.K. 3rd, and Pope, R.M. (2001). Rheumatoid arthritis synovial macrophages express the Fas-associated death domain-like interleukin-1beta-converting enzyme-inhibitory protein and are refractory to Fas-mediated apoptosis. *Arthritis Rheum. 44,* 21–30.

Pettit, A.R., Ji, H., von Stechow, D., Muller, R., Goldring, S.R., Choi, Y., Benoist, C., and Gravallese, E.M. (2001). TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. *Am. J. Pathol. 159,* 1689–1699.

Pilling, D., Akbar, A.N., Girdlestone, J., Orteu, C.H., Borthwick, N.J., Amft, N., Scheel-Toellner, D., Buckley, C.D., and Salmon, M. (1999). Interferon-beta mediates stromal cell rescue of T cells from apoptosis. *Eur. J. Immunol. 29,* 1041–1050.

Pincus, T., Ferraccioli, G., Sokka, T., Larsen, A., Rau, R., Kushner, I., and Wolfe, F. (2002). Evidence from clinical trials and long-term observational studies that disease-modifying anti-rheumatic drugs slow radiographic progression in rheumatoid arthritis: updating a 1983 review. *Rheumatology 41,* 1346–1356.

Prakken, B.J., Roord, S., van Kooten, P.J., Wagenaar, J.P., van Eden, W., Albani, S., and Wauben, M.H. (2002). Inhibition of adjuvant-induced arthritis by interleukin-10-driven regulatory cells induced via nasal administration of a peptide analog of an arthritis-related heat-shock protein 60 T cell epitope. *Arthritis Rheum. 46,* 1937–1946.

Qin, S., Rottman, J.B., Myers, P., Kassam, N., Weinblatt, M., Loetscher, M., Koch, A.E., Moser, B., and Mackay, C.R. (1998). The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J. Clin. Invest. 101,* 746–754.

Quintana, F.J., Carmi, P., Mor, F., and Cohen IR. (2003). DNA fragments of the human 60-kDa heat shock protein (HSP60) vaccinate against adjuvant arthritis: identification of a regulatory HSP60 peptide. *J. Immunol. 171,* 3533–3541.

Reparon-Schuijt, C.C., van Esch, W.J., van Kooten, C., Schellekens, G.A., de Jong, B.A., van Venrooij, W.J., Breedveld, F.C., and Verweij, C.L. (2001). Secretion of anti-citrulline-containing peptide antibody by B lymphocytes in rheumatoid arthritis. *Arthritis Rheum. 44,* 41–7.

Romas, E., Gillespie, M.T., and Martin, T.J. (2002a). Involvement of receptor activator of NFkappaB ligand and tumor necrosis factor-alpha in bone destruction in rheumatoid arthritis. *Bone 30,* 340–346.

Romas, E., Sims, N.A., Hards, D.K., Lindsay, M., Quinn, J.W., Ryan, P.F., Dunstan, C.R., Martin, T.J., and Gillespie, M.T. (2002b). Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. *Am. J. Pathol. 161,* 1419–1427.

Roosnek, E., and Lanzavecchia, A. (1991). Efficient and selective presentation of antigen–antibody complexes by rheumatoid factor B cells. *J. Exp. Med. 173,* 487–489.

Ruddy, S., and Austen, K.F. (1975). Activation of the complement and properdin systems in rheumatoid arthritis. *Ann. N.Y. Acad. Sci. 256,* 96–104.

Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., and Sakaguchi, S. (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature 426,* 454–460.

Salmon, M., Scheel-Toellner, D., Huissoon, A.P., Pilling, D., Shamsadeen, N., Hyde, H., DAngeac, A.D., Bacon, P.A., Emery, P., and Akbar, A.N. (1997). Inhibition of T cell apoptosis in the rheumatoid synovium. *J. Clin. Invest. 99,* 439–446.

Saraux, A., Bourdon, V., Devauchelle, V., and Le Goff, P. (2001). Is hypocomplementemia useful for diagnosing or predicting extra-articular manifestations in patients with rheumatoid arthritis? *Joint Bone Spine 68,* 487–92.

Schellekens, G.A., Visser, H., de Jong, B.A., van den Hoogen, F.H., Hazes, J.M., Breedveld, F.C., and van Venrooij, W.J. (2000). The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum. 43,* 155–63.

Schett, G., Redlich, K., Xu, Q., Bizan, P., Groger, M., Tohidast-Akrad, M., Kiener, H., Smolen, J., and Steiner, G. (1998). Enhanced expression of heat shock protein 70 (hsp70) and heat shock factor 1 (HSF1) activation in rheumatoid arthritis synovial tissue. Differential regulation of hsp70 expression and hsf1 activation in synovial fibroblasts by proinflammatory cytokines, shear stress, and antiinflammatory drugs. *J. Clin. Invest. 102,* 302–311.

Schett, G., Tohidast-Akrad, M., Smolen, J.S., Schmid, B.J., Steiner, C.W., Bitzan, P., Zenz, P., Redlich, K., Xu, Q., and Steiner, G. (2000). Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-JUN N-terminal kinase, and p38 mitogen-activated protein kinase, in synovial tissue and cells in rheumatoid arthritis. *Arthritis Rheum. 43,* 2501–2512.

Schnitzer, T.J., Yocum, D.E., Michalska, M., Balius, R., Snider, M.E., Hays, A., Thurmond, L.M., and Johnston, J.M. (1997). Subcutaneous administration of CAMPATH-1H: clinical and biological outcomes. *J. Rheumatol. 24,* 1031–1036.

Sebbag, M., Simon, M., Vincent, C., Masson-Bessiere, C., Girbal, E., Durieux, J.J., and Serre, G. (1995). The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. *J. Clin. Invest. 95,* 2672–9.

Sedgwick, J.D., Riminton, S., Cyster, J.G., and Körner, H. (2000). Tumor necrosis factor: a master-regulator of leukocyte movement. *Immunol. Today 21,* 110–113.

Seemayer, C.A., Kuchen, S., Kuenzler, P., Rihoskova, V., Rethage, J., Aicher, W.K., Michel, B.A., Gay, R.E., Kyburz, D., Neidhart, M., and Gay, S. (2003). Cartilage destruction mediated by synovial fibroblasts does not depend on proliferation in rheumatoid arthritis. *Am. J. Pathol. 162,* 1549–1557.

Seibl, R., Birchler, T., Loeliger, S., Hossle, J.P., Gay, R.E., Saurenmann, T., Michel, B.A., Seger, R.A., Gay, S., and Lauener, R.P. (2003). Expression and regulation of Toll-like receptor 2 in rheumatoid arthritis synovium. *Am. J. Pathol. 162,* 1221–1227.

Sen, M., Lauterbach, K., El-Gabalawy, H., Firestein, G.S., Corr, M., and Carson, D.A. (2000). Expression and function of wingless and frizzled homologs in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A. 97,* 2791–2796.

Shadidi, K.R., Aarvak, T., Henriksen, J.E., Natvig, J.B., and Thompson, K.M. (2003). The chemokines CCL5, CCL2 and CXCL12 play significant roles in the migration of Th1 cells into rheumatoid synovial tissue. *Scand. J. Immunol. 57,* 192–198.

Simon, A.K., Seipelt, E., and Sieper, J. (1994). Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc. Natl. Acad. Sci. U.S.A. 91,* 8562–8566.

Smeets, T.J., Kraan, M.C., Versendaal, J., Breedveld, F.C., and Tak, P.P. (1999). Analysis of serial synovial biopsies in patients with rheumatoid arthritis: description of a control group without clinical improvement after treatment with interleukin 10 or placebo. *J. Rheumatol. 26,* 2089–2093.

Snowden, J.A., Passweg, J., Moore, J.J., Milliken, S., Cannell, P., Van Laar, J., Verburg, R., Szer, J., Taylor, K., Joske, D., Rule, S., Bingham, S.J., Emery, P., Burt, R.K., Lowenthal, R.M., Durez, P., McKendry, R.J., Pavletic, S.Z., Espigado, I., Jantunen, E., Kashyap, A., Rabusin, M., Brooks, P., Bredeson, C., and Tyndall, A. (2004). Autologous hemopoietic stem cell transplantation in severe rheumatoid arthritis: a report from the EBMT and ABMTR. *J. Rheumatol. 31,* 482–488.

Steiner, G., Tohidast-Akrad, M., Witzmann, G., Vesely, M., Studnicka-Benke, A., Gal, A., Kunaver, M., Zenz, P., and Smolen, J.S. (1999). Cytokine production by synovial T cells in rheumatoid arthritis. *Rheumatology (Oxford) 38,* 202–213.

Steiner, G., and Smolen, J. (2002). Autoantibodies in rheumatoid arthritis and their clinical significance. *Arthritis Res. 4*(Suppl 2), S1–5.

Stolt, P., Bengtsson, C., Nordmark, B., Lindblad, S., Lundberg, I., Klareskog, L., Alfredsson, L.; EIRA study group. (2003). Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann. Rheum. Dis. 62,* 835–841.

Sturrock, R.D., Sharma, J.N., and Buchanan, W.W. (1977). Evidence of rheumatoid arthritis in ancient India. *Arthritis Rheum. 20,* 42–44.

Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y., and Yamamoto, K. (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet. 34,* 395–402.

Symmons, D.P., Jones, M.A., Scott, D.L., and Prior, P. (1998). Longterm mortality outcome in patients with rheumatoid arthritis: early presenters continue to do well. *J. Rheumatol. 25,* 1072–1077.

Symmons, D., Turner, G., Webb, R., Asten, P., Barrett, E., Lunt, M., Scott, D., and Silman, A. (2002). The prevalence of rheumatoid arthritis in the United Kingdom: new estimates for a new century. *Rheumatology 41,* 793–800.

Szekanecz, Z., Kim, J., and Koch, A.E. (2003). Chemokines and chemokine receptors in rheumatoid arthritis. *Semin. Immunol. 15,* 15–21.

Szodoray, P., Jellestad, S., Nakken, B., Brun, J.G., and Jonsson, R. (2003). Programmed cell death in rheumatoid arthritis peripheral blood T-cell subpopulations determined by laser scanning cytometry. *Lab. Invest. 83,* 1839–1848.

Tan, A.L., Tanner, S.F., Conaghan, P.G., Radjenovic, A., OConnor, P., Brown, A.K., Emery, P., and McGonagle, D. (2003). Role of metacarpophalangeal joint anatomic factors in the distribution of synovitis and bone erosion in early rheumatoid arthritis. *Arthritis Rheum. 48,* 1214–1222.

Taylor, P.C. (2002). VEGF and imaging of vessels in rheumatoid arthritis. *Arthritis Res. 4*(Suppl 3), S99–107.

Thomas, R. and Lipsky, P.E. (1996). Presentation of self peptides by dendritic cells: possible implications for the pathogenesis of rheumatoid arthritis. *Arthritis Rheum. 39,* 183–190.

Thompson, M., and Bywaters, E.G.L. (1961). Unilateral rheumatoid arthritis following hemiplegia. *Ann. Rheum. Dis. 21,* 370–377.

van den Broek, M.F., van Bruggen, M.C., Stimpson, S.A., Severijnen, A.J., van de Putte, L.B., and van den Berg, W.B. (1990). Flare-up reaction of streptococcal cell wall incluced arthritis in Lewis and F344 rats: the role of T lymphocytes. *Clin. Exp. Immunol. 79*, 297–306.

Van Eden, W., and Waksman, B.H. (2003). Immune regulation in adjuvant-induced arthritis: possible implications for innovative therapeutic strategies in arthritis. *Arthritis Rheum. 48,* 1788–1796.

van Gaalen, F.A., Toes, R.E., Ditzel, H.J., Schaller, M., Breedveld, F.C., Verweij, C.L., and Huizinga, T.W. (2004a). Association of autoantibodies to glucose-6-phosphate isomerase with extraarticular complications in rheumatoid arthritis. *Arthritis Rheum. 50,* 395–399.

van Gaalen, F.A., van Aken, J., Huizinga, T.W., Schreuder, G.M., Breedveld, F.C., Zanelli, E., van Venrooij, W.J., Verweij, C.L., Toes, R.E., and de Vries, R.R. (2004b). Association between HLA class II genes and autoantibodies to cyclic citrullinated peptides (CCPs) influences the severity of rheumatoid arthritis. *Arthritis Rheum. 50,* 2113–2121.

Wang, C.R., Liu, M.F., Huang, Y.H., and Chen, H.C. (2004). Up-regulation of XCR1 expression in rheumatoid joints. *Rheumatology 43,* 569–573.

Weyand, C.M., Fulbright, J.W., and Goronzy, J.J. (2003). Immunosenescence, autoimmunity, and rheumatoid arthritis. *Exp. Gerontol. 38,* 833–841.

Wiles, N., Symmons, D.P., Harrison, B., Barrett, E., Barrett, J.H., Scott, D.G., and Silman, A.J. (1999). Estimating the incidence of rheumatoid arthritis: trying to hit a moving target? *Arthritis Rheum. 42,* 1339–1346.

Wipke, B.T., Wang, Z., Nagengast, W., Reichert, D.E., and Allen, P.M. (2004). Staging the initiation of autoantibody-induced arthritis: a critical role for immune complexes. *J. Immunol. 172,* 7694–7702.

Wolfe, F. (1998). A comparison of IgM rheumatoid factor by nephelometry and latex methods: clinical and laboratory significance. *Arthritis Care Res. 11,* 89–93.

Zvaifler, N.J. and Firestein, G.S. (1994). Pannus and pannocytes. Alternative models of joint destruction in rheumatoid arthritis. *Arthritis Rheum. 37,* 783–789.

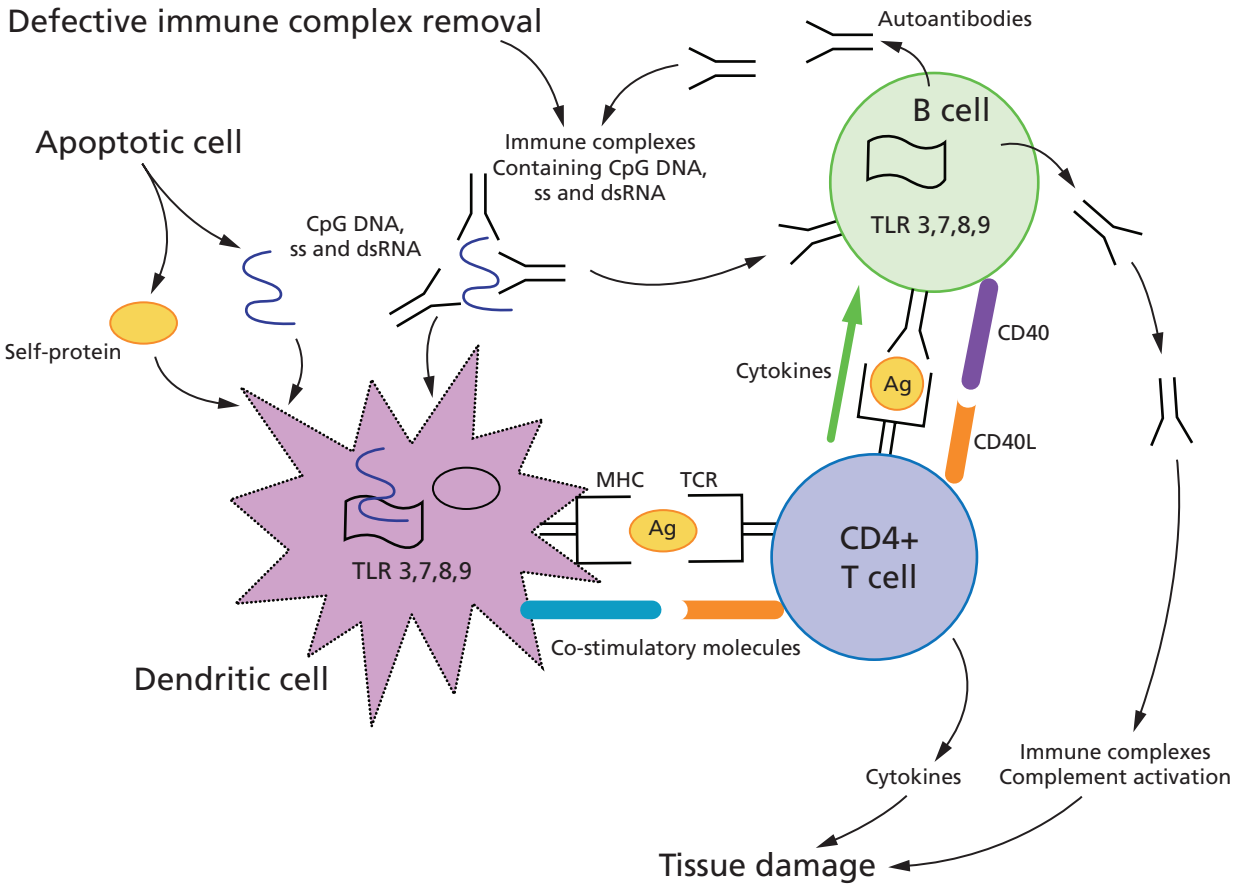


FIGURE 28.1 Immunologic pathogenesis of systemic lupus erythematosus (SLE). The figure depicts potential immunologic mechanisms involved in the development of SLE. These include defects in immune complex removal, apoptosis, and antigen presentations by antigen presenting cells (APCs) such as dendritic cells, as well as altered T- and B-cell interactions and their cytokine and autoantibody production. Nucleic acids (CpG DNA and ssRNA) from self and from microorganisms can also participate in stimulating APCs and B cells through Toll-like receptors (TLRs). Ag, antigen; ds, double stranded; MHC, major histocompatibility complex; ss, single stranded; TCR, T-cell receptor.

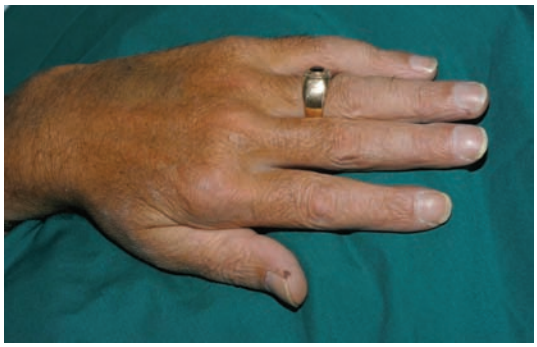


FIGURE 32.1 Rheumatoid arthritis with nailfold vasculitis.

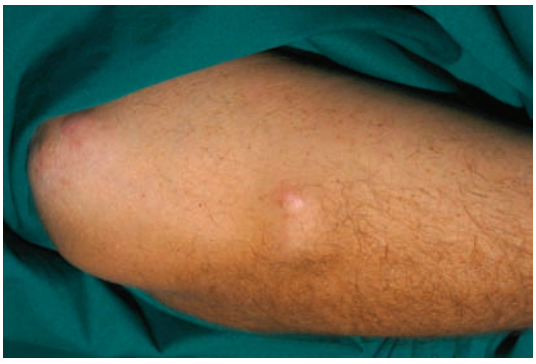


FIGURE 32.2 Rheumatoid nodule on extensor aspect of forearm.

CHAPTER

33

Spondyloarthritis and Chronic Idiopathic Arthropathies

GERD-RÜDIGER BURMESTER, THOMAS DÖRNER, AND JOACHIM SIEPER

Department of Rheumatology and Clinical Immunology, Charité Central Campus; Department of Transfusion Medicine, Charité Central Campus; Department of Gastroenterology, Infection Medicine and Rheumatology, Charité Campus Benjamin Franklin; Charité University Medicine Berlin, Humboldt University and Free University of Berlin, Berlin, Germany

SPONDYLOARTHRITIDES

Definition, Epidemiology, Clinical Manifestations, and Treatment

The spondyloarthritis (SpA) diseases comprise ankylosing spondylitis (AS), reactive arthritis (ReA), arthritis/spondylitis with inflammatory bowel disease and arthritis/spondylitis with psoriasis. The main links between each of these is the association with HLA-B27, similar clinical symptoms such as inflammatory back pain, and similar patterns of peripheral joint involvement with an asymmetric arthritis predominantly of the lower limbs, and the possible occurrence of sacroiliitis, spondylitis, enthesitis, and uveitis. Most striking is the direct relationship between the prevalence of SpA and the prevalence of HLA-B27 in the general population. This strong correlation suggests that environmental or genetic factors that are necessary in addition to HLA-B27 to get SpA must be ubiquitous (Braun and Sieper, 2003).

Ankylosing spondylitis is regarded as the SpA with the most severe outcome. Its prevalence has been estimated to be between 0.2 and 0.9% and the disease normally starts in the second decade of life. The male-to-female ratio has more recently been estimated to be around 2 : 1. Back pain is the leading clinical symptom in these patients, which is characterized by morning stiffness and improvement by exercise. The disease starts in 90% or more cases with a sacroiliitis. Further in the course of the disease the whole spine can be affected with spondylitis, spondylodiscitis, and arthritis of the small intervertebral joints. As a reaction to the inflammation, ankylosis occurs, which can involve the whole spine. Relapsing uveitis, peripheral asymmetric arthritis predominantly of the lower limbs, and enthesitis are the most

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

frequent extraspinal manifestations. HLA-B27 is found to be positive in 90–95% of patients, and inflammatory bowel disease, psoriasis, or preceding reactive arthritis can be found in about 10% of AS patients.

Diagnosis is made by a combination of clinical symptoms (such as inflammatory back pain or limitation of spinal mobility) and the demonstration of radiologic sacroiliitis, according to the modified New York criteria (van der Linden et al., 1984). More recently, we have proposed an approach on how to make an early diagnosis before evidence of radiologic sacroiliitis by combining clinical, laboratory, and imaging parameters such as magnetic resonance imaging (MRI) (Rudwaleit et al., 2004). A new approach to an earlier diagnosis is also mandatory because of the major delay of 5–7 years between the occurrence of the first symptoms and making the diagnosis. In the last decades, only nonsteroidal anti-inflammatory drugs (NSAIDs) were used, together with physiotherapy, as an effective treatment. Rather surprisingly, disease-modifying antirheumatic drugs (DMARDs) and corticosteroids, which are highly effective in other chronic inflammatory diseases such as rheumatoid arthritis, show only a small or no effect in AS. On this background, the finding that blockers of tumor necrosis factor (TNF) such as infliximab and etanercept are highly effective means a breakthrough in the treatment of this disease (Braun and Sieper, 2004). At least 50% of active AS patients refractory to treatment with NSAIDs show a 50% or more improvement when treated either with the monoclonal anti-TNF-α antibody infliximab (Braun et al., 2002; van der Heijde et al., 2005) or the soluble TNF-receptor construct etanercept (Davis et al., 2003).

Reactive arthritis occurs after a preceding infection of the urogenital tract with *Chlamydia trachomatis* or of the gut with enterobacteriae such as *Yersinia*, *Salmonella*, *Campylobacter jejuni*, or *Shigella*, usually after a few days up to 4–6 weeks (Sieper et al., 2000). The arthritis is normally an oligoarthritis, predominantly of the lower limbs, but in about 20% this will manifest as a polyarthritis. Other manifestations can be an enthesitis, conjunctivitis/uveitis, or inflammatory back pain. Between 30 and 60% of these patients with ReA are positive for HLA-B27; arthritis occurs in approximately 4% of the general population, but in about 25% of HLA-B27$^+$ individuals after one of these infections. Usually, the patients recover in 3–6 months; however, up to 20% can run a chronic course longer than 12 months. Bacterial antigen and, in the case of *Chlamydia*, also DNA and RNA have been detected in the joint, indicating that bacterial antigens persist in the joint and drive the local immune response. For making a diagnosis of ReA, a combination of clinical manifestations (preceding infection, typical pattern of arthritis) and laboratory evidence of previous or present bacterial infection is necessary (Sieper et al., 2002). While antibiotic treatment of urogenital tract infection with antibiotics prevents the occurrence of arthritis, this is not the case for bacterial enteritis. Once an arthritis is established, long-term therapy with antibiotics does not seem to influence the arthritis, suggesting that either the antibiotics used are not effective enough or that the once-triggered hypersensitive immune response cannot be interrupted by antibiotics (Sieper et al., 2000).

Approximately 10–20% of patients with inflammatory bowel disease (IBD) exhibit an often transient peripheral arthritis, which often occurs concurrently with gut inflammation. The frequency of HLA-B27 is only slightly elevated among patients with peripheral arthritis, but is present among 50–70% of patients with IBD and AS. About 5% of IBD patient, mostly those who are HLA-B27$^+$, will develop AS. Treatment should primarily be directed against the gut inflammation. Up to 50% of patients with psoriatic arthritis show a clinical picture compatible with SpA, such as oligoarthritis of the lower limbs and/or spinal inflammation. Among these patients, HLA-B27 is positive in approximately 25% (peripheral arthritis) to 60% (spinal manifestations). Treatment is similar to that for other forms of SpA.

Bacterial Trigger and Autoimmunity in the Pathogenesis of the Spondyloarthritides

Central to the discussion about the pathogenesis of the SpA is the interaction between bacteria and HLA-B27 (Kuon and Sieper, 2003). The best evidence for this comes from ReA, which is triggered usually by a genitourinary infection with *C. trachomatis* or an enteritis due to certain gram-negative enterobacteria, such as *Shigella*, *Salmonella*, *Yersinia*, or *Campylobacter*. The demonstration of microbial antigens within the synovium suggests that ReA may be related to the persistence of microbial antigens at the sites of inflammatory arthritis. Approximately 20–40% of HLA-B27$^+$ ReA patients develop the full clinical picture of AS after 10–20 years (Leirisalo-Repo, 1998). Thus, exposure of the immune system to bacteria seems also to be an important initial triggering event for AS. Although clinically diagnosed ReA arthritis precedes AS only in less than 10%, this figure may be much higher because many of the gut or urogenital infections preceding the clinical manifestation of ReA can be asymptomatic. The central role of bacteria in the pathogenesis of SpA is further supported by the relationship between Crohn's disease, HLA-B27 positivity and the occurrence of AS: in one study, in 13 out of 24 (54%) HLA-B27$^+$ patients with Crohn's disease, AS could also be diagnosed, but only in 5 out of 189 (2.6%) such patients who were HLA-B27$^-$ (Purrmann et al., 1988). In the case of Crohn disease, the leakage in the gut mucosa, as a consequence of the related inflammation, presumably allows an interaction of the immune system with the normal gut bacteria. It has also become clear that in about 50% of patients with so-called idiopathic AS, macroscopic or microscopic

mucosal chronic lesions resembling Crohn disease can be detected in the gut by colonoscopy (Mielants et al., 1988).

Finally, there is also evidence for the importance of B27-bacteria interaction from animal models. HLA-B27 transgenic rats develop features of ReA, including gut inflammation, peripheral arthritis, and psoriasiform skin and nail changes. The importance of environmental factors is emphasized by the observation that many of these features, including gut inflammation and arthritis, do not develop in HLA-B27 transgenic rats born and bred in a germ-free environment. Germ-free animals rapidly develop inflammatory disease on removal from the sterile environment. This can be partially prevented by treatment with antibiotics (Taurog et al., 1999).

Therefore, AS can probably be regarded as the long-term outcome for patients most of whom are HLA-B27$^+$ and who have been exposed to ReA-associated bacteria, bacteria in the gut, or other as yet unidentified bacteria.

Although bacteria appear to play a crucial role as an initial event for the pathogenesis of AS, there is no evidence that they are also directly responsible for the immunopathology in AS. It seems to be unlikely that bacteria or bacterial antigens do persist in such diverse structures as the sacroiliac joint, the enthesis, and in the eye. Furthermore, biopsies from the sacroiliac joint were investigated for the presence of bacteria and have been proven to be negative (Braun et al., 1997). Therefore, the induction of autoimmunity by bacteria is the more likely event in AS. Especially in the light of the strong association with major histocompatibility complex (MHC), research has concentrated in the past on the role of T cells in SpA. Currently, there is no evidence for any role for the humoral immune response in the pathogenesis of SpA.

Cytokines in the Pathogenesis of Reactive Arthritis

As it has been shown that bacteria persist *in vivo* in patients with ReA, most likely in the joint in the case of *Chlamydia* and in the gut mucosa or lymph nodes of the gut in the case of the enterobacteria, the questions raised are 1) why do these bacteria persist in some patients but not in others; and 2) why do some patients (although the minority) develop chronic courses of their arthritis. The ReA-associated bacteria are obligate (such as *Chlamydia*) or facultative intracellular bacteria. T-helper 1 (Th1) cytokines such as TNF-α and interferon-γ (IFN-γ) are crucial for an effective elimination of these bacteria, while Th2 cytokines such as interleukin 4 (IL-4) or Th3 cytokines such as IL-10 might inhibit an effective elimination. We and others showed that there is a relative deficiency of Th1-cytokines in ReA, especially of TNF-α but also of IFN-γ, both locally in synovial fluid and synovial membrane and systemically in peripheral blood (Yin et al., 1997a; Braun et al., 1999). Furthermore, we could demonstrate a correlation between a low TNF-α production in peripheral blood and a longer duration of arthritic symptoms (Braun et al., 1999). Thus, a relative lack of Th1 cytokines appears to be relevant for the occurrence and persistence of ReA, probably mediated by a persistence of bacteria. If this assumption is correct, immunopathology would then be caused by a "hypersensitive" immune response against bacterial antigens.

IL-10 has received increasing interest recently as a potentially immunosuppressive cytokine. While upregulation of such a cytokine would be wanted in autoimmune diseases such as rheumatoid arthritis we could show that it is relatively upregulated in ReA, and thus that it might contribute to bacterial persistence in ReA, possibly by downregulation of the Th1 cytokines, IFN-γ and TNF-α (Yin et al., 1999b).

Cytokines in the Pathogenesis of Ankylosing Spondylitis

Data are few on cytokines in AS. We measured cytokine-positive CD4$^+$ and CD8$^+$ T cells derived from peripheral blood after mitogenic *in vitro* stimulation by flow cytometry (Rudwaleit et al., 2001). Patients with AS had a significantly lower percentage of IFN-γ$^-$ or TNF-α$^+$ CD4$^+$ T cells compared with HLA-B27$^-$ controls, while the results for a HLA-B27$^+$ healthy control group were intermediate between the two groups. For IL-10$^+$ T cells, we found a significant increase in the CD8$^+$ T-cell subpopulation from HLA-B27$^+$ AS patients compared with B27$^+$ and B27$^-$ controls, but this did not pertain for the CD4$^+$ subpopulation. This relative (small) lack of Th1 cytokines in the peripheral blood of AS patients is in contrast to the presence of abundant TNF-α in biopsies taken from the sacroiliac joint from AS patients (Braun et al., 1995) and the very strong therapeutic response to treatment with TNF-blockers in AS (Braun and Sieper, 2004), suggesting that TNF-α and possibly also IFN-γ are important in the pathogenesis of AS and other SpA. This discrepancy is not easy to explain, but measuring cytokines in peripheral blood might not accurately reflect the situation at the local site of inflammation.

Cytokine Gene Polymorphisms in Reactive Arthritis and Ankylosing Spondylitis

Cytokine genes have also been investigated as possible candidate genes (Sieper et al., 2000). Most data in SpA patients are currently available for TNF-α. Investigating TNF-α microsatellites, an association of ReA with a TNF-α6-allele has been described; this allele has previously been associated with a low TNF-α secretion. Since, in this study from Finland, TNF-α6 was also associated with HLA-B27, the association of TNF-α6 with ReA was thought to be

secondary to B27. Two promoter polymorphisms of the TNF-α gene at positions –308 (308.1 and 308.2) and –238 (238.1 and 238.2) have been investigated in AS. The 308.2 genotype was found significantly less frequently in AS than in controls. In some studies 308.2 was associated with higher transcriptional activity (Hoehler et al., 1998). Thus, there is some evidence that TNF-α genotypes that may be associated with a low TNF-α production are present in a higher percentage in patients with ReA or AS. However, clear proof of an association of these genotypes with low TNF-α production is lacking.

Looking for IL-10 gene polymorphism in another study, there was a significant decrease in the promoter alleles G12 and G10 in the ReA group compared with HLA-B27$^+$ controls, indicating that these alleles might have a protective effect against the occurrence of ReA (Kaluza et al., 2001). Although it is not yet clear whether these alleles are associated with a higher production of IL-10, these data suggest that the relative increase of IL-10 found in ReA might be, at least partially, genetically determined.

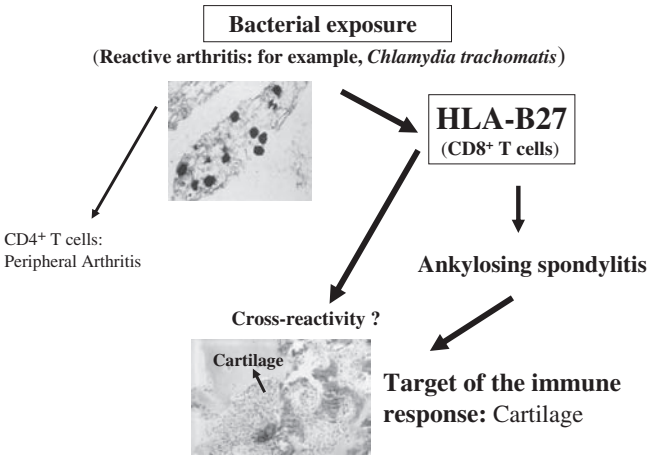


FIGURE 33.1 Hypothesis how bacterial exposure induces a peripheral arthritis, probably via a CD4$^+$ T cell response. However, axial manifestations might be mediated by CD8$^+$ T cells because of the high association with HLA-B27. The cartilage might become the primary target of the immune response through cross-reactivity with bacterial antigens.

The Role of HLA-B27 in the Pathogenesis of Spondyloarthritis

The association of HLA-B27 with SpA is the highest known MHC association for human diseases and the most relevant single factor for the pathogenesis of SpA. There are now considerable data from epidemiologic studies and transgenic animals to indicate a direct effect of HLA-B27, rather than that of a closely linked gene, in disease pathogenesis. It is also clear now that one copy of HLA-B27 (heterozygosity) is sufficient for the disease. The susceptibility to AS has been estimated to be more than 90% genetically determined, and so it has been suggested that there is a rather ubiquitous environmental factor. Besides HLA-B27, other MHC genes such as HLA-B60 and HLA-DR1 seem to be associated, but are of minor importance. However, although MHC is the major susceptibility locus, it has been suggested that it contributes only approximately 36% to the overall genetic risk (Brown et al., 2002).

Since the main function of HLA class I molecules is to present peptide antigens to cytotoxic T cells, the antigen-presenting properties of HLA-B27 could be crucial in the pathogenesis of spondyloarthritides leading to the so-called arthritogenic peptide hypothesis (Kuon and Sieper, 2003). Thus, some HLA-B27 subtypes, due to their unique amino-acid residues, can bind specific arthritogenic peptide, and so become recognized by CD8$^+$ T cells. Furthermore, in response to these bacterial peptides, autoreactive T cells recognizing antigens with sufficient structural similarity between bacteria and self, might become activated by self-peptides that are present particularly in spinal joints (Figure 33.1).

Major support for this hypothesis comes from studies in humans showing the differential association of some of the HLA-B27 subtypes with AS. While B*2705, B*2702, B*2704, and B*2707 are strongly associated with the disease, the HLA-B27 subtypes B*2709 in whites and B*2706 in Southeast Asians are not at all or only rarely associated. Most interestingly, B*2709 differs from the disease-associated B*2705 by only one amino acid substitution, the exchange of Asp116 to His116. B*2706 differs by only two amino acid substitutions from the disease-associated B*2704 by exchange of His114 to Asp114 and Asp116 to Tyr116 (Khan, 2000).

In studies on patients, both bacteria-specific and autoreactive CD8$^+$ T cells have been demonstrated in AS and ReA. Recently, a synovial CD8$^+$ T-cell response to a peptide from *Yersinia* heat shock protein 60 in patients with *Yersinia*-induced ReA and also an HLA-B27-restricted CD8$^+$ T-cell response to peptides derived from several chlamydial proteins in patients with *Chlamydia*-induced ReA have been described (Kuon and Sieper, 2003). In the latter study, there was a novel approach in searching the whole chlamydial proteome to identify peptides which stimulate CD8$^+$ T cells from patients in an HLA-B27 restricted manner. Recently, there was a report of a CD8$^+$ T-cell response to an Epstein–Barr virus (EBV) epitope derived from the LMP2 protein, and to a sequence-related self-peptide from the autoantigen vasoactive intestinal peptide (VIP) receptor 1 (Fiorillo et al., 2000). However, the exact identity of a potentially arthritogenic peptide has yet to be determined.

An oligoclonal expansion of T cells has also been demonstrated for CD4$^+$ and CD8$^+$ T cells in AS and for CD8$^+$ T cells in ReA. Synovial T cells derived from different

HLA-B27$^+$ patients suffering from ReA and triggered by different bacteria revealed an astonishingly high homology of T-cell receptors (May et al., 2002). These results led to the suggestion that similar antigens are recognized by these oligoclonally expanded CD8$^+$ T cells. This implies that under certain conditions a specific arthritogenic peptide might indeed be produced and presented to the host's immune system.

Beside the "classical" arthritogenic peptide theory, other hypotheses have emerged. One interesting concept, the HLA-B27 misfolding hypothesis states that HLA-B27 itself is directly involved in the pathologic process of SpA. That is, HLA-B27 can be misfolded, which might have implications for pathogenesis (Colbert, 2004). The misfolding is suggested to be due to a particular feature of the HLA-B27 molecule: e.g., newly synthesized HLA-B*2705 seems to fold and associate with β2-microglobulin more slowly compared with other MHC class I molecules. Allen et al. (1999) reported that as a consequence of HLA-B27 misfolding, free HLA-B27 heavy chains can form abnormal heavy-chain homodimers. This homodimer formation could be facilitated by unpaired free cysteine residues at position 67 (Cys67) of the HLA-B27 heavy chain α1 helix. Furthermore, Boyle et al. (2004) have proposed that these HLA-B27 homodimers might mimic MHC class II molecules, and they provided evidence that HLA-B27 can be recognized by CD4$^+$ instead of CD8$^+$ T cells (Boyle et al., 2001). Hence, they postulate a role for HLA-B27 reactive CD4$^+$ T cells in the pathogenesis of AS and other SpA. All in all, despite the high frequency of HLA-B27 in these diseases, its role in pathogenesis has not yet been clarified.

What is the Immune Target in Ankylosing Spondylitis?

If it is indeed unlikely that persistent bacterial infection drives the immunopathology in AS (see above), the question is the nature of the target of the immune response. Studies using MRI have shown that the most relevant inflammatory site in SpA is an osteitis occurring at the bone/cartilage interface (McGonagle et al., 1999). This finding has been supplemented by histologic investigations in SpA from the sacroiliac joint and other structures where, especially in the early phases, mononuclear cells invade and erode the cartilage at different sites (Bollow et al., 2000); such findings have suggested that the cartilage is the primary target of the immune response in SpA (Makymowych, 2000).

Aggrecan G1 domain has been implicated as one source of a possible T-cell autoantigen in AS and similar rheumatic diseases, based both on results from animal models and on studies in patients. A specific CD4$^+$ T-cell response to peptides derived from the G1 domain of aggrecan was found in animal models and also in patients with AS. Here, in 60% of patients, a CD4$^+$ response against the whole G1 protein and against a set of overlapping peptides derived from this G1 protein was evident (Zou et al., 2003).

Because of the type of tissue-specific damage in AS, other extracellular matrix proteins apart from aggrecan, derived from human cartilage and enthesis, could also be targets of an autoimmune response. Recently, a CD8$^+$ T-cell response to a nonameric peptide from collagen type VI was detected in the synovial fluid from patients with AS (Atagunduz et al., 2005). Further work is in progress and will, therefore, have to focus on T-cell responses against cartilage-derived antigens.

PSORIATIC ARTHRITIS

Clinical, Pathologic, and Epidemiologic Features

Psoriatic arthritis is a fascinating disease in combining features of an autoimmune skin disease (see Chapter 58) with those of a seronegative arthritis. The term "psoriatic arthritis" defines a heterogeneous group of arthritides characterized by peripheral monoarticular, oligoarticular, or polyarticular disease, potentially including axial skeletal involvement. Despite the quite different clinical expressions, common features include skin lesions of psoriasis, absence of rheumatoid factor (RF), similar HLA associations and characteristic radiologic findings.

Approximately 2% of the white population in Europe and North America suffer from psoriasis. Of these, some 5–10% have an inflammatory arthritis. Men and women are affected with equal frequency, and the peak incidence is in the fourth through sixth decades. Psoriatic skin disease precedes the onset of arthritis in some 80% of cases, occurs coincidentally with arthritis in some 15% of cases, and after the onset in about 5% of cases. There are even some (rare) cases in which overt psoriasis never develops; yet, the disease may have the typical clinical (usually radiologic) features of psoriatic arthritis ("psoriatic arthritis sine psoriase"). Especially in these cases, a thorough family history is of prime importance.

Clinical Manifestations

Although new attempts are being made to subclassify psoriatic arthritis, the following five different patterns of psoriatic arthritis are currently recognized, and these may shift from one to another.

1. **Asymmetric mono- and oligoarticular arthritis** in 30–50% of cases is the most common initial presentation of psoriatic arthritis.

2. **Symmetric polyarticular arthritis** in 30–50% of patients is the final form of psoriatic arthritis.
3. **Distal interphalangeal (DIP) joint involvement** in 25% of cases is nearly always associated with nail manifestations.
4. **Arthritis mutilans** in 5% of cases is characterized by resorption of the phalangeal bones leading to rapid invalidism.
5. **Axial arthritis** in 30–35% of cases can differ from AS, especially in its radiologic features, can present as sacroiliitis, which may be asymmetric and asymptomatic, or as spondylitis which may occur without sacroiliitis, can affect any level of the spine, and displays typical "parasyndesmophyte" lesions.

Even though, like all inflammatory rheumatic disorders, psoriatic arthritis is a systemic rheumatic disease, involvement of extra-articular sites excluding the skin is rare, but involvement of the eyes (uveitis), heart, lungs, and kidneys with amyloidosis (very infrequently) may occur. Patients with psoriatic arthritis can also develop inflammation of the tendons, especially Achilles tendonitis, and around cartilaginous tissue. Inflammation of the chest wall and of the parasternal cartilage can lead to painful costochondritis. In addition to the cutaneous psoriatic plaques, other characteristic features include nail involvement with pitting, ridging, and onycholysis, dactylitis with so-called "sausage fingers" or "sausage toes" in which there is diffuse swelling of the entire digit due to pronounced tenosynovitis (Figures 33.2 and 33.3).

Radiographically, psoriatic arthritis is a typical unique mixture of bone destruction and proliferation: "destructive and productive" lesions (Figure 33.4). Manifestations may include erosive arthritis giving rise to the classic "pencil-in-cup" deformity in the phalanges, osteolysis, articular ankylosis, sacroiliitis, spondylitis, enthesitis, and periostitis. There is no laboratory test to diagnose psoriatic arthritis, and, interestingly, unlike in rheumatoid arthritis, assays such as erythrocyte sedimentation rate (ESR) or CRP values can give values in the normal range despite severe joint inflammation.

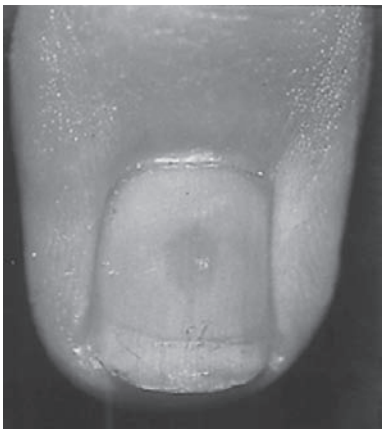


FIGURE 33.2 Typical nail changes in psoriatic arthritis.

Genetic Features and Environmental Influences

As for psoriasis itself, the etiology of psoriatic arthritis is currently unknown and, as for all inflammatory rheumatic diseases, may involve a combination of genetic and (auto)immune, as well as environmental factors. An important link to the spondyloarthritides is the association among psoriatic arthritis patients with spine involvement with HLA-B27 in 70–80% of cases. Several other genes also have been described at higher frequency among patients with psoriatic arthritis. Thus, alleles associated with an adverse outcome, apart from HLA-B27, include HLA DR3 or DR4. There is a very high concordance for psoriasis in monozygotic twins (70%) and a 50-fold increased risk of developing psoriatic arthritis in first-degree relatives of patients with this disease. Interestingly, there is a two-fold increased risk of disease "transmission" by an affected father compared with an affected mother.

Alterations in the immune system also appear important in the development of psoriatic arthritis. A possible clue is provided by the finding that the decline in the number of $CD4^+$ helper T cells in patients with acquired immune deficiency syndrome (AIDS) often results in the development and progression of psoriasis. The importance of infectious agents and other environmental factors as a cause of psoriatic arthritis is a major focus of current research. Streptococcal infection can precipitate the development of guttate psoriasis. In addition, psychological stress and physical trauma has been reported to induce the development of arthritis, suggesting that psoriatic arthritis is the manifestation of a "deep Koebner" type of response.

Treatment and Outcome

Treatment generally starts with NSAIDs, which may suffice in mild cases. In patients with active and potentially destructive disease, DMARDs need to be added early. In contrast to rheumatoid arthritis, only a few studies have addressed effects of conventional DMARDs in psoriatic arthritis, and these have shown limited efficacy. The following drugs should be considered:

- **Methotrexate** is effective for both the cutaneous and peripheral articular manifestations of psoriasis, and is generally the first choice of DMARD due to its efficacy

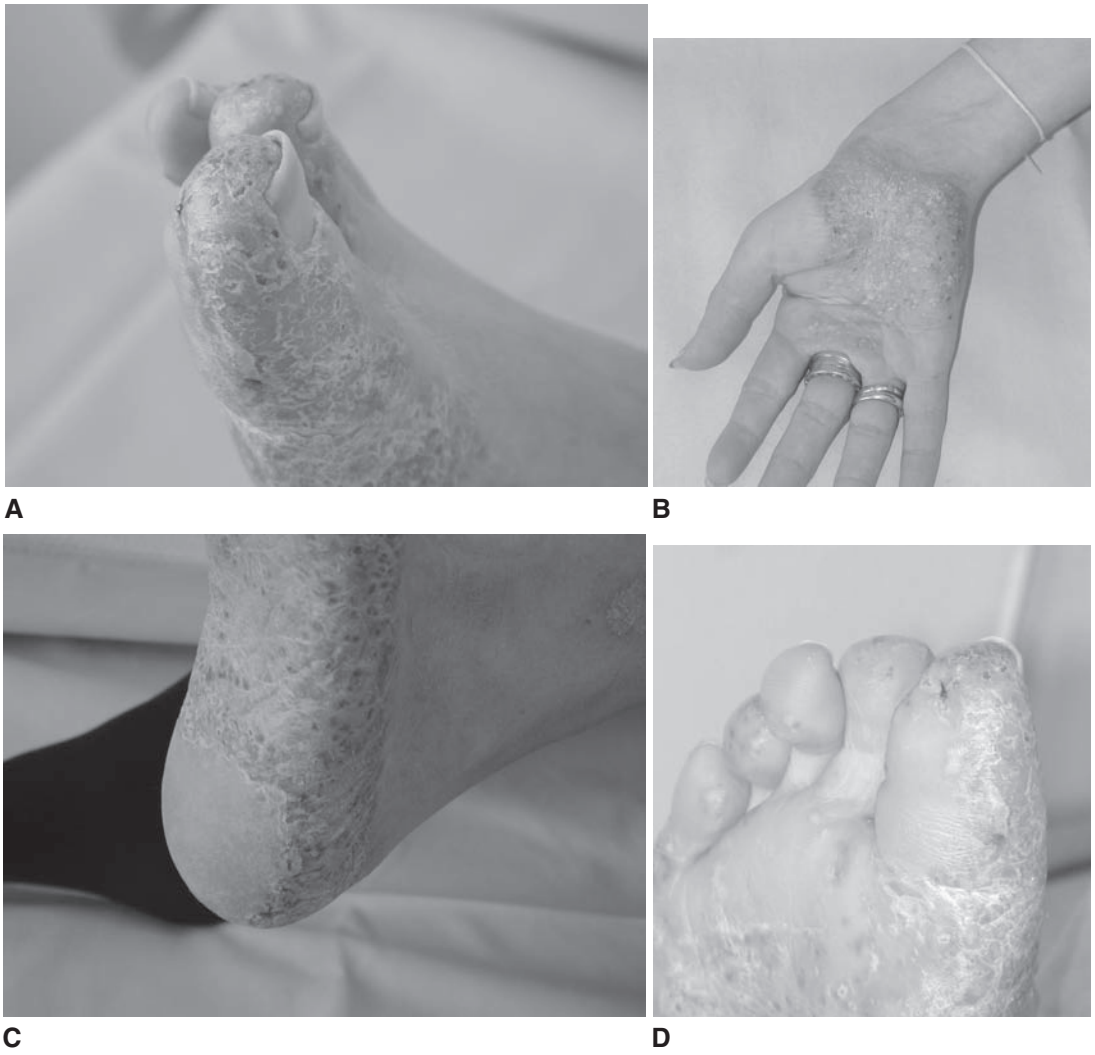


FIGURE 33.3 Characteristic skin changes in psoriatic arthritis.

and tolerability, but liver function should be assessed frequently (Willkens et al., 1984; Espinoza et al., 1992).

- **Sulfasalazine** is only moderately active and does not influence the cutaneous lesions.
- **Cyclosporine** may be effective for both cutaneous and articular disease (Gupta et al., 1989; Salvarani et al., 2001; Sarzi-Puttini et al., 2002), but with risk of hypertension and nephrotoxicity.
- The TNF inhibitors **etanercept**, **infliximab** and, recently, **adalimumab**, in contrast to conventional DMARDs, have proven very effective and well tolerated in the treatment of both psoriasis and psoriatic arthritis (Mease et al., 2000; Mease, 2001; Antoni et al., 2002; Cauza et al., 2002; Iyer et al., 2002) and are currently recommended when any of the above-mentioned DMARDs (usually methotrexate) is ineffective.
- Intra-articular and low-dose systemic glucocorticoids have been used as "bridging" therapy when treatment with a DMARD is instituted, but, as a note of caution, tapering of high-dose corticosteroids has been associated with the development of generalized and sometimes life-threatening pustular psoriasis; hence, systemic glucocorticoid treatment is best avoided when possible.

SAPHO SYNDROME

"SAPHO" is an acronym for a syndrome in which there is a combination of pathologic changes of the skeleton and skin. It is characterized by osteoarticular and dermatologic symptoms compiled by French rheumatologists after a national survey in 1987 (Chamot et al., 1987). Even more

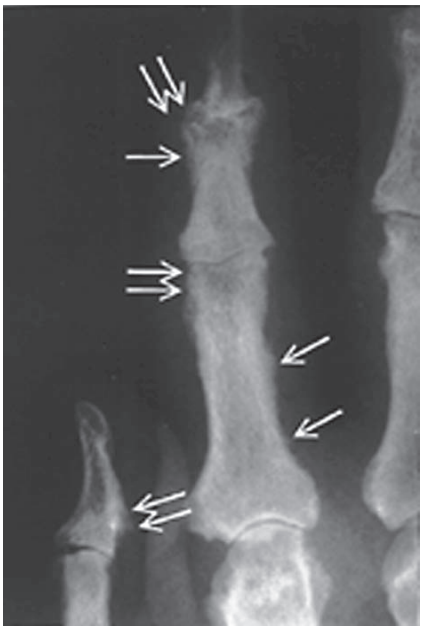


FIGURE 33.4 Radiological changes in psoriatic arthritis. Both destructive and proliferative changes are characteristic.

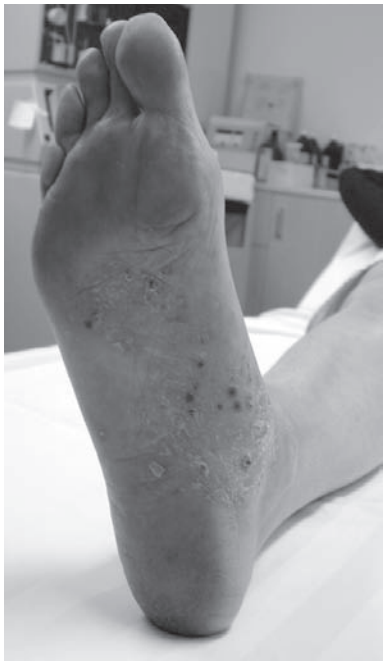


FIGURE 33.5 Palmo-plantar pustulosis in SAPHO syndrome.

than psoriatic arthritis, this unique disease entity provides a striking link between disease expressions of bones, joints, entheses, and the skin.

Clinical, Pathologic, and Epidemiologic Features

All entities forming the SAPHO syndrome are linked by a frequent albeit non-obligate aseptic pustulous "dermatoskeletal" association that includes **s**ynovitis (inflammation of the joints), **a**cne (acne conglobata or fulminans), **p**ustulosis of the skin (purulent blisters), often psoriatic, mainly on the palms of hands and on the soles of feet (palmo-plantar pustulosis) (Figure 33.5), **h**yperostosis (increase in bone substance), **o**steitis (inflammation of the bones).

SAPHO has a clinical course that is marked by relapses and remissions. The major sites of involvement are the anterior chest wall, especially the sternoclavicular joint, the spine, long bones, flat bones, and large and small joints. The distribution and severity of involvement varies from the adult to the pediatric form referred to as chronic recurrent multifocal osteomyelitis (CRMO), which is closely associated with or a component of SAPHO, and is a systemic aseptic inflammation of the bone marrow (osteitis) (Björkstén et al., 1978; Chamot and Kahn, 1994; Handrick et al., 1998). It can occur in many sites, and there may be an association with pustulous acne-like dermatitis. The histopathology of the bone marrow lesions is pathognomonic with a lymphocytic/plasma cell infiltration without evidence of suppurative bacterial infection. Possibly because of its multifactorial nature, the incidence and prevalence of the SAPHO syndrome are still unknown (Chow et al., 1999). The diagnostic features lead to the diagnosis of CRMO being made more frequently, suggesting a frequency approaching that of some systemic autoimmune diseases, for example, scleroderma estimated to be 0.04%. Cases of "enteropathic CRMO" have been described showing resemblances to the enteropathic spondyloarthritides (Huber et al., 2002). Both diseases show osteoarticular manifestations, often preceding the gastrointestinal disease. Crohn's disease-like lesions may occur throughout the gastrointestinal tract (Greenstein et al., 1976; Schilling and Märker-Herrmann, 2003).

The number of affected areas in bones and joints in SAPHO decreases with increasing age of onset. In general, in half of the cases, patients are affected by an inflammatory thoracic wall syndrome with an inflammation of the sternum and neighboring joints (Dihlmann et al., 1997). A primary chronic osteomyelitis of the clavicle is very characteristic and can occur as a single lesion. The axial type of CRMO affects one or several vertebrae as in sterile spondylitis, possibly including the disks as a spondylodiscitis. The mandible may also be affected leading to facial swelling.

An aseptic inflammation of the bone marrow is revealed by typical MRI appearances showing inflammatory bone marrow edema at characteristic bony areas: sternum, clavicle, metaphyses of lamellar bones, pelvis bones, vertebrae, calcaneus, lower jaw, and others (Kohler et al., 1975; Kawei et al., 1988; Kahn and Khan, 1994; Kirchhoff et al., 1998), but the process is not always multifocal. Though rare, unifocal bone lesions may be observed most often at the clavicle or mandible. The results of laboratory tests are not characteristic, with variable indications of inflammation at low levels of activity.

Visceral complications occasionally occur in soft tissues surrounding the affected bones, clavicle, vertebrae and pelvis, the area being inflamed, edematous and fibrotic, possibly resulting in vascular stenosis, for example, subclavicular in osteitis of the clavicle, pelvic veins due to retroperitoneal fibrosis caused by osteitis of the iliac bone, vasculitis due to aortitis between spondylitis and sternal osteitis, neural inflammation due to brachial plexus neuritis in spondylitis of the lower cervical vertebrae or intercostal neuralgia or pleurisy and pericarditis in upper chest osteitis (Greenstein et al., 1976; Kerem et al., 1989; Kaiser, 1996; Schilling and Kessler, 2000).

Genetic and Environmental Features

The etiology of the SAPHO syndrome or CRMO is not known, and it is unclear if any single agent or factor is involved. Cutaneous lesions common to most patients may be an etiologic component. Defined genetic factors are still unclear, especially since neither HLA-B27 nor any other HLA allele is more prevalent (Kahn and Khan, 1994; Schilling and Kessler, 1998). Pathogenesis may involve enthesopathic alterations and has been attributed to immunologic effects (see below). No infectious agent has been found in the osteomyelitis of CRMO. However, *Propionibacterium acnes* (Kotilainen et al., 1996) is claimed to be important as a potential antigenic trigger, since in several cases anaerobic hypovirulent bacteria normally located on the skin have been observed. Thus, under certain circumstances, these organisms may trigger a bone marrow inflammation so leading to lymphoplasmacellular infiltrates with a sclerosing and hyperostosis reaction that may lead to sclerosing osteomyelitis. This process may determine CRMO, and so characterize this primarily chronic osteomyelitis as a reactive osteomyelitis. A "*cmo-mouse*" (chronic multifocal osteomyelitis) has been established as an experimental animal model for CRMO and will provide further insights into the immunopathogenesis.

Treatment

No specific drug exists for SAPHO syndrome. Treatment is mainly with NSAIDs or sulfasalazine (Otte et al., 1982). Glucocorticoids are indicated only in severe cases. Some trials have been carried out with various immunomodulating treatments including anti-TNF-α agents (Wagner et al., 2002). Methotrexate is frequently tried as an immunosuppressive treatment. Antibiotics, especially macrolides, have also been tried for the treatment of CRMO based on the assumption that this type of osteomyelitis is caused by an infection, for example, by *P. acnes* (Kotilainen et al., 1996). A frequently observed positive effect of azithromycin in the treatment of CRMO has suggested that this macrolide also may have anti-inflammatory and even immunomodulating effects (Schilling and Wagner, 2000). Therefore, long-term therapy with azithromycin as the first-line treatment of CRMO has been proposed. Biphosphonates have also been used either orally or as an infusion of pamidronat (Guignard et al., 2002; Marshall et al., 2002).

JUVENILE IDIOPATHIC ARTHRITIS

Juvenile idiopathic arthritis (JIA) is a chronic inflammatory disorder primarily affecting the joints. Diagnosis can be difficult because of diversity of symptoms at onset and course of diseases, and laboratory tests may not be helpful. Different diagnostic criteria used in the past have hindered consensus approaches to this disease. However, recently, JIA has been classified into seven categories, shown below (Petty et al., 2004). In North America, the term "juvenile rheumatoid arthritis" was common, whilst European rheumatologists used "juvenile chronic arthritis." Hence, the term "juvenile idiopathic arthritis" (JIA) was introduced as a diagnostic term generally accepted.

Epidemiology

An incidence for JIA of 13.9 cases per 100,000 per year was reported, with a female-to-male ratio of 2 : 1. African-American children are more likely to have a polyarticular onset than white children, who most often have a pauciarticular onset, indicating that genetic factors are likely to contribute to these differences. Overall, JIA appears to be less common in African-American and Asian populations than in whites.

Classification

A recent classification of JIA (Petty et al., 2004) in childhood defined separate categories of childhood arthritides. Notably, the common characteristics of JIA have an onset before age 16 years, and duration of symptoms of at least 6 weeks. In particular, exclusion criteria have been nominated and are applied as indicated in each of the seven categories below. These exclusions are:

a) Psoriasis including psoriasis in a first degree relative;
b) Arthritis in an HLA-B27$^+$ male before the age of 6 years;
c) Ankylosing spondylitis, enthesitis-associated arthritis, sacroiliitis in IBD, reactive arthritis, or acute anterior uveitis in a first degree relative;
d) Repeated positive tests of RF at least 3 months apart;
e) Systemic arthritis.

The subtype categories of JIA are as follows:

1. **RF$^+$ polyarthritis** with five joints affected in the first 6 months, and exclusion of a, b, c, e;
2. **RF$^-$ polyarthritis** with more than five joints affected in the first 6 months, and with exclusion of a–e;
3. **Systemic arthritis** with arthritis and fever (at least 3 days reaching 39°C) and one of the following criteria: exanthema, generalized lymphadenopathy, hepato/splenomegaly, serositis, and with exclusion criteria a, b, c, d;
4. **Oligoarthritis** is present, with between one and four joints affected within the first 6 months of illness, and with exclusion criteria a–e;
5. **Enthesitis-related arthritis** with two or more of the following involved: sacroiliac joint tenderness; inflammatory spinal pain; HLA-B27$^+$ family history of anterior uveitis with pain, spondyloarthritis, or inflammatory bowel disease; anterior uveitis associated with pain, redness, or photophobia; and with exclusion criteria a, d, e;
6. **Psoriatic arthritis** with arthritis and/or psoriasis and at least two of the following: positive family history of psoriasis in a first degree relative, and dactylitis or characteristic fingernail abnormalities, e.g., pitting or onycholysis, and with exclusion criteria: b–e;
7. **Other arthritides** which comprises patients who do not fulfill criteria for categories 1–6.

It is obvious that this nomenclature is far from complete, or informative on pathogenesis. Thus, there are children who do not fit into any particular category, or whose features overlap those of more than one category, and, therefore, category 7 has been introduced. Careful explanations should avoid any confusion, and the children with overlapping manifestations should be informed of the diagnosis of JIA.

Pathogenesis

The etiology and pathogenesis of JIA remains uncertain, although genetic factors, immune mechanisms and environmental influences are considered to be of importance.

Genetics

Familial predisposition

It is of interest, and in contrast to accepted autoimmune disorders, that there is no family clustering with multiple affected members.

Major histocompatibility genes

- Early-onset oligoarticular JIA is associated with various HLA class II DR and DP alleles, such as HLA-DR8, DR11, DR13, DPw2, whereas HLA-DR4 and HLA-DR7 were found very infrequently. Patients who are heterozygous for HLA-DR5/HLA-DR8 have an enhanced risk for uveitis;
- Polyarticular onset, RF$^-$ JIA has risk conferred by HLA-DPw3;
- RF$^+$, polyarticular JIA has an HLA-DR4 association: HLA-DRB1*0401 and HLA-DRB1*0101;
- Systemic onset JIA is associated with HLA-DR4;
- HLA-B27 associated forms of JIA can be triggered by bacteria.

Environment

Environmental agents are suspected to trigger JIA subtypes but none has been identified so far. Infectious agents have been suggested in particular, but there is also no solid evidence for any particular bacterial or viral trigger.

Immunologic Features

Immunoglobulins

The serum level of immunoglobulins is elevated in many patients with JIA, and those with polyarticular and systemic disease usually have higher levels than those with oligoarticular JIA.

Rheumatoid factor

Rheumatoid factor is infrequently detected in patients with early JIA, but its frequency increases in cases with later onset of the disease, and in longstanding JIA.

Antinuclear antibodies

The reported range of frequency of antinuclear antibodies (ANAs) in JIA patients is very wide: 5–90%, depending on the clinical subtype. Antinuclear antibodies are uncommon in systemic-onset disease but occur in over 50% of cases in oligoarticular JIA. Positivity for ANAs indicates a higher risk of chronic anterior uveitis. Whether the fine specificity of ANAs in uveitis is closely correlated with uveitis, remains uncertain. Overall antibodies to identified nuclear proteins, including Ro, La, centromeric proteins, topoisomerase (Scl-70), or double-stranded DNA are not characteristic of JIA and do not show an increased incidence.

Immune Complexes

Abnormal antibody production and defects in clearance by the reticuloendothelial system may result in an increased concentration of circulating immune complexes in serum and synovial fluid, possibly resulting in the induction of autoantibodies.

Complement Activation

No association has been identified between clinical activity of JIA and complement activation. However, it is considered to be involved in tissue damage. Here the complement activation cascade (C1–C5) participates in a number of inflammatory processes by releasing anaphylatoxins, while the terminal cascade results in the formation of the cytolytic macromolecular complex.

Cytokines

Tumor necrosis factor-α, IL-1β, IL-6, and IL-15 have been identified as important proinflammatory cytokines in JIA synovium indicating their pivotal role in the disease, and the likely role of T-cell activation, but the provocation for this is unknown.

LYME DISEASE (LYME BORRELIOSIS)

The disease was named after the town of Old Lyme, Connecticut, where in 1975 an accumulation of arthritis cases occurred in children of a rural community. These originally were suspected to be juvenile chronic polyarthritis, but a surveillance study revealed that 25% of patients had had an *erythema migrans* and recalled a tick bite prior to the development of arthritis. Erythema migrans had been known to be caused by an infective organism transmitted by ticks since 1910 (Afzelius, 1910). Moreover, an association of erythema migrans with neurologic features including a sensory radiculitis (Garin-Bujadoux, 1922) was also long known—features described by Bannwarth (1944) with pain, paresthesias, Bell's palsy, and lymphocytic pleocytosis of cerebrospinal fluid. In addition to arthritis, skin, and neurologic symptoms, the novel symptoms of the disease included cardiac conduction abnormalities. In 1981, Burgdorfer isolated a spirochete from ticks in an endemic area. This spirochete could also be cultured from skin, blood, and cerebrospinal fluid from patients with Lyme disease and was named *Borrelia burgdorferi* (Burgdorfer et al., 1982).

Clinical, Pathologic, and Epidemiologic Features

Epidemiology

Lyme borreliosis is the most common vector-borne disease in the USA, Europe, and parts of Asia, with annual reported incidences exceeding 100 cases per 100,000 inhabitants in certain areas (Huppertz et al., 1999). Approximately 15,000 cases of Lyme disease are reported in the USA annually (CDC 2000; Steere, 2001).

Etiology

Lyme disease is caused by *B. burgdorferi* sensu lato, a gram-negative spirochete. *B. burgdorferi* undergoes enzootic cycles between ixoid ticks, *Ixodes ricinus* in Europe and *Ixodes scapularis* and *Ixodes pacificus* in the USA, and small mammalian reservoirs. *B. burgdorferi* sensu lato is quite heterogeneous, and currently 11 different *Borrelia* species have been isolated from ticks. At least three species are pathogenic in humans: *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*. In the USA, only *B. burgdorferi sensu stricto* occurs (Fraser et al., 1997; Steere, 2001), whereas all three species can be found in Europe (Berglund et al., 1995; Huppertz et al., 1995; 1999; Steere, 2001; Lünemann et al., 2001). These different species preferentially invade different organs (organotropism), which may partly explain the different clinical features of Lyme disease in the USA compared with Europe.

Clinical Manifestations

Although Lyme disease is usually grouped into three stages (early, stage 1; weeks after the tick bite, stage 2; or months-to-years after the tick bite, stage 3), earlier stages may be skipped, missed by the patients, or may overlap. Nevertheless, pragmatically, it is valid to allocate patients to these stages and to discriminate between early localized disease (erythema migrans), acute disseminated disease (neurologic or non-neurologic with carditis) and chronic disease (arthritis, acrodermatitis).

Early Lyme disease has two features: first, the characteristic erythema migrans (Figure 33.6), and second, systemic constitutional signs with low-grade fever, 'flu'-like symptoms, arthralgias, malaise, headaches, and paresthesias, which may accompany erythema migrans or occur without a recognized skin lesion. Erythema migrans starts as a red macula or papule at the site of the tick bite. After an incubation period of a few days up to 8 weeks, the lesions gradually expand, sometimes reaching a large size. Even without antibiotic treatment, erythema migrans will resolve within 4–12 weeks; however, in many cases bacteria will persist at other sites and will cause subsequent Lyme manifestations. An additional *dermatologic manifestation* in the early phase is the lymphocytoma cutis, especially in Europe, usually presenting as a purplish nodule at the ear lobe, the nose, the forehead, or the nipple.

In a minority of patients (about 15%), typical *neurologic symptoms* develop within weeks or months after the tick bite (Eskow et al., 2001); these are cranial neuropathy most

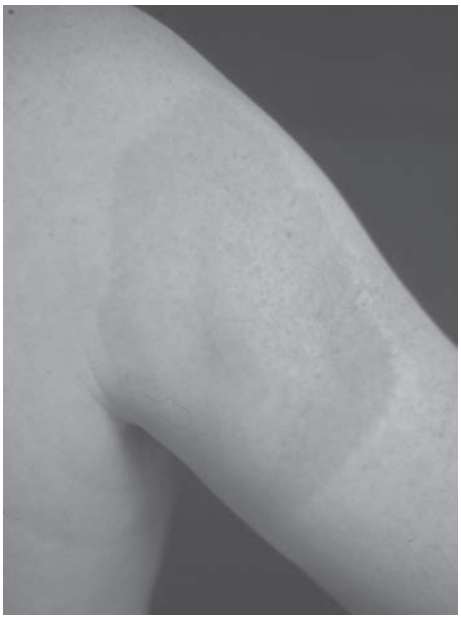


FIGURE 33.6 Erythema migrans.

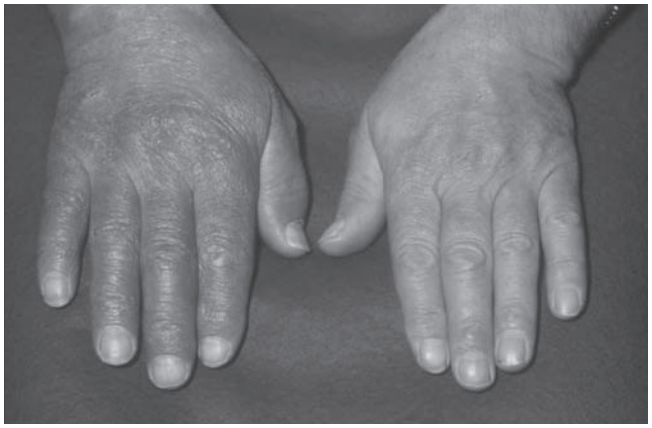


FIGURE 33.7 Acrodermatitis chronica atrophicans of the right hand.

commonly involving the facial nerve, meningitis especially in children, and radiculoneuropathy alone or in combination. The rare manifestations of chronic neurologic Lyme disease occurring months to years after infection include demyelinating encephalopathy or myelopathy, chronic encephalopathy, peripheral polyneuropathy and transverse myelitis (Pachner et al., 1989; Eskow et al., 2001; Steere, 2001).

Lyme *carditis* is a rather rare entity occurring in 5–8% of patients (Steere et al., 1980). It belongs to stage 2 of the disease, frequently occurring after a preceding erythema migrans. Many patients also have neurologic symptoms and arthralgias. Typical manifestations are conduction abnormalities with varying degrees of atrioventricular block, right or left bundle block, atrial fibrillations, or tachycardias. Rare incidences of a chronic Lyme carditis have been described resulting in cardiomyopathy even with a fatal outcome (Steere, 2001).

In the USA, about 60%, and in Europe a much lesser proportion of untreated patients, develop arthritis usually weeks to years after the initial infection. Nevertheless, the onset of arthritis is acute, making discrimination from other acutely occurring arthritides difficult. The clinical course is usually intermittent with acute attacks, with transient apparent "remissions" and left untreated may go on for months and years (Shadick et al., 1999; Kalish et al., 2001; Karkkonen et al., 2001). In most cases, there is a mono- or oligoarticular course predominantly affecting the knees, ankles, and sometimes elbows, usually with massive effusions. There may be no overt arthritis with the disease characterized by nonspecific arthralgias, myalgias, and periarticular pain, with distinction difficult from functional syndromes and fibromyalgia, especially in seropositive individuals living in endemic areas (Shapiro and Gerber, 2000; Burmester et al., 2002).

Chronic borreliosis of the skin is rare in the USA, but frequent in untreated European patients. Acrodermatitis chronica atrophicans (ACA) occurs more than 12 months after the initial infection and is characterized by a unilateral extended distal atrophic skin lesion frequently preceded by an edematous violaceous stage (Figure 33.7).

Pathogenic Mechanisms

Upon transmission, the spirochetes *B. burgdorferi* invade several host tissues. They first encounter cells of the innate immune system predominantly polymorphonuclear cells and macrophages, but also innate T cells, natural killer (NK) and γδ-T cells as a first line of defense (Kamradt and Mitchison, 2001; Burmester et al., 2002). Binding of *B. burgdorferi* lipoproteins to Toll-like receptors induces a variety of pro-inflammatory mediators that account for the inflammatory reaction in the infected host tissue. There is also a strong but delayed adaptive immune response by B and T cells. Despite these innate and adaptive immune responses, about 80% of infected persons develop a systemic disease, with several properties of the spirochetes as well as host factors involved.

The pathogenesis of chronic persistent Lyme disease is still a matter of debate. Most patients with Lyme arthritis recover completely after antibiotic therapy; however, in some 10% of patients, inflammation persists even after

repeated courses of antibiotic therapy. Both persistent infection and induction of immunopathology by the spirochetes are currently discussed. A persistent infection is favored by isolation of spirochetes even at late stages of disease from involved tissues, for example, from acrodermatitis chronica atrophicans tissue after 10 years. Also by polymerase chain reaction (PCR), the *B. burgdorferi* genome could be detected in synovial tissue (Priem et al., 1998; Lünemann et al., 2001). Further, ultrastructural studies of synovial membranes from patients with chronic arthritis, and use of a three-dimensional *in vitro* model of Lyme arthritis, showed spirochetes not only in tissues with a low turnover rate, such as within bundles of collagen fibers, but also within macrophages and resident synovial fibroblasts (Franz et al., 2001).

On the other hand, in chronic therapy-resistant Lyme arthritis, the histologic lesions of affected synovial tissues resemble those of rheumatoid arthritis. Thus, together with the rare isolation of viable spirochetes from patients with chronic disease, the unresponsiveness to antibiotic treatment and the association with HLA DR4 and DR2 point towards an immunopathogenesis induced by the spirochetes. Hence, cross-reactivity between the outer surface protein A (OspA) of the spirochete and a self-antigen is discussed as cause of treatment-resistant Lyme arthritis. An alternative explanation for the development of treatment-resistant Lyme arthritis would be a hypersensitivity reaction that develops to traces of persistent antigen. Cytokines produced by cells of the innate immune system, including IL-1, IL-6, IL-10, IL-11, IL-12, IL-17, and TNF-α have all been implicated in the generation of arthritis severity in patients or animal models. Altogether, it is likely that non-antigen-specific mechanisms mediate, perhaps in synergy with antigen-specific mechanisms, the immunopathology that finally leads to treatment-resistant Lyme arthritis in susceptible patients.

Treatment and Outcome

All clinical manifestations of Lyme borreliosis should be treated with antibiotics as early as possible to shorten the clinical course and prevent the progression of the disease (for guidelines see URL http://www.dis.strath.ac.uk/vie/LymeEU). Amoxicillin, doxycycline and third-generation cephalosporins are the drugs of choice (Nadelmann and Wormser, 1998; Sigal, 1998; Shapiro and Gerber, 2000; Steere, 2001). In patients with persistent symptoms, a second parenteral antibiotic should be given, again following recommended guidelines. Using such approaches, the prognosis of Lyme disease is excellent, with almost all early infections cured rapidly without sequelae. In later disease stages, especially in Lyme arthritis, symptoms often disappear slowly, and clinical improvement is observed only several weeks after therapy. However, approximately 10% of patients with Lyme arthritis in the USA and probably a smaller percentage of patients in Europe do not respond sufficiently even to repeated courses of antibiotics. The pathogenesis of this so-called treatment-resistant Lyme arthritis is not fully elucidated but infection-induced immunopathology seems most likely. It is not easily claimed that persistent Lyme disease is truly autoimmune in its origin.

CONCLUDING REMARKS—FUTURE PROSPECTS

The spondyloarthritides and related diseases including psoriatic arthritis and the SAPHO syndrome are among the most fascinating rheumatic diseases, since they clearly demonstrate the close interplay of manifestations of the skin, the gut, the musculoskeletal system, and the immune system, especially with regard to the HLA complex. Long neglected, particularly because rheumatologists had little to offer, increasing awareness has been raised because of new treatment options, utilizing biologic therapy. We now recognize that these diseases are no longer rare, that they represent an enormous burden to the patients affected, and that they offer excellent opportunities to learn more about disease mechanisms in general. Despite, however, several decades of research, the riddles of the role of the HLA-B27 molecule and the triggering events of infections still remain. Even in Lyme borreliosis with its clearly defined causative microbial organism, important questions still remain, such as why only a minority of patients develop the disease after infection and why the courses differ so tremendously in individual patients. The next years will witness important new developments addressing these problems certainly leading to better lives for our patients.

References

Afzelius, A. (1910). Verhandlungen der Gesellschaft zu Stockholm on October 29, 1909. *Arch. Dermatol. Syph. 20,* 7–17.

Allen, R.L., O'Callaghan, C.A., McMichael, A.J., Bowness, P. (1999). Cutting edge: HLA-B27 can form a novel beta 2-microglobulin-free heavy chain homodimer structure. *J. Immunol.* 162:5045–5048.

Antoni, C., Dechant, C., Lorenz, P.D.H.M., Wendler, J., Ogilvie, A., Lueftl, M., Kalden-Nemeth, D., Kalden, J. R., and Manger, B. (2002). Open-label study of infliximab treatment for psoriatic arthritis: Clinical and magnetic resonance imaging measurements of reduction of inflammation. *Arthritis Rheum. Arthritis Care Res. 47,* 506–512.

Atagunduz, P., Appel, H., Kuon, W., Wu, P., Thiel, A., Kloetzel, P.M., and Sieper, J. (2005). HLA-B27 restricted CD8+ T cell response to cartilage-derived self-peptides in ankylosing spondylitis. *Arthritis Rheum. 52,* 892–901

Bannwarth, A. (1944). Zur Klinik und Pathogenese der 'chronischen lymphozytären meningitis'. *Arch. Psychiat. Nervenkr. 117,* 161–185.

Berglund, J., Eitrem, R., Ornstein, K., Lindberg, A., Ringner, A., Elmrud, H., Carlsson, M., Runehagen, A., Svanborg, C., and Norrby, R. (1995). An epidemiologic-study of lyme-disease in southern sweden. *N. Engl. J. Med. 333*(20), 1319–1324.

Björksten, B., Gustavson, K.H., Eriksson, B., Lindholm, A., and Nordstrom, S. 1978) Chronic recurrent multifocal osteomyelitis and pustulosis palmoplantaris. *J. Pediat. 93*(2), 227–231.

Bollow, M., Fischer, T., Reisshauer, H., Backhaus, M., Sieper, J., Hamm, B., and Braun, J. (2000). Quantitative analyses of sacroiliac biopsies in spondyloarthropathies: T cells and macro phages predominate in early and active sacroiliitis—cellularity correlates with the degree of enhancement detected by magnetic resonance imaging. *Ann. Rheum. Dis. 59,* 135–140.

Boyle, L.H., Goodall, J.C., Opat, S.S., Gaston, J.S. (2001). The recognition of HLA-B27 by human CD4(+) T lymphocytes. *J. Immunol. 167,* 2619–2624.

Boyle, L.H., Goodall, J.C., Gaston, J.S. (2004). The recognition of abnormal forms of HLA-B27 by CD4+ T cells. *Curr. Mol. Med. 4,* 51–58.

Braun, J., Bollow, M., Neure, L., Seipelt, E., Seyrekbasan, F., Herbst, H., Eggens, U., Distler, A., and Sieper, J. (1995). Use of immunohistologic and in-situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing-spondylitis. *Arthritis Rheum. 38,* 499–505.

Braun, J., Tuszewski, M., Ehlers, S., Haberle, J., Bollow, M., Eggens, U., Distler, A., and Sieper, J. (1997). Nested polymerase chain reaction strategy simultaneously targeting DNA sequences of multiple bacterial species in inflammatory joint diseases. II. Examination of sacroiliac and knee joint biopsies of patients with spondyloarthropathies and other arthritides. *J. Rheumatol. 24(6)*, 1101–1105.

Braun, J., Yin, Z.N., Spiller, I., Siegert, S., Rudwaleit, M., Liu, L.Z., Radbruch, A., and Sieper, J. (1999). Low secretion of tumor necrosis factor alpha, but no other Th1 or Th2 cytokines, by peripheral blood mononuclear cells correlates with chronicity in reactive arthritis. *Arthritis Rheum. 42,* 2039–2044.

Braun, J., Brandt, J., Listing, J., Rudwaleit, M., and Sieper, J. (2003). Biologic therapies in the spondyloarthritis: new opportunities, new challenges. *Curr. Opin. Rheumatol. 15,* 394–407.

Braun, J. and Sieper, J. (2003). Spondyloarthritides and related arthritides. In D.A. Warrel, T.M. Cox, J.D. Firth, E.J. Benz (eds), *Oxford Textbook of Medicine*, 4th ed. (pp. 43–53). New York: Oxford University Press.

Braun, J. and Sieper, J. (2004). Biological therapies in the spondyloarthritides—the current state. *Rheumatology (Oxford). 43,* 1072–1084

Brown, M.A., Wordsworth, B.P., and Reveille, J.D. (2002). Genetics of ankylosing spondylitis. *Clin. Exp. Rheumatol. 20,* S43–S49.

Burgdorfer, W., Barbour, A.G., Hayes, S.F., Benach, J.L., Grunwaldt, E., and Davis, J.P. (1982). Lyme-disease—a tick-borne spirochetosis. *Science 216,* 1317–1319.

Burmester G.R., Kamradt T., and Krause A. Lyme Borreliosis. (2004). In Isenberg, D., Maddison, P., Woo, P., Glass, D., Breedveld, F. (eds.) *The Oxford Textbook of Rheumatology*.

Cauza, E., Spak, M., Cauza, K., Hanusch-Enserer, U., Dunky, A., and Wagner, E. (2002). Treatment of psoriatic arthritis and psoriasis vulgaris with the tumor necrosis factor inhibitor infliximab. *Rheumatol. Intl. 22,* 227–232.

CDC (2000). Lyme disease—United States. *Morb. Mortal. Wkly. Rep. 51,* 29–31.

Chamot, A.M., Benhamou, C.L., Kahn, M.F., Beraneck, L., Kaplan, G., and Prost, A. (1987). The Syndrome Acne-Pustulosis-Hyperostosis-Osteitis (Sapho)—results of a national investigation—85 Case-Reports. *Rev. Rhumatisme 54,* 187–196.

Chamot, A.M. and Kahn, M.F. (1994) Sapho-syndrome. *Z. Rheumatol. 53,* 234–242.

Chow, L.T.C., Griffith, J.F., Kumta, S.M., and Leung, P.C. (1999). Chronic recurrent multifocal osteomyelitis: A great clinical and radiologic mimic in need of recognition by the pathologist. *Apmis 107,* 369–379.

Colbert, R.A. (2004) The immunobiology of HLA-B27: Variations on a theme. *Curr. Mol. Med. 4,* 21–30.

Davis, J.C. Jr, Van Der Heijde, D., Braun, J., Dougados, M., Cush, J., Clegg, D.O., Kivitz, A., Fleischmann, R., Inman, R., and Tsuji, W. (2003). Enbrel Ankylosing Spondylitis Study Group. Recombinant human tumor necrosis factor receptor (etanercept) for treating ankylosing spondylitis: a randomized, controlled trial. *Arthritis Rheum. 48,* 3230–3236.

Dihlmann, W., Dihlmann, S.W., and Hering, L. (1997). Acquired hyperostosis syndrome—AHYS—(Sternocostoclavicular hyperostosis, pustulotic arthro-osteitis, SAPHO-syndrome): Bone scintigraphy of the anterior chest wall. *Clin. Rheumatol. 16,* 13–24.

Eskow, E., Rao, R.V.S., and Mordechai, E. (2001). Concurrent infection of the central nervous system by *Borrelia burgdorferi* and *Bartonella henselae*—Evidence for a novel tick-borne disease complex. *Arch. Neurol. 58,* 1357–1363.

Espinoza, L.R., Zakraoui, L., Espinoza, C.G., Gutierrez, F., Jara, L.J., Silveira, L.H., Cuellar, M. L., and Martinezosuna, P. (1992). Psoriatic-arthritis—clinical-response and side-effects to methotrexate therapy. *J. Rheumatol. 19,* 872–877.

Fiorillo, M.T., Maragno, M., Butler, R., Dupuis, M.L., Sorrentino, R. 2000, CD8+ T-cell autoreactivity to an HLA-B27-restricted self-epitope correlates with ankylosing spondylitis. *J. Clin. Invest. 106,* 47–53.

Franz, J.K., Fritze, O., Rittig, M., Keysser, G., Priem, S., Zacher, J., Burmester, G.R., and Krause, A. (2001). Insights from a novel three-dimensional *in vitro* model of lyme arthritis—Standardized analysis of cellular and molecular interactions between *Borrelia burgdorferi* and synovial explants and fibroblasts. *Arthritis Rheum. 44,* 151–162.

Fraser, C.M., Casjens, S., Huang, W.M., Sutton, G.G., Clayton, R., Lathigra, R., White, O., Ketchum, K.A., Dodson, R., Hickey, E.K., Gwinn, M., Dougherty, B., Tomb, J.F., Fleischmann, R.D., Richardson, D., Peterson, J., Kerlavage, A.R., Quackenbush, J., Salzberg, S., Hanson, M., vanVugt, R., Palmer, N., Adams, M.D., Gocayne, J., Weidman, J., Utterback, T., Watthey, L., McDonald, L., Artiach, P., Bowman, C., Garland, S., Fujii, C., Cotton, M.D., Horst, K., Roberts, K., Hatch, B., Smith, H.O., and Venter, J.C. (1997). Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature 390,* 580–586.

Garin-Bujadoux, C. (1922). Paralysis par les tiques. *J. Med. Lyon 71,* 765–767.

Greenstein, A.J., Janowitz, H.D., and Sachar, D.B. (1976). Extra-intestinal complications of Crohns-disease and ulcerative-colitis—Study of 700 Patients. *Medicine 55,* 401–412.

Guignard, S., Job-Deslandre, C., Sayag-Boukris, V., and Kahan, A. (2002). Pamidronate treatment in SAPHO syndrome. *Joint Bone Spine 69,* 392–396.

Gupta, A.K., Matteson, E.L., Ellis, C.N., Ho, V.C., Tellner, D.C., Voorhees, J.J., and McCune, W.J. (1989). Cyclosporine in the treatment of psoriatic-arthritis. *Arch. Dermatol. 125,* 507–510.

Handrick, W., Hormann, D., Voppmann, A., Schille, R., Reichardt, P., Trobs, R.B., Moritz, R. P., and Borte, M. (1998) Chronic recurrent multifocal osteomyelitis—report of eight patients. *Pediat. Surg. Intl. 14,* 195–198.

Hoehler, T., Schaper, T., Schneider, P.M., zum Buschenfelde, K.H.M., and Marker-Hermann, E. (1998). Association of different tumor necrosis factor alpha promoter allele frequencies with ankylosing spondylitis in HLA-B27 positive individuals. *Arthritis Rheum. 41,* 1489–1492.

Huber, A.M., Lam, P.Y., Duffy, C.M., Yeung, R.S.M., Ditchfield, M., Laxer, D., Cole, W.G., Graham, H.K., Allen, R.C., and Laxer, R.M. (2002). Chronic recurrent multiflocal osteomyelitis: Clinical outcomes after more than five years of follow-up. *J. Pediat. 141,* 198–203.

Huppertz, H.I., Karch, H., Suschke, H.J., Doring, E., Ganser, G., Thon, A., and Bentas, W. (1995). Lyme arthritis in European children and adolescents. *Arthritis Rheum. 38,* 361–368.

Huppertz, H.I., Bohme, M., Standaert, S.M., Karch, H., and Plotkin, S.A. (1999) Incidence of Lyme borreliosis in the Wurzburg region of Germany. *Eur. J. Clin. Microbiol. Infect. Dis. 18,* 697–703.

Iyer, S., Yamauchi, P., and Lowe, N.J. (2002). Etanercept for severe psoriasis and psoriatic arthritis: observations on combination therapy. *Br. J. Dermatol. 146,* 118–121.

Kahn, M.F. and Khan, M.A. (1994). The Sapho syndrome. *Baillières Clin. Rheumatol. 8,* 333–362.

Kaiser, H. (1996). Update on SAPHO-syndrome—Reply. *Z. Rheumatol. 55,* 75.

Kalish, R.A., Kaplan, R.F., Taylor, E., Woodward, L.J., Workman, K., and Steere, A.C. (2001). Evaluation of study patients with Lyme disease, 10–20-year follow-up. *J. Infect. Dis. 183,* 453–460.

Kaluza, W., Leirisalo-Repo, M., Marker-Hermann, E., Westman, P., Reuss, E., Hug, R., Mastrovic, K., Stradmann-Bellinghausen, B., Granfors, K., Galle, P.R., and Hohler, T. (2001). IL10.G microsatellites mark promoter haplotypes associated with protection against the development of reactive arthritis in Finnish patients. *Arthritis Rheum. 44,* 1209–1214.

Kamradt, T. and Mitchison, N.A. (2001). Advances in immunology: Tolerance and autoimmunity. *N. Engl. J. Med. 344,* 655–664.

Karkkonen, K., Stiernstedt, S.H., and Karlsson, M. (2001). Follow-up of patients treated with oral doxycycline for Lyme neuroborreliosis. *Scand. J. Infect. Dis. 33,* 259–262.

Kawai, K., Doita, M., Tateishi, H., and Hirohata, K. (1988). Bone and joint lesions associated with pustulosis palmaris et plantaris—a clinical and histological study. *J. Bone Joint Surg. Br. Vol. 70,* 117–122.

Kerem, E., Manson, D., Laxer, R.M., Levison, H., and Reilly, B.J. (1989). Pulmonary association in a case of chronic recurrent multifocal osteomyelitis. *Pediat. Pulmonol. 7,* 55–58.

Khan, M.A. (2000). Update: the twenty subtypes of HLA-B27. *Curr. Opin. Rheumatol. 12,* 235–238.

Kirchhoff, T.D., Merkesdal, S.A., Rosenthal, H., Prokop, M., Wagner, A., and Galanski, M.W. (1998). Importance of MRI and CT-guided bone-biopsy in the management of patients with SAPHO-syndrome. *Radiology 209,* 501.

Kohler, H., Uehlinger, E., Kutzner, J., Weihrauch, T.R., Wilbert, L., and Schuster, R. (1975). Sterno-costo-clavicular hyperostosis—hitherto undescribed entity. *Deutsche Medizinische Wochenschrift 100,* 1519–1523.

Kotilainen, P., MerilahtiPalo, R., Lehtonen, O.P., Manner, I., Helander, I., Mottonen, T., and Rintala, E. (1996). *Propionibacterium acnes* isolated from sternal osteitis in a patient with SAPHO syndrome. *J. Rheumatol. 23,* 1302–1304.

Kuon, W. and Sieper, J. (2003). Identification of HLA-B27-restricted peptides in reactive arthritis and other spondyloarthropathies: computer algorithms and fluorescent activated cell sorting analysis as tools for hunting of HLA-B27-restricted chlamydial and autologous crossreactive peptides involved in reactive arthritis and ankylosing spondylitis. *Rheum. Dis. Clin. N. Am. 29,* 595–611.

Leirisalo-Repo, M. (1998). Prognosis, course of disease, and treatment of the spondyloarthropathies. *Rheum. Dis. Clin. N. Am. 24,* 737.

Lünemann, J.D., Zarmas, S., Priem, S., Franz, J., Zschenderlein, R., Aberer, E., Klein, R., Schouls, L., Burmester, G.R., and Krause, A. (2001). Rapid typing of *Borrelia burgdorferi* sensu lato species in specimens from patients with different manifestations of lyme borreliosis, *J. Clin. Microbiol. 39,* 2041.

Maksymowych, W.P. (2000). Ankylosing spondylitis—at the interface of bone and cartilage. *J. Rheumatol. 27,* 2295–301.

Marshall, H., Bromilow, J., Thomas, A.L., and Arden, N.K. (2002). Pamidronate: a novel treatment for the SAPHO syndrome? *Rheumatology 41,* 231–2U3.

May, E., Dulphy, N., Frauendorf, E., Duchmann, R., Bowness, P., de Castro, J.A.L., Toubert, A., and Marker-Hermann, E. (2002). Conserved TCR beta chain usage in reactive arthritis; evidence for selection by a putative HLA-B27-associated autoantigen. *Tissue Antigens 60,* 299–308.

McGonagle, D., Gibbon, W., O'Connor, P., Green, M., Pease, C., Ridgway, J., and Emery, P. (1999). An anatomical explanation for good-prognosis rheumatoid arthritis. *Lancet 353,* 123–124.

Mease, P.J., Goffe, B.S., Metz, J., VanderStoep, A., Finck, B., and Burge, D.J. (2000). Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet 356,* 385–390.

Mease, P.J. (2001). Cytokine blockers in psoriatic arthritis. *Ann. Rheum. Dis. 60,* III37-III40.

Mielants, H., Veys, E.M., Cuvelier, C., and Devos, M. (1988). Ileocolonoscopic findings in seronegative spondylarthropathies. *Br. J. Rheumatol. 27,* 95–105.

Nadelman, R.B. and Wormser, G.P. (1998). Lyme borreliosis. *Lancet 352,* 557–565.

Neure, L., Braun, J., and Sieper, J. (1999). A higher ratio of IL-10/TNFα-positive cells in reactive arthritis (REA) synovial membrane is found for all cell types. *Arthritis Rheum. 42,* S402.

Otte, P., Schiller, H., and Schilling, F. (1982). Arthritis and spondyloarthritis in acne fulminans. *Aktuelle Rheumatol. 7,* 33–35.

Pachner, A.R., Duray, P., and Steere, A.C. (1989). Central nervous-system manifestations of Lyme-disease. *Arch. Neurol. 46,* 790–795.

Petty, R.E., Southwood, T.R., Manners, P., Baum, J., Glass, D.N., Goldenberg, J., He, X., Maldonado-Cocco, J., Orozco-Alcala, J., Prieur, A.M., Suarez-Almazor, M.E., and Woo, P. (2004). International League of Associations for Rheumatology. *J. Rheumatol. 31,* 390–392.

Priem, S., Burmester, G.R., Kamradt, T., Wolbart, K., Rittig, M.G., and Krause, A. (1998). Detection of *Borrelia burgdorferi* by polymerase chain reaction in synovial membrane, but not in synovial fluid from patients with persisting Lyme arthritis after antibiotic therapy. *Ann. Rheum. Dis. 57,* 118–121.

Purrmann, J., Zeidler, H., Bertrams, J., Juli, E., Cleveland, S., Berges, W., Gemsa, R., Specker, C., and Reis, H.E. (1988). Hla antigens in ankylosing-spondylitis associated with Crohns-disease—increased frequency of the Hla phenotype B27,B44. *J. Rheumatol. 15,* 1658–1661.

Rudwaleit, M., Siegert, S., Yin, Z., Eick, J., Thiel, A., Radbruch, A., Sieper, J., and Braun, J. (2001). Low T cell production of TNF alpha and IFN gamma in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. *Ann. Rheum. Dis. 60,* 36–42.

Rudwaleit, M., van der Heijde, D., Khan, M.A., Braun, J., and Sieper, J. (2004). How to diagnose axial spondyloarthritis early. *Ann. Rheum. Dis. 63,* 535–543.

Salvarani, C., Macchioni, P., Olivieri, I., Marchesoni, A., Cutolo, M., Ferraccioli, G., Cantini, F., Salaffi, F., Padula, A., Lovino, C., Dovigo, L., Bordin, G., Davoli, C., Pasero, G., and Alberighi, O.D.C. (2001) A comparison of cyclosporine, sulfasalazine, and symptomatic therapy in the treatment of psoriatic arthritis. *J. Rheumatol. 28,* 2274–2282.

Sarzi-Puttini, P., Cazzola, M., Panni, B., Turiel, M., Fiorini, T., Belai-Beyene, N., and Cherie-Ligniere, E.L. (2002). Long-term safety and efficacy of low-dose cyclosporin A in severe psoriatic arthritis. *Rheumatol. Intl. 21,* 234–238.

Schilling, F. and Kessler, S. (1998). Spondarthritis hyperostotica pustulopsoriatica—Review and a clinical-radiological study with special emphasis on SAPHO syndrome. *Rofo-Fortschritte Auf dem Gebiet der Rontgenstrahlen und der Bildgebenden Verfahren 169,* 576–584.

Schilling, F. and Kessler, S. (2000). The SAPHO syndrome: Clinical and radiological differentiation and classification on the basis of 86 cases. *Z. Rheumatol. 59,* 1–28.

Schilling, F. and Wagner, A.D. (2000). Azithromycin: An anti-inflammatory activity applicable to chronic recurrent multifocal osteomyelitis? Some thoughts. *Z. Rheumatol. 59,* 352–353.

Schilling, F. and Märker-Hermann, E. (2003). Chronic recurrent multifocal osteomyelitis in association with chronic inflammatory bowel disease: entheropathic CRMO. *Z. Rheumatol. 62,* 527–538.

Shadick, N.A., Phillips, C.B., Sangha, O., Logigian, E.L., Kaplan, R.F., Wright, E.A., Fossel, A.H., Fossel, K., Berardi, V., Lew, R.A., and Liang, M.H. (1999). Musculoskeletal and neurologic outcomes in patients with previously treated Lyme disease. *Ann. Intern. Med. 131,* 919.

Shapiro, E.D. and Gerber, M.A. (2000). Lyme disease. *Clin. Infect. Dis. 31,* 533–542.

Sieper, J., Braun, J., and Kingsley, G.H. (2000). Report on the Fourth International Workshop on Reactive Arthritis. *Arthritis Rheum. 43,* 720–734.

Sieper, J., Rudwaleit, M., Braun, J., and van der Heijde, D. (2002). Diagnosing reactive arthritis—Role of clinical setting in the value of serologic and microbiologic assays. *Arthritis Rheum. 46,* 319–327.

Sigal, L.H. (1998) Musculoskeletal manifestations of Lyme arthritis. *Rheum. Dis. Clin. N. Am. 24,* 323.

Steere, A.C., Batsford, W.P., Weinberg, M., Alexander, J., Berger, H.J., Wolfson, S., and Malawista, S.E. (1980). Lyme carditis—cardiac abnormalities of Lyme-disease. *Ann. Intern. Med. 93,* 8–16.

Steere, A.C. (2001). Medical progress: Lyme disease. *N. Engl. J. Med. 345,* 115–125.

Taurog, J.D., Maika, S.D., Satumtira, N., Dorris, M.L., Mclean, I.L., Yanagisawa, H., Sayad, A., Stagg, A.J., Fox, G.M., O'Brien, A.L., Rehman, M., Zhou, M., Weiner, A.L., Splawski, J.B., Richardson, J.A., and Hammer, R.E. (1999). Inflammatory disease in HLA-B27 transgenic rats. *Immunol. Rev. 169,* 209–223.

van der Heijde, D., Dijkmans, B., Geusens, P., Sieper, J., Dewoody, K., Williamson, P. and Braun, J. (2005). Efficacy and safety of infliximab in patients with ankylosing spondylitis: Results of a randomized, placebo-controlled trial (ASSERT). *Arthritis Rheum. 52,* 582–591.

van der Linden, S., Cats, A., Valkenburg, H.A., and Khan, M.A. (1984). Evaluation of the diagnostic-criteria for ankylosing-spondylitis—a proposal for modification of the New-York criteria. *Br. J. Rheumatol. 23,* 148–149.

Wagner, A.D., Andresen, J., Jendro, M.C., Hulsemann, J.L., and Zeidler, H. (2002). Sustained response to tumor necrosis factor alpha-blocking agents in two patients with SAPHO syndrome. *Arthritis Rheum. 46,* 1965–1968.

Wang, G.Q., van Dam, A.P., Schwartz, I., and Dankert, J. (1999). Molecular typing of *Borrelia burgdorferi* sensu lato: Taxonomic, epidemiological, and clinical implications. *Clin. Microbiol. Rev. 12,* 633.

Willkens, R.F., Williams, H.J., Ward, J.R., Egger, M.J., Reading, J.C., Clements, P.J., Cathcart, E.S., Samuelson, C.O., Solsky, M.A., Kaplan, S.B., Guttadauria, M., Halla, J.T., and Weinstein, A. (1984). Randomized, double-blind, placebo controlled trial of low-dose pulse methotrexate in psoriatic-arthritis. *Arthritis Rheum. 27,* 376–381.

Yin, Z., Braun, J., Grolms, M., Spiller, I., Radbruch, A., and Sieper, J. (1997a). IFN gamma, IL-4 and IL-10 positive cells in the CD4+ and CD8+ T cell population of peripheral blood in untreated patients with early rheumatoid arthritis and early reactive arthritis. *Arthritis Rheum. 40,* 41.

Yin, Z., Neure, L., Grolms, M., Eggens, U., Radbruch, A., Braun, J., and Sieper, J. (1997b). TH1/TH2 cytokine pattern in the joint rheumatoid arthritis and reactive arthritis patients: Analysis at the single cell level. *Arthritis Rheum. 40,* 40.

Zou, J., Zhang, Y., Thiel, A., Rudwaleit, M., Shi, S.L., Radbruch, A., Poole, R., Braun, J., and Sieper, J. (2003). Predominant cellular immune response to the cartilage autoantigenic G1 aggrecan in ankylosing spondylitis and rheumatoid arthritis. *Rheumatology 42,* 846–855.

CHAPTER

34

Polymyositis and Dermatomyositis

REINHARD HOHLFELD*

**Institute for Clinical Neuroimmunology, Klinikum Grosshadern, University of Munich, Marchioninistr. 15, Munich, Germany*

ANDREW G. ENGEL†

†Department of Neurology, Mayo Clinic and Mayo Foundation, Rochester, MN, USA

Polymyositis (PM) and dermatomyositis (DM) are often categorized as "dysimmune myopathies" or "idiopathic inflammatory myopathies." The third member of this heterogeneous group of diseases is inclusion-body myositis (IBM). It is presently thought that PM is a T-cell mediated, presumably autoimmune disorder, whereas DM is an antibody-mediated vascular disorder. In contrast, it appears that the inflammatory changes observed in IBM are secondary to an unknown primary (degenerative, metabolic, infectious, or other) process. Therefore, while IBM was included in the Third Edition of this book, we elected not to include it in this chapter. The authors have drawn on material published in review form by Engel and Hohlfeld (2004) in assembling this chapter.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

In both PM and DM, proximal muscles are usually symmetrically affected. Respiratory, pharyngeal and neck muscles may be involved during later stages (Dalakas and Hohlfeld, 2003; Engel and Hohlfeld, 2004). Up to 50% of patients suffer from muscle pain or arthralgia. The history, clinical symptoms and signs, elevated serum levels of muscle enzymes, electrophysiological changes, and histologic findings together provide the basis for the diagnosis. The main diagnostic criteria and features of the different inflammatory myopathies are compared and summarized in Table 34.1. Some characteristic histologic features of DM and PM are shown in Figures 34.1 and 34.2, respectively.

Pure polymyositis is probably an overdiagnosed entity. At one extreme, represented by a recently published series of Dutch cases, only very few cases (less than 5%) were diagnosed as PM (van der Meulen et al., 2003). At the other extreme, application of the now obsolete Bohan and Peter criteria (Bohan and Peter, 1975a; 1975b) will lead to an overdiagnosis of PM, because these criteria ignore the immunohistologic characteristics of PM, DM, and IBM (Dalakas and Hohlfeld, 2003).

Characteristic skin changes may accompany the muscle weakness in DM. These include heliotropic erythema of the eyelids, cheeks, and trunk; chronic skin lesions with depigmentation and hyperpigmentation; painfully dilated capillaries at the base of the fingernails; erosions of the knuckles (Gottron's sign); dry and cracked skin of palms and fingers ("mechanic's hands"); subcutaneous calcifications in later stages (mainly juvenile DM); and cutaneous or intestinal ulcerations as a sign of generalized vascular injury (mainly juvenile DM).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 34.1 Clinical and diagnostic criteria of polymyositis, dermatomyositis and inclusion-body myositis

	Polymyositis	Dermatomyositis	Inclusion Body Myositis
Age at manifestation	>18 years	Any age, two peaks: 5–15 and 45–65 years	>50 years
Female : male ratio	2 : 1	2 : 1	1 : 3
Muscle involvement	Proximal symmetrical	Proximal symmetrical	Distal to proximal, asymmetrical
Atrophy	+	(+)	++
Muscle pain	(+)	+	(+)
Serum creatine kinase	Elevated up to 50-fold	Normal to 50-fold elevated	Normal to 10-fold elevated
Electromyography	Myopathic	Myopathic	Myopathic and mixed large units
Muscle biopsy	Peri- and endomysial infiltrate, invasion of MHC I^+ fibers	Perifascicular atrophy +/– infiltrate (perivascular and perifascicular)	Prominent endomysial infiltrate, atrophic fibers, "rimmed vacuoles", congophilic inclusions
Immunohistochemistry	Autoinvasive $CD8^+$ T cells, macrophages	B cells, macrophages $CD4^+$ T cells	Autoinvasive $CD8^+$ T cells, β-amyloid, prion-protein, and others in vacuolated fibers; enhanced αB-crystallin expression in numerous nonvacuolated fibers
Electronmicroscopy		Tubulovesicular inclusions in capillary endothelium; necrotic endothelial cells; platelet-fibrin thrombi in capillaries	Helical filaments, fibrils

+, present; –, absent; (+), weak or inconsistent.

Laboratory Markers of Muscle Fiber Injury

Of the enzymes released as a consequence of muscle fiber injury, the serum concentration of creatine kinase (CK) provides the best estimate of the extent of muscle damage and clinical activity. Both BB and MM isozymes may be elevated in myositis. Creatine kinase levels can be elevated up to 50 times normal in active disease phases of PM and adult DM. In contrast, the erythrocyte sedimentation rate is not a reliable parameter for disease activity and is normal in half of the patients.

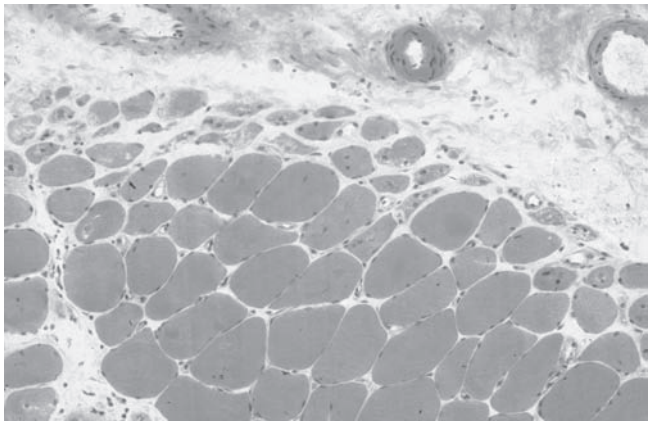
Systemic Involvement

In both PM and DM, cardiac involvement with electrocardiography (ECG) changes, pericarditis, cardiomyopathy, or heart failure can occur during all stages of the disease. Pulmonary complications can be secondary to aspiration or a restrictive ventilatory defect (if the pharyngeal or respiratory muscles are affected). Further, approximately 10% of patients with PM or DM develop interstitial lung disease. Approximately 50% of these patients produce autoantibodies directed against histidyl transfer RNA (tRNA) synthetase, so-called Jo-1 antibodies. Interstitial lung disease predicts a severe course and poor prognosis (see section on "Immunologic markers").

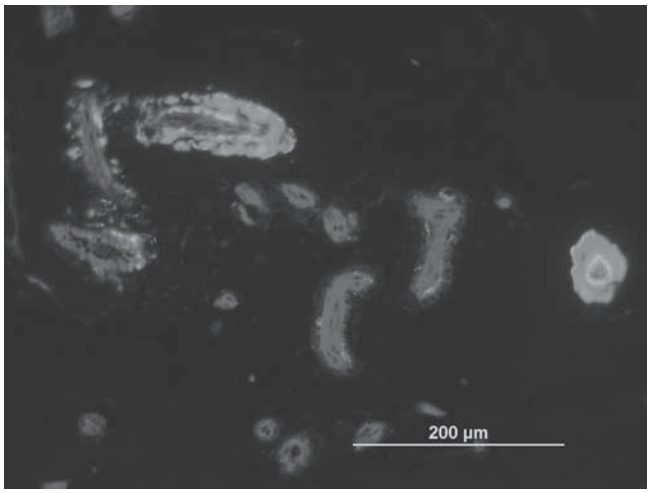
Association with Malignancy

The exact nature of the relationship between malignancy and myositis has created continued controversy. Most of the evidence points toward an association between DM and malignancy. The relation with PM seems to be weaker (Yazici and Kagen, 2000). The weight of the available information suggests that a temporal association exists between DM/PM and malignancy over a period of about 2 years, and that the risk of malignancy in that period is increased several-fold. Although the types of malignancy seen in DM and polymyositis are similar to those in the general population, ovarian cancer seems to be more common in DM. Most experts recommend that otherwise asymptomatic patients with DM/PM have an age-specific examination for occult malignancy. All suggestive symptoms or clinical clues should be evaluated thoroughly (Yazici and Kagen, 2000).

In the absence of malignancy, 5-year survival rates of 70–90% have been reported (reviewed by Engel and Hohlfeld, 2004). Indicators for a poor prognosis include increased age, extramuscular organ involvement (heart, lung, pharyngeal muscles), acute onset of the disease, malignancy, and late or insufficient treatment. Functional recovery is best if treatment is started within the first 6 months of the disease.



A



B

FIGURE 34.1 *A,* Perifascicular atrophy in dermatomyositis muscle. Atrophic muscle fibers are located at the periphery of a muscle fascicle. This is a typical, even diagnostic feature of dermatomyositis. It reflects chronic ischemia of peripheral muscle fibers, due to an underlying vasculopathy (H&E stain). *B,* C5b9 membrane attack complex (MAC) deposits (green) on capillaries (red) in dermatomyositis. The capillaries are visualized with the lectin *Ulex Europaeus* agglutinin 1 and MAC with a monoclonal antibody. The MAC deposits appear on and surround the capillaries. The capillary density is markedly decreased and the remaining capillaries are dilated. See color plate section.

Principles of Treatment

The aims of therapy are to improve the ability to carry out activities of daily living. There have been relatively few controlled clinical trials. Overall, DM responds better to immunosuppressive treatment than PM. Corticosteroids remain the first-line immunomodulatory treatment both in DM and PM. Often it becomes necessary to add an immunosuppressive drug like azathioprine or methotrexate. Intravenous immunoglobulin was shown to be effective in DM and may be effective in a proportion of patients with PM.

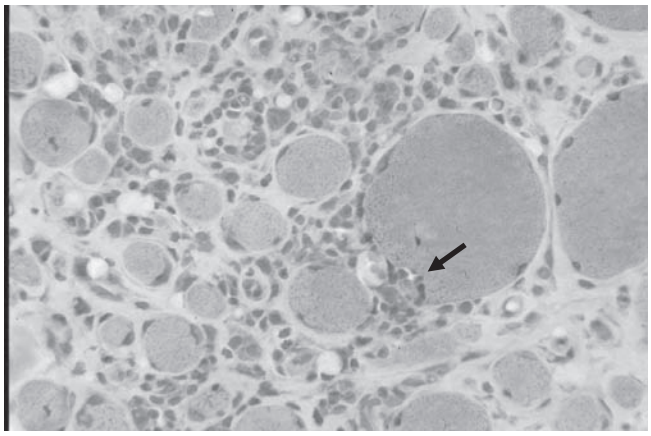


FIGURE 34.2 Dense endomysial inflammatory infiltrate in polymyositis muscle. Invasion of non-necrotic muscle fibers by $CD8^+$ T cells and macrophages is one of the hallmarks of polymyositis. The large fiber just right from the middle is invaded by a cluster of inflammatory cells (*arrow*; Gomori trichrome stain). See color plate section.

In severe, otherwise treatment-resistant cases, additional immunosuppressive agents, including cyclosporine, chlorambucil, and cyclophosphamide need to be considered. A more detailed discussion of the treatment of PM and DM may be found in Dalakas and Hohlfeld (2003) and Engel and Hohlfeld (2004).

AUTOIMMUNE FEATURES

The pathophysiological concepts of PM and DM rest mainly on morphologic, especially immunohistochemical studies. The latter are described in more detail in the next section. In essence, in PM there is a conspicuous endomysial inflammatory exudate containing mainly $CD8^+$ T cells and macrophages that surround and focally invade non-necrotic muscle fibers (Figure 34.3). Immunoelectron microscopy demonstrated that $CD8^+$ T cells and macrophages traverse the basal lamina; focally compress the fiber; and ultimately replace entire segments of muscle fiber (Arahata and Engel, 1986). All of the invaded fibers and some noninvaded fibers, express increased amounts of HLA-class I, but not class II molecules (reviewed in Hohlfeld and Engel, 1994; Engel and Hohlfeld, 2004). By contrast, normal muscle fibers do not express detectable amounts of HLA class I or class II antigens. Taken together, these observations are consistent with an HLA class I-restricted CTL-mediated response against antigen(s) expressed on muscle fibers in PM. Consistent with this hypothesis, $CD8^+$ T cells expanded from muscle of patients with different inflammatory myopathies may show low but significant cytotoxicity against autologous cultured myotubes (Hohlfeld and Engel, 1994).

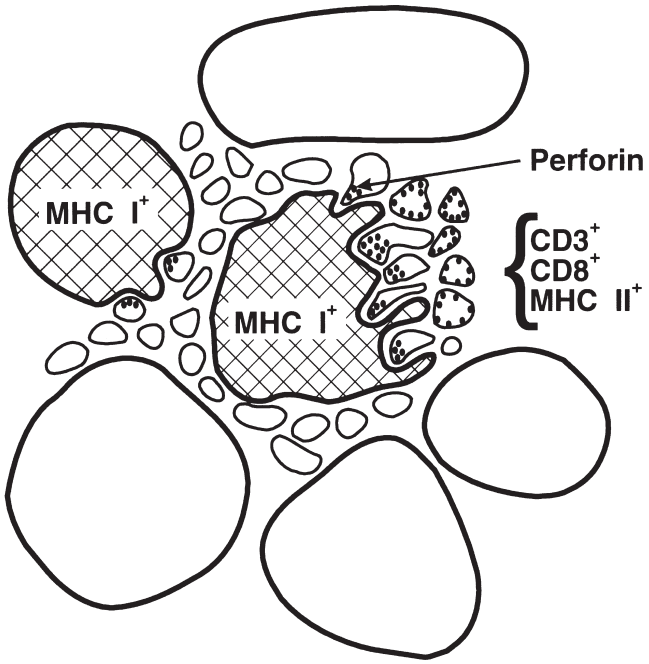


FIGURE 34.3 Schematic representation of a focal endomysial inflammatory infiltrate in polymyositis muscle. T cells focally surround and invade a non-necrotic muscle fiber. The majority of the autoinvasive T cells are $CD3^+CD8^+$. Many of the autoinvasive T cells are activated, as indicated by their expression of MHC class II (HLA-DR) antigen (MHC II^+). All invaded muscle fibers, and some that are noninvaded, show surface reactivity for MHC class I. The autoinvasive T cells express the cytotoxic effector molecule perforin, which is oriented towards the zone of contact with the invaded muscle fiber.

In DM, perifascicular atrophy is a highly characteristic microscopic feature, which is due to degeneration of muscle fibers at the periphery of muscle fascicles secondary to microvascular damage (see Figure 34.1). Quantitative morphologic analyses suggest that the depletion of capillaries is one of the earliest changes in DM. Immunofluorescence studies revealed the deposition of complement in or around microvascular endothelium in a significant proportion of capillaries (reviewed by Mendell et al., 1996). These observations support the concept that an antibody- or immune-complex-mediated response against a vascular-endothelial component is a primary pathogenetic mechanism in DM.

In juvenile inflammatory myopathies, especially juvenile DM, maternal chimerism has been implicated in the pathogenesis (Artlett et al., 2000; Reed et al., 2000). In one study, the families of 15 boys with DM were investigated for chimerism by polymerase chain reaction (PCR). Chimerism was noted in 13 of the 15 affected children with DM, compared with 5 of 35 siblings (Reed et al., 2000). Maternal cells among peripheral blood mononuclear cells were detected in 11 of the 15 boys, compared with 5 of 17 unaffected controls, and in muscle tissue of 12 of 15 compared with 2 of 10 unaffected siblings (Reed et al., 2000). Very similar results were obtained in another study (Artlett et al., 2000). Microchimerism could induce graft-versus-host reactions, which could manifest as autoimmune disease. For example, chimeric cells of fetal origin were identified in skin lesions and peripheral blood of women with systemic sclerosis (Artlett et al., 1998).

Immunohistologic Features of Polymyositis and Dermatomyositis

A number of studies have investigated the expression of inflammatory molecules such as cytokines, matrix metalloproteases, adhesion molecules, and costimulatory molecules in the different inflammatory myopathies (reviewed in Hohlfeld et al., 1997). Despite some discrepancies between the different studies, which are probably explained by methodologic aspects, it is safe to conclude that inflammatory cells, muscle fibers and endothelial cells express a complex array of different inflammatory mediators, adhesion and costimulatory molecules (Figarella-Branger et al., 2003). The local production of soluble mediators likely induces the expression of cell interaction and adhesion molecules in various cellular components of muscle tissue.

A crucial prerequisite for immunologic interaction between muscle fibers and inflammatory T cells is the expression of major histocompatibility complex (MHC, also called HLA) molecules. Muscle fibers normally do not express detectable amounts of MHC class I or II molecules. However, classical studies by the groups of George Karpati (Karpati et al., 1988; Karpati and Carpenter, 1993) and Andrew Engel demonstrated that MHC class I is strongly upregulated in pathologic conditions, especially inflammatory myopathies. In principle, muscle fibers also seem to be capable of expressing MHC class II (HLA-DR) antigen, although to a lesser extent (Bartoccioni et al., 1994; Inukai et al., 2000). Recently, the expression of a "nonclassical" HLA class I molecule (HLA-G) has been demonstrated in muscle of patients with PM, DM, and IBM (Wiendl et al., 2000).

In PM, the endomysial inflammatory infiltrate is typically dominated by $CD8^+$ T lymphocytes, which surround, invade and eventually destroy muscle fibers (see Figure 34.3). In a rare subtype of PM, the infiltrate consists of $\gamma\delta$ T lymphocytes (Hohlfeld et al., 1991). In contrast to non-inflamed muscle, the invaded muscle fibers express HLA class I molecules. This is a prerequisite for the immunologic interaction with $CD8^+$ T cells. The different stages of CTL-mediated myocytotoxicity were analyzed by immunoelectron microscopy (Arahata and Engel, 1986). Initially, $CD8^+$ cells and macrophages abut on and send spike-like processes into non-necrotic muscle fibers. Subsequently, an increasing number of $CD8^+$ cells and macrophages traverse the basal lamina and focally replace the fiber.

Approximately one-third of all autoinvasive cells and about one-half of the $CD8^+$ autoinvasive T cells is HLA-DR^+, suggesting that they have been activated (Engel and Arahata, 1984). The vast majority of the inflammatory $CD4^+$ and $CD8^+$ T cells display the phenotype of memory T cells, that is, they express the RO isoform of the leukocyte common antigen CD45 (De Bleecker and Engel, 1995). The intensity of the CD45RO signal was similar in all $CD8^+$ T cells regardless of their position relative to the invaded muscle fiber surface. A similar expression pattern was noted for the leukocyte function-associated antigen 1 (LFA-1) (De Bleecker and Engel, 1994; Iannone et al., 1996). The LFA-1 (CD11a/CD18) is a β2 integrin that has a key role in mediating leukocyte adhesion to endothelium and T-cell adhesion to target cells. Intercellular adhesion molecule 1 (ICAM-1), a ligand of LFA-1, was upregulated especially on T cells in the vicinity of invaded muscle fibers, suggesting that the expression of CD45RO and ICAM-1 is differentially regulated (De Bleecker and Engel, 1994). Indeed, LFA-1 is mainly constitutively expressed, whereas ICAM-1 is widely inducible on B and T cells. Taken together, these results establish that the autoaggressive (autoinvasive) T cells in the inflammatory lesions of PM and IBM muscle represent activated $CD8^+$ memory T cells.

In contrast to PM, perivascular and perifascicular infiltrates consisting predominantly of B lymphocytes, macrophages, and $CD4^+$ T lymphocytes prevail in DM. Immunohistochemistry shows immune complexes and C5b9-complement (membrane attack complex) on small blood vessels, suggesting a humoral immune effector mechanism (Mendell et al., 1996; Emslie-Smith and Engel, 1990). The immune processes affecting the muscle microvasculature lead to a reactive proliferation of endothelial cells and a reduction of muscle capillaries. Focal capillary depletion, with marked reduction of the capillary density, can be observed in DM specimens with minimal structural alterations, suggesting that the capillaries are an early and specific target of the disease process (Emslie-Smith and Engel, 1990). Electron microscopy demonstrates tubulovesicular inclusions in endothelial cells. Capillary changes are thought to be the cause of the characteristic perifascicular muscle fiber atrophy in DM. Perifascicular atrophy is diagnostic for DM, even in the absence of an inflammatory infiltrate. As in PM, the molecular target of the presumed humoral autoimmune reaction in DM has not yet been defined.

T-Cell Repertoire Studies

The characteristic lesion of PM has several features that make it an ideal paradigm to study $CD8^+$ T-cell-mediated immunopathology (see Figure 34.3). First of all, the muscle fiber target cells can be readily distinguished from the effector T cells. Secondly, different populations of inflammatory T cells can be discerned: one population, which deeply invades muscle fibers (the autoaggressive or autoinvasive T cells), and another, which remains in interstitial areas and, therefore, seems to represent regulatory or bystander cells (the interstitial T cells).

To address the question whether the two morphologically defined T-cell populations represent distinct clones, Bender et al. (1995) combined two independent PCR techniques with immunohistochemistry to characterize the T-cell receptor repertoire. The results show that the T-cell repertoire of the autoinvasive T cells is distinct from the repertoire of the interstitial T cells (see Figure 34.3). These findings are consistent with the results of previous studies, which however did not combine sequence with histologic analysis of T-cell receptor (TCR) Vβ expression (Mantegazza et al., 1993; Lindberg et al., 1994; O'Hanlon et al., 1994). Differences in TCR usage in the different studies presumably reflect differences between individual patients, for example, different HLA types.

More recently, clonal expansions of $CD8^+$ T cells were identified in muscle and blood of polymyositis patients by PCR techniques including TCR complementarity determining region 3 (CDR3) length analysis (spectratyping). To examine a possible pathogenic role of these clonally expanded T cells, CDR3 spectratyping was combined with laser-microdissection and single-cell PCR of individual myocytotoxic T cells that contact, invade, and destroy a skeletal muscle fiber (Hofbauer et al., 2003). First, cDNA from muscle biopsy specimens was screened by CDR3-spectratyping for expanded TCR BV sequences. To pinpoint the corresponding T cells in tissue, cryostat sections were stained with appropriate anti-TCR BV monoclonal antibodies (mAbs), isolated single BV^+ T cells that directly contacted or invaded a muscle fiber by laser-assisted microdissection and amplified their TCR BV chain sequences from rearranged genomic DNA. In this way the oligoclonal peaks identified by CDR3 spectratype screening could be related to morphologically characterized microdissected T cells. In one patient, a large fraction of the microdissected T cells carried a common TCR-BV amino-acid CDR3 motif and conservative nucleotide exchanges in the CDR3 region, suggesting an antigen-driven response. In several cases, these T-cell clones could be tracked for several years in $CD8^+$ (but not $CD4^+$) blood lymphocytes, and, in two patients, also in consecutive muscle biopsy specimens. During immunosuppressive therapy, oligoclonal CDR3 spectratype patterns tended to revert to more polyclonal, Gaussian distribution-like patterns (Hofbauer et al., 2003). The findings demonstrate that CDR3 spectratyping and single-cell analysis can be combined to identify and track autoaggressive T-cell clones in blood and target tissue.

A possible clue to the nature of the suspected autoantigen(s) was provided by the discovery of a rare variant of PM. In this variant, $CD3^+CD4^-CD8^-TCR\gamma\delta^+$ T cells surrounded and invaded non-necrotic muscle fibers in the same

way as $CD3^+CD8^+TCR\alpha\beta^+$ T cells in the more common forms of PM (Hohlfeld et al., 1991). The autoaggressive myocytotoxic γδ T cells were essentially monoclonal, and expressed an unusual Vγ3Jγ1Cγ1-Vδ2Jδ3Cδ disulfide-linked TCR (Pluschke et al., 1992). In γδ T-cell-mediated PM, all muscle fibers expressed MHC class I antigen and showed intense reactivity with a monoclonal antibody specific for the 65 kDa heat shock protein (hsp) (Hohlfeld et al., 1991). One possible implication of the striking co-localization of γδ T cells with the 65 kDa hsp is that the autoinvasive γδ T cells recognize hsp determinants on muscle fibers. Presently, however, the target autoantigens of this autoreactive human γδ T-cell receptor remain unknown, although it has been formally demonstrated that the TCR recognizes a muscle-associated autoantigen in a CDR3-dependent, MHC non-restricted way (Wiendl et al. 2002).

GENETIC FEATURES

There are numerous reports on the association of certain HLA haplotypes with subgroups of myositis (reviewed in Engel and Hohlfeld, 2004). In white people, there is an association with HLA-B8 with childhood DM and adult PM. This is attributed to the association of HLA-B8 with HLA-DR3 (which is linkage disequilibrium with HLA-B8). Childhood DM is also associated with HLA-DQA1*0501 on non-DR3 haplotypes. Different HLA associations have been documented in different ethnic groups. Interestingly, when patients are analyzed according to patterns of autoantibody production, additional associations emerge (Engel and Hohlfeld, 2004). Despite these observations, however, the evidence is still too fragmentary to identify primary susceptibility HLA alleles for specific types of inflammatory myopathy, and likely the HLA associations only partially account for the genetic risk.

IN VIVO AND *IN VITRO* MODELS

Animal Models of Myositis

Inflammatory and necrotizing lesions of muscle were experimentally induced in various animal species by the injection of Freund's complete adjuvant and homogenates of muscle or muscle protein preparations (Dawkins 1965; 1975; Uemura 1969; Webb, 1970a; 1970b; Currie, 1971; Morgan et al., 1971; Esiri and MacLennan, 1974; 1975). The majority of these models show only limited resemblance to human polymyositis. The pathologic changes in muscle usually consist of necrotic and regenerating fibers and mononuclear infiltrates in the perimysium and endomysium, particularly at perivascular sites. In some studies, the changes of experimental myositis were transferred with lymphocytes or serum. These early experiments were conducted when little or nothing was known about the mechanisms of antigen recognition and cytotoxicity. Their relevance to the pathogenesis of any human inflammatory muscle disease is uncertain.

Several reports described the induction of myositis in the SJL mouse (Rosenberg et al., 1987; Rosenberg and Kotzin, 1989; Matsubara et al., 1993; Matsubara and Okumura, 1996). However, SJL/J mice spontaneously develop a necrotizing myopathy (Hohlfeld et al., 1988). This myopathy is now known to be caused by a deletion in the dysferlin gene, defining a natural model for limb girdle muscular dystrophy (Bittner et al., 1999; Vafiadaki et al., 2001). Inflammatory changes are not uncommon in some human hereditary myopathies and muscular dystrophies, including facioscapulohumoral muscular dystrophy and dysferlinopathies. One important lesson from these observations is that, in any myopathy, the presence of inflammatory changes does not necessarily imply an (auto)immune pathogenesis but may be secondary to a metabolic or genetic defect.

A novel transgenic mouse model of myositis was recently described (Nagaraju et al., 2000b). The authors used a controllable muscle-specific promoter system to upregulate MHC class I expression in skeletal muscles of mice. The transgenic mice developed an inflammatory myopathy accompanied by autoantibodies against histidyl-tRNA synthetase, the most commonly observed antibody specificity in human myositis (Nagaraju et al., 2000b). This model indicates that the sustained upregulation of MHC class I antigen in a tissue that normally lacks MHC class I may be sufficient to induce T-cell and antibody responses to antigens expressed in the target tissue.

In another transgenic model, $CD8^+$ T-cell-dependent myositis develops in non-obese diabetic (NOD) mice made Th1 cytokine-deficient by expression of an interferon-γ receptor β chain transgene. This suggests that a disturbance of the normal cytokine balance might be sufficient to trigger myositis in susceptible animals (Serreze et al., 2003).

An infectious model of myositis has been described in CBA/J mice infected with *Trypanosoma cruzi* (Andersson et al., 2003). In this model, inflammatory infiltrates were predominantly found in the endomysium and, to a lesser extent, in perivascular areas. Furthermore, $CD8^+$ T cells invaded non-necrotic muscle fibers. In this regard, this infectious mouse model closely resembles human PM.

In Vitro Models using Cultured Myoblasts

Numerous studies have shown that cultured myoblasts (myogenic stem cells) can express a variety of immunologically important molecules (Table 34.2). Furthermore, myoblasts express a surprising number of cytokines and chemokines (reviewed in Hohlfeld et al., 1997; Figarella-Branger et al., 2003). For these and other reasons, it seems

TABLE 34.2 Immunologic properties of human myoblasts

Surface antigen	Constitutive expression	Interferon-γ-stimulated expression
Differentiation antigen		
NCAM-1 (CD56)	+	+
HLA-molecules		
Classical HLA class I	(+)	+
HLA-DR	–	+
HLA-DP	–	+
HLA-DQ	–	(+)
HLA-G	–	+
Adhesion molecules		
ICAM-1 (CD54)	–	+
LFA-3 (CD58)	(+)	(+)
Costimulatory molecules		
B7.1 (CD80)	–	–
B7.2 (CD86)	–	–
ICOS-L	(+)	(+)
B7-H1	–	+
CD40	+	+

+, present; –, absent; (+), weak or inconsistent.

Based on data from Goebels et al., 1992; Michaelis et al., 1993; Behrens et al., 1998b; Hohlfeld and Engel, 1990; Wiendl et al., 2000; 2003a; 2003b; 2003c.

likely that in myositis muscle cells play an active immunologic role in the affected tissue (Hohlfeld and Engel, 1994). Myoblasts are, therefore, extremely useful for functional studies of various aspects of the pathogenesis of myositis. For example, cultured human myoblasts can serve as targets for cytotoxic $CD8^+$ T cells and other cytotoxic effector cells. Perhaps even more interesting, myoblasts can present antigens to $CD4^+$ T cells (see below).

Myoblasts can be isolated and purified from muscle biopsy specimens and expanded in culture (Goebels et al., 1992). In contrast to fibroblasts, myoblasts express the cytoskeletal protein desmin and the neural cell adhesion molecule NCAM (CD56/Leu 19/NKH-1) (Hohlfeld and Engel, 1994). Myoblasts constitutively express HLA class I antigens and a low level of lymphocyte function-associated molecule 3 (LFA-3, CD58). Tumor necrosis factor α (TNF-α), a cytokine secreted by macrophages, T cells, and natural killer (NK) cells induces myoblasts to express the ICAM-1 (CD54) (Goebels et al., 1992). Interferon-γ, a cytokine secreted by T cells and NK cells, induces myoblasts to express HLA-DR and ICAM-1 (Hohlfeld and Engel, 1990; Goebels et al., 1992). HLA-DP and HLA-DQ are also inducible by interferon-γ but the kinetics of induction and the levels of expression vary with the different HLA class II molecules (Goebels et al., 1992).

Cultured myotubes and myoblasts express HLA class I molecules. This qualifies them as potential targets of $CD8^+$ CTL. Lysis of myotubes by CTL was shown in different experimental situations. On the one hand myotubes were lysed by allogeneic $CD8^+$ CTL lines raised against the allogeneic HLA antigens expressed by the myotubes (Hohlfeld and Engel, 1994). Autologous control myotubes were not lysed. Lysis involved the recognition of allogeneic class I HLA antigens, since it was completely blocked by a monoclonal antibody against a monomorphic determinant of HLA class I (Hohlfeld and Engel, 1994). Furthermore, myotubes were lysed by autologous polyclonal $CD8^+$ T-cell lines directly expanded from muscle of patients with different inflammatory myopathies (Hohlfeld and Engel, 1994). The results obtained in this model system clearly establish that cultured myotubes are fully susceptible to HLA-class I restricted lysis by $CD8^+$ CTL. The autoreactive myocytotoxicity is consistent with the hypothesis that some of the CTL isolated from muscle recognize the same antigen on myotubes *in vitro* that they recognize on muscle fibers *in vivo*.

Antigen presentation to $CD4^+$ T cells depends on the constitutive or induced expression of HLA class II on the presenting cell. Myoblasts can be induced to express HLA class II by interferon-γ (Hohlfeld and Engel, 1990). Highly purified human myoblasts were tested for their ability to present various protein antigens to autologous $CD4^+$ T-cell lines specific for tuberculin, tetanus toxoid or myelin basic protein (Goebels et al., 1992). Non-induced myoblasts or myoblasts treated with TNF-α alone could not present any of these antigens to T cells. However, interferon-γ-treated myoblasts induced antigen-specific T-cell proliferation and were killed by the T cells only in the presence of the relevant antigen (Goebels et al., 1992). Antigen-specific lysis was reduced to a background level by adding the anti-HLA-DR monoclonal antibody L-243. These results suggest that HLA class II-positive human myoblasts can act as facultative local antigen-presenting cells in muscle by providing the signals necessary to trigger both antigen-specific lysis and T-cell proliferation. In addition to presentation of exogenous antigens processed in the classical MHC class II-restricted pathway, human myoblasts seem to be capable of presenting endogenous antigen to MHC class II restricted $CD4^+$ T cells (Curnow et al., 2001).

It is clear from these studies that myoblasts have more than sufficient immunologic "potential" to qualify them for local (re)stimulation of memory and effector T cells. It is not clear, however, to what extent myoblasts can stimulate naïve (unprimed T cells). Myoblasts do not express the classical costimulatory molecules CD80 (B7.1) and CD86 (B7.2) (Behrens et al., 1998), but they do express CD40, another important costimulatory molecule (Behrens et al., 1998; Sugiura et al., 2000). Furthermore, a subpopulation of myoblasts express BB-1, a CD80 (B7)-related molecule that has not been molecularly defined (Behrens et al., 1998). In addition, myoblasts can express the CD80/CD86 (B7.1/2) related inducible costimulatory molecule ICOS-L (Wiendl et al. 2003c) and the B7-related molecule B7-H1 (Wiendl

et al., 2003b). The presence of these costimulatory molecules further emphasizes the important immunologic role of myoblasts.

In addition to the classical MHC class I molecules (HLA-A, -B, and -C), myoblasts can be induced to express the nonclassical HLA-G (Wiendl et al., 2000). HLA-G expression was first observed in cytotrophoblasts of the human placenta. The full functional potential of HLA-G is not yet known. Like the classical HLA class I molecules, HLA-G binds antigenic peptides and CD8. It should, therefore, be capable of presenting antigenic peptides to T cells in a way similar to the classical HLA class I molecules. This raises the possibility that in polymyositis, some of the autoaggressive T cells recognize their antigen in the molecular context of HLA-G. In addition, HLA-G interacts with different receptors expressed on various types of lymphocytes, monocytes, and dendritic cells. It is thought that the recognition of HLA-G induces immunoregulatory functions in these cells, raising the possibility that HLA-G protects muscle fibers from attack by certain types of immune cells, including T cells and NK cells (Wiendl et al., 2003a). Whether HLA-G has a protective (anti-inflammatory) or proinflammatory role in myositis is unknown.

PATHOGENIC EFFECTOR MECHANISMS

The precise mechanisms by which muscle fibers are injured in the different inflammatory myopathies remain to be defined. In PM (and IBM), there is strong evidence that muscle fibers are directly attacked by cytotoxic T cells. An additional possibility is that locally produced soluble inflammatory mediators and cytokines exert toxic effects on muscle fibers.

Cytotoxic T cells can kill by a variety of different mechanisms (Barry and Bleackley, 2002). A main pathway of cytotoxicity is mediated by the secretion of the pore-forming protein perforin by the cytotoxic T cell. An alternative, nonsecretory pathway relies on the interaction of the Fas ligand that is upregulated during T-cell activation with the apoptosis-inducing Fas receptor on the target cell. In PM, there is evidence that a perforin- and secretion-dependent mechanism contributes to the muscle fiber injury. Perforin has been localized in inflammatory T cells by immunohistochemistry (Orimo et al., 1994; Goebels et al., 1996) and by *in situ* hybridization (Cherin et al., 1996). In PM but not DM, the auto-invasive T cells orient their perforin-containing cytotoxic granules towards the target muscle fiber (Goebels et al., 1996), suggesting that secretion of this cytotoxic effector molecule contributes to muscle fiber injury.

Perforin is not the only potentially cytotoxic molecule expressed in inflammatory myopathies. For example, muscle fibers in myositis display distinct upregulation both of inducible and neuronal nitric oxide synthase (NOS) (Tews and Goebel, 1998). One may speculate that the enhanced expression of NOS with production of nitric oxide contributes to oxidative stress, mediating muscle fiber damage. Furthermore, different matrix metalloproteinases (MMPs) are expressed in PM and DM muscle (Choi and Dalakas, 2000; Kieseier et al., 2001). Matrix metalloproteinases are zinc-dependent endopeptidases capable of degrading extracellular matrix. MMP-1 (interstitial collagenase) is localized around the sarcolemma of injured muscle fibers and to fibroblast-like cells, whereas MMP-9 (gelatinase B) is localized mainly in inflammatory T lymphocytes (Kieseier et al., 2001). The matrix-degrading action of the MMPs could directly contribute to muscle fiber injury, or it could facilitate the access of cytotoxic factors and cells to muscle fibers.

If perforin is indeed involved in muscle fiber injury, why does the surface membrane of muscle fibers appear intact at the light microscopic (Engel and Arahata, 1984) and electron microscopic (Arahata and Engel, 1986) levels in the early stages of muscle fiber invasion? Pore-like structures could not be detected in the sarcolemma of muscle fibers attacked by T cells in PM (Arahata and Engel, 1986). One possible explanation is that perforin pores/channels on nucleated cells *in vivo* are smaller in size than the pores generated *in vitro* on erythrocytes and other target cells by the addition of purified perforin. Perforin pores containing less than 10–20 monomers would escape detection by electron microscopy (Liu et al., 1995). Another explanation for the lack of morphologically visible muscle cell damage is that the surface membrane of the muscle fiber is rapidly repaired at least during the early stages of muscle fiber invasion. Repair could occur, for example, by shedding or endocytosis of pore-damaged membrane (reviewed in Henkart, 1994).

It is interesting to note that the volume of a 25-mm-long and 50-μm-wide muscle fiber is nearly 28,000-fold larger than, for example, that of a spherical 15-μm tumor cell (Arahata and Engel, 1986). Perforin pores would allow the influx of calcium. Consistent with this assumption is the observation that invaded muscle fibers show signs of focal myofibrillar degeneration near invading cells (Arahata and Engel, 1986). These changes could be a consequence of membrane insertion of perforin and focal protease activation (Arahata and Engel, 1986). Another indirect sign of muscle fiber damage is the intense focal regenerative activity noted in areas immediately adjacent to autoinvasive T cells (Arahata and Engel, 1986).

In addition to the perforin-mediated killing mechanism, cytotoxic T cells can kill by a non-secretory, ligand-mediated mechanism (Barry and Bleackley, 2002). This second killing mechanism requires the interaction between Fas (expressed on the target cell) and Fas-ligand (expressed on the T cell). Fas-mediated cytotoxicity is thought to induce programmed cell death—apoptosis.

Ligation of Fas (CD95) recruits adaptor molecules (such as procaspase-8 and -10) to the receptor. By being brought into proximity with one another, these procaspases cleave their nearest neighbors and active, mature caspases form (Green, 2000). The active caspases efficiently cleave procaspase-3 and other executioner caspases, and apoptosis proceeds.

Different groups of investigators found no evidence that apoptosis is a mechanism of muscle fiber injury in human inflammatory myopathies (Schneider et al., 1996; Behrens et al., 1997; Fyhr and Oldfors, 1998). On the other hand, in PM, DM, and IBM many muscle fibers express Fas (Behrens et al., 1997; Fyhr and Oldfors, 1998). What could explain the discrepancy between the expression of the Fas "death receptor" on muscle fibers and the absence of signs of apoptosis? One possibility is that muscle fibers are intrinsically resistant to Fas-mediated classical apoptosis, at least *in vivo*. Resistance could be related to the peculiar properties of syncytial muscle fibers discussed above, or the expression of specific inhibitory factors such as Bcl-2, or both. Indeed, the majority of Fas^+ fibers coexpress Bcl-2 (Behrens et al., 1997; Vattemi et al., 2000). Further, muscle fibers constitutively express the human IAP (inhibitor of apoptosis)-like protein hILP (human IAP-like protein) (also called XIAP) (Li and Dalakas, 2000), which was found to be expressed in the sarcolemmal region, co-localizing with dystrophin (Li and Dalakas, 2000). The staining pattern for hILP was essentially identical in normal, PM, and IBM muscle. Furthermore, muscle fibers in PM muscle express the anti-apoptotic molecule FLIP (FLICE-inhibitory protein) (Nagaraju et al., 2000a). These authors provided evidence that FLIP indeed protects muscle cells from apoptosis in functional experiments with cultured human myoblasts (Nagaraju et al., 2000a).

In conclusion, the observation that the autoinvasive T cells express and orient perforin towards target muscle fibers is consistent with a secretion- and perforin-dependent cytotoxic mechanism of muscle fiber injury in PM. On the other hand, although many muscle fibers and inflammatory cells express Fas, the nuclear changes typical for apoptosis are essentially absent in the inflammatory myopathies (Schneider et al., 1996; Behrens et al., 1997). Resistance to Fas-mediated injury is probably related to the expression of protective (anti-apoptotic) factors in muscle fibers and inflammatory cells.

AUTOANTIBODIES AS POTENTIAL IMMUNOLOGIC MARKERS

Autoantibodies against nuclear or cytoplasmic antigens, directed against ribonucleoproteins involved in protein synthesis ("anti-synthetase") or translational transport (anti-signal-recognition particle), are detectable in about 20% of patients (Miller, 1993; Rider and Miller, 2000). Although the pathogenetic relevance of these autoantibodies has yet to be defined, they are associated with certain disease characteristics and HLA haplotypes (Miller, 1993; Engel and Hohlfeld, 2004). Antibodies against histidyl-tRNA synthetase, anti-Jo-1, accounts for 80% of all the anti-aminoacyl tRNA-synthetases, and seem to confer specificity for identifying a disease subset that combines myositis, non-erosive arthritis, and Raynaud phenomenon. A more detailed discussion of these antibodies may be found in Dalakas and Hohlfeld (2003) and Engel and Hohlfeld (2004).

Interestingly, histidyl-tRNA synthetase and asparaginyl-tRNA synthetase can activate chemokine receptors on T cells and dendritic cells (Howard et al., 2002). Thus, autoantigenic aminoacyl-tRNA synthetases, liberated from damaged muscle cells, might perpetuate the development of myositis by recruiting mononuclear cells. One could, therefore, speculate that the anti-aminoacyl-tRNA synthetase antibodies might not necessarily be harmful, but might inhibit the chemotactic properties of the aminoacyl-tRNA synthetases. Presently, however, there is no evidence that would support this possibility.

CONCLUDING REMARKS—FUTURE PROSPECTS

Much has been learned in the past two decades about the pathogenesis of the inflammatory myopathies. Different effector mechanisms have been attributed to PM (mainly T-cell-mediated) and DM (mainly antibody-mediated), and a unique $\gamma\delta$ T-cell-mediated subtype of PM has been described. Challenges for future research include the identification of the elusive (auto?)antigens and the triggering mechanisms of the immune reactions against muscle fibers. Eventually, progress in these areas should lead to advances in therapy.

Acknowledgment

The Institute for Clinical Neuroimmunology is supported by the Hermann and Lilly Schilling Foundation.

References

Andersson, J., Englund, P., Sunnemark, D., Dahlstedt, A., Westerblad, H., Nennesmo, I., Orn, A., and Lundberg, I.E. (2003). CBA/J mice infected with *Trypanosoma cruzi*: an experimental model for inflammatory myopathies. *Muscle Nerve 27,* 442–448.

Arahata, K. and Engel, A.G. (1986). Monoclonal antibody analysis of mononuclear cells in myopathies. III. Immunoelectron microscopy aspects of cell-mediated muscle fiber injury. *Ann. Neurol. 19,* 112–125.

Artlett, C.M., Ramos, R., Jiminez, S.A., Patterson, K., Miller, F.W., and Rider, L.G. (2000). Chimeric cells of maternal origin in juvenile idiopathic inflammatory myopathies. Childhood Myositis Heterogeneity Collaborative Group. *Lancet 356,* 2155–2156.

Artlett, C.M., Smith, J.B., and Jimenez, S.A. (1998). Identification of fetal DNA and cells in skin lesions from women with systemic sclerosis. *N. Engl. J. Med. 338,* 1186–1191.

Barry, M. and Bleackley, R.C. (2002). Cytotoxic T lymphocytes: all roads lead to death. *Nat. Rev. Immunol. 2,* 401–409.

Bartoccioni, E., Gallucci, S., Scuderi, F., Ricci, E., Servidei, S., Broccolini, A., and Tonali, P. (1994). MHC class I, MHC class II and intercellular adhesion molecule-1 (ICAM-1) expression in inflammatory myopathies. *Clin. Exp. Immunol. 95,* 166–172.

Behrens, L., Bender, A., Johnson, M.A., and Hohlfeld, R. (1997). Cytotoxic mechanisms in inflammatory myopathies. Co-expression of Fas and protective Bcl-2 in muscle fibers and inflammatory cells. *Brain 120,* 929–938.

Behrens, L., Kerschensteiner, M., Misgeld, T., Goebels, N., Wekerle, H., and Hohlfeld, R. (1998). Human muscle cells express a functional costimulatory molecule distinct from B7.1 (CD80) and B7.2 (CD86) *in vitro* and in inflammatory lesions. *J. Immunol. 161,* 5943–5951.

Bender, A., Ernst, N., Iglesias, A., Dornmair, K., Wekerle, H., and Hohlfeld, R. (1995). T cell receptor repertoire in polymyositis: Clonal expansion of autoaggressive $CD8^+$ T cells. *J. Exp. Med.* 181, 1863–1868.

Bittner, R.E., Anderson, L.V., Burkhardt, E., Bashir, R., Vafiadaki, E., Ivanova, S., Raffelsberger, T., Maerk, I., Hoger, H., Jung, M., Karbasiyan, M., Storch, M., Lassmann, H., Moss, J.A., Davison, K., Harrison, R., Bushby, K.M., and Reis, A. (1999). Dysferlin deletion in SJL mice (SJL-Dysf) defines a natural model for limb girdle muscular dystrophy 2B. *Nat. Genet. 23,* 141–142.

Bohan, A. and Peter, J.B. (1975a). Polymyositis and dermatomyositis (first of two parts). *N. Engl. J. Med. 292,* 344–347.

Bohan, A. and Peter, J.B. (1975b). Polymyositis and dermatomyositis (second of two parts). *N. Engl. J. Med. 292,* 403–407.

Cherin, P., Herson, S., Crevon, M.C., Hauw, J.-J., Cervera, P., Galanaud, P., and Emilie, D. (1996). Mechanisms of lysis by activated cytotoxic cells expressing perforin and gransyme-B genes and the protein TIA-1 in muscle biopsies of myositis. *J. Rheumatol. 23,* 1135–1142.

Choi, Y.C. and Dalakas, M.C. (2000). Expression of matrix metalloproteinases in the muscle of patients with inflammatory myopathies. *Neurology 54,* 65–71.

Curnow, J., Corlett, L., Willcox, N., and Vincent, A. (2001). Presentation by myoblasts of an epitope from endogenous acetylcholine receptor indicates a potential role in the spreading of the immune response. *J. Neuroimmunol. 115,* 127–134.

Currie, S. (1971). Experimental myositis: the in-vivo and in-vitro activity of lymph-node cells. *J. Pathol. 105,* 169–185.

Dalakas, M.C. and Hohlfeld, R. (2003). Polymyositis and dermatomyositis. *Lancet 362,* 971–982.

Dawkins, R.L. (1965). Experimental myositis associated with hypersensitivity to muscle. *J. Pathol. Bacteriol. 90,* 619–625.

Dawkins, R.L. (1975). Experimental autoallergic myositis, polymyositis and myasthenia gravis. Autoimmune muscle disease associated with immunodeficiency and neoplasia. *Clin. Exp. Immunol. 21,* 185–201.

De Bleecker, J.L. and Engel, A.G. (1994). Expression of cell adhesion molecules in inflammatory myopathies and Duchenne dystrophy. *J. Neuropathol. Exp. Neurol. 53,* 369–376.

De Bleecker, J.L. and Engel, A.G. (1995). Immunocytochemical study of CD45 T cell isoforms in inflammatory myopathies. *Am. J. Pathol. 146,* 1178–1187.

Emslie-Smith, A.M. and Engel, A.G. (1990). Microvascular changes in early and advanced dermatomyositis: A quantitative study. *Ann. Neurol. 27,* 343–356.

Engel, A.G. and Arahata, K. (1984). Monoclonal antibody analysis of mononuclear cells in myopathies. II. Phenotypes of autoinvasive cells in polymyositis and inclusion body myositis. *Ann. Neurol. 16,* 209–216.

Engel, A.G. and Hohlfeld, R. (2004). The polymyositis and dermatomyositis syndromes. In: A.G. Engel and C. Franzini-Armstrong (eds), *Myology,* 3rd ed. (pp. 1321–1366). New York: McGraw Hill.

Esiri, M.M. and MacLennan, I.C. (1974). Experimental myositis in rats. I. Histological and creatine phosphokinase changes, and passive transfer to normal syngeneic rats. *Clin. Exp. Immunol. 17,* 139–150.

Esiri, M.M. and MacLennan, I.C. (1975). Experimental myositis in rats. II. The sensitivity of spleen cells to syngeneic muscle antigen. *Clin. Exp. Immunol. 19,* 513–520.

Figarella-Branger, D., Civatte, M., Bartoli, C., and Pellissier, J.F. (2003). Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies. *Muscle Nerve 28,* 659–682.

Fyhr, I.M. and Oldfors, A. (1998). Upregulation of Fas/Fas ligand in inclusion body myositis. *Ann. Neurol. 43,* 127–130.

Goebels, N., Michaelis, D., Engelhardt, M., Huber, S., Bender, A., Pongratz, D., Johnson, M.A., Wekerle, H., Tschopp, J., Jenne, D., and Hohlfeld, R. (1996). Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. *J. Clin. Invest. 97,* 2905–2910.

Goebels, N., Michaelis, D., Wekerle, H., and Hohlfeld, R. (1992). Human myoblasts as antigen presenting cells. *J. Immunol. 149,* 661–667.

Green, D.R. (2000). Apoptotic pathways: paper wraps stone blunts scissors. *Cell 102,* 1–4.

Henkart, P.A. (1994). Lymphocyte-mediated cytotoxicity: Two pathways and multiple effector molecules. *Immunity 1,* 343–346.

Hofbauer, M., Wiesener, S., Babbe, H., Roers, A., Wekerle, H., Dornmair, K., Hohlfeld, R., and Goebels, N. (2003). Clonal tracking of autoaggressive T cells in polymyositis by combining laser microdissection, single-cell PCR, and CDR3- spectratype analysis. *Proc. Natl. Acad. Sci. U.S.A. 100,* 4090–4095.

Hohlfeld, R. and Engel, A.G. (1990). Induction of HLA-DR expression on human myoblasts with interferon-gamma. *Am. J. Pathol. 136,* 503–508.

Hohlfeld, R. and Engel, A.G. (1994). The immunobiology of muscle. *Immunol. Today 15,* 269–274.

Hohlfeld, R., Engel, A.G., Goebels, N., and Behrens, L. (1997). Cellular immune mechanisms in inflammatory myopathies. *Curr. Opin. Rheumatol. 9,* 520–526.

Hohlfeld, R., Engel, A.G., Ii, K., and Harper, M.C. (1991). Polymyositis mediated by T lymphocytes that express the g/d receptor. *N. Engl. J. Med. 324,* 877–881.

Hohlfeld, R., Muller, W., and Toyka, K.V. (1988). Necrotizing myopathy in SJL mice. *Muscle Nerve 11,* 184–185.

Howard, O.M., Dong, H.F., Yang, D., Raben, N., Nagaraju, K., Rosen, A., Casciola-Rosen, L., Hartlein, M., Kron, M., Yang, D., Yiadom, K., Dwivedi, S., Plotz, P.H., and Oppenheim, J.J. (2002). Histidyl-tRNA synthetase and asparaginyl-tRNA synthetase, autoantigens in myositis, activate chemokine receptors on T lymphocytes and immature dendritic cells. *J. Exp. Med. 196,* 781–791.

Iannone, F., Cauli, A., Yanni, G., Kingsley, G., Isenberg, D.A., Corrigall, V.M., and Panayi, G.S. (1996). T lymphocyte immunophenotyping in polymyositis and dermatomyositis. *Br. J. Rheumatol. 35,* 839–845.

Inukai, A., Kuru, S., Liang, Y., Takano, A., Kobayashi, Y., Sakai, M., Doyu, M., and Sobue, G. (2000). Expression of HLA-DR and its enhancing molecules in muscle fibers in polymyositis. *Muscle Nerve 23,* 385–392.

Karpati, G. and Carpenter, S. (1993). Pathology of the inflammatory myopathies. In: Mastaglia, F.L. (ed.), *Baillière's Clinical Neurology.* (pp. 527–556). London: Baillière Tindall.

Karpati, G., Pouliot, Y., and Carpenter, S. (1988). Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. *Ann. Neurol. 23,* 64–72.

Kieseier, B.C., Schneider, C., Clements, J.M., Gearing, A.J., Gold, R., Toyka, K.V., and Hartung, H.P. (2001). Expression of specific matrix metalloproteinases in inflammatory myopathies. *Brain 124,* 341–351.

Li, M. and Dalakas, M.C. (2000). Expression of human IAP-like protein in skeletal muscle: a possible explanation for the rare incidence of muscle fiber apoptosis in T-cell mediated inflammatory myopathies. *J. Neuroimmunol. 106,* 1–5.

Lindberg, C., Oldfors, A., and Tarkowski, A. (1994). Restricted use of T cell receptor V genes in endomysial infiltrates of patients with inflammatory myopathies. *Eur. J. Immunol. 24,* 2659–2663.

Liu, C.C., Walsh, C.M., and Young, J.D.E. (1995). Perforin: Structure and function. *Immunol. Today 16,* 194–201.

Mantegazza, R., Andreetta, F., Bernasconi, P., Baggi, F., Oksenberg, J.R., Simoncini, O., Mora, M., Cornelio, F., and Steinman, L. (1993). Analysis of T cell receptor repertoire of muscle-infiltrating T lymphocytes in polymyositis. *J. Clin. Invest. 91,* 2880–2886.

Matsubara, S. and Okumura, S. (1996). Experimental autoimmune myositis in SJL/J mice produced by immunization with syngeneic myosin B fraction. Transfer by both immunoglobulin G and T cells. *J. Neurol. Sci. 144,* 171–175.

Matsubara, S., Shima, T., and Takamori, M. (1993). Experimental allergic myositis in SJL/J mice immunized with rabbit myosin B fraction: immunohistochemical analysis and transfer. *Acta Neuropathol. (Berl) 85,* 138–144.

Mendell, J.R., Garcha, T.S., and Kissel, J.T. (1996). The immunopathogenic role of complement in human muscle disease. *Curr. Opin. Neurol. 9,* 226–234.

Michaelis, D., Goebels, N., and Hohlfeld, R. (1993). Constitutive and cytokine-induced expression of human leukocyte antigens and cell adhesion molecules by human myotubes. *Am. J. Pathol. 143,* 1142–1149.

Miller, F.W. (1993). Myositis specific autoantibodies. Touchstones for understanding the inflammatory myopathies. *JAMA 270,* 1846–1849.

Morgan, G., Peter, J.B., and Newbould, B.B. (1971). Experimental allergic myositis in rats. *Arthritis Rheum. 14,* 599–609.

Nagaraju, K., Casciola-Rosen, L., Rosen, A., Thompson, C., Loeffler, L., Parker, T., Danning, C., Rochon, P.J., Gillespie, J., and Plotz, P. (2000a). The inhibition of apoptosis in myositis and in normal muscle cells. *J. Immunol. 164,* 5459–5465.

Nagaraju, K., Raben, N., Loeffler, L., Parker, T., Rochon, P.J., Lee, E., Danning, C., Wada, R., Thompson, C., Bahtiyar, G., Craft, J., Hooft, V.H., and Plotz, P. (2000b). From the cover: conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis- specific autoantibodies. *Proc. Natl. Acad. Sci. U.S.A 97,* 9209–9214.

O'Hanlon, T.P., Dalakas, M.C., Plotz, P.H., and Miller, F.W. (1994). Predominant TCR-ab variable and joining gene expression by muscle-infiltrating lymphocytes in the idiopathic inflammatory myopathies. *J. Immunol. 152,* 2569–2576.

Orimo, S., Koga, R., Nakamura, K., Arai, M., Tamaki, M., Sugita, H., Nanaka, I., and Arahata, K. (1994). Immunohistochemical analysis of perforin and granzyme A in inflammatory myopathies. *Neuromusc. Disord. 4,* 219–226.

Pluschke, G., Rüegg, D., Hohlfeld, R., and Engel, A.G. (1992). Auto-aggressive myocytotoxic T lymphocytes expressing an unusual γ/δ T cell receptor. *J. Exp. Med.* 176, 1785–1789.

Reed, A.M., Picornell, Y.J., Harwood, A., and Kredich, D.W. (2000). Chimerism in children with juvenile dermatomyositis. *Lancet 356,* 2156–2157.

Rider, L.G. and Miller, F.W. (2000). Idiopathic inflammatory muscle disease: clinical aspects. *Baillières Best. Pract. Res. Clin. Rheumatol. 14,* 37–54.

Rosenberg, N.L. and Kotzin, B.L. (1989). Aberrant expression of class II MHC antigens by skeletal muscle endothelial cells in experimental autoimmune myositis. *J. Immunol. 142,* 4289–4294.

Rosenberg, N.L., Ringel, S.P., and Kotzin, B.L. (1987). Experimental autoimmune myositis in SJL/J mice. *Clin. Exp. Immunol. 68,* 117–129.

Schneider, C., Gold, R., Dalakas, M.C., Schmied, M., Lassmann, H., Toyka, K.V., and Hartung, H.P. (1996). MHC class I mediated cytotoxicity does not induce apoptosis in muscle fibers nor in inflammatory cT cells: Studies in patients with polymyositis, dermatomyositis and inclusion body myositis. *J. Neuropathol. Exp. Neurol. 55,* 1205–1209.

Serreze, D.V., Pierce, M.A., Post, C.M., Chapman, H.D., Savage, H., Bronson, R.T., Rothman, P.B., and Cox, G.A. (2003). Paralytic autoimmune myositis develops in nonobese diabetic mice made Th1 cytokine-deficient by expression of an IFN-gamma receptor beta-chain transgene. *J. Immunol. 170,* 2742–2749.

Sugiura, T., Kawaguchi, Y., Harigai, M., Takagi, K., Ohta, S., Fukasawa, C., Hara, M., and Kamatani, N. (2000). Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: role of CD40–CD40 ligand interaction in IL-6, IL-8, IL-15, and monocyte chemoattractant protein-1 production. *J. Immunol. 164,* 6593–6600.

Tews, D.S. and Goebel, H.H. (1998). Cell death and oxidative damage in inflammatory myopathies. *Clin. Immunol. Immunopathol. 87,* 240–247.

Uemura, N. (1969). Histological and histochemical studies on experimental allergic myositis and human polymyositis. *Nagoya J. Med. Sci. 31,* 357–377.

Vafiadaki, E., Reis, A., Keers, S., Harrison, R., Anderson, L.V., Raffelsberger, T., Ivanova, S., Hoger, H., Bittner, R.E., Bushby, K., and Bashir, R. (2001). Cloning of the mouse dysferlin gene and genomic characterization of the SJL-Dysf mutation. *Neuroreport 12,* 625–629.

van der Meulen, M.F., Bronner, I.M., Hoogendijk, J.E., Burger, H., van Venrooij, W.J., Voskuyl, A.E., Dinant, H.J., Linssen, W.H., Wokke, J.H., and de Visser, M. (2003). Polymyositis: an overdiagnosed entity. *Neurology 61,* 316–321.

Vattemi, G., Tonin, P., Filosto, M., Spagnolo, M., Rizzuto, N., and Tomelleri, G. (2000). T-cell anti-apoptotic mechanisms in inflammatory myopathies. *J. Neuroimmunol. 111,* 146–151.

Webb, J.N. (1970a). Experimental immune myositis in guinea pigs. *J. Reticuloendothel. Soc. 7,* 305–316.

Webb, J.N. (1970b). *In vitro* transformation of lymphocytes in experimental immune myositis. *J. Reticuloendothel. Soc. 7,* 445–452.

Wiendl, H., Behrens, L., Maier, S., Johnson, M.A., Weiss, E.H., and Hohlfeld, R. (2000). Muscle fibers in inflammatory myopathies and cultured myoblasts express the nonclassical major histocompatibility antigen HLA-G. *Ann. Neurol. 48,* 679–684.

Wiendl, H., Malotka, J., Holzwarth, B., Weltzien, H.U., Wekerle, H., Hohlfeld, R., and Dornmair, K. (2002). An autoreactive gd TCR derived from a polymyositis lesion. *J. Immunol. 169,* 515–521.

Wiendl, H., Mitsdoerffer, M., Hofmeister, V., Wischhusen, J., Weiss, E.H., Dichgans, J., Lochmüller, H., Hohlfeld, R., Melms, A., and Weller, M. (2003a). The non-classical MHC molecule HLA-G protects human muscle cells from immune-mediated lysis: implications for myoblast transplantation and gene therapy. *Brain 126,* 176–185.

Wiendl, H., Mitsdoerffer, M., Schneider, D., Chen, L.P., Lochmuller, H., Melms, A., and Weller, M. (2003b). Human muscle cells express a B7-related molecule, B7-H1, with strong negative immune regulatory potential: a novel mechanism of counterbalancing the immune attack in idiopathic inflammatory myopathies. *FASEB J. 17,* 185–192.

Wiendl, H., Mitsdoerffer, M., Schneider, D., Melms, A., Lochmüller, H., Hohlfeld, R., and Weller, M. (2003c). Muscle fibres and cultured muscle cells express the B7.1/2-related inducible co-stimulatory molecule, ICOSL: implications for the pathogenesis of inflammatory myopathies. *Brain 126,* 1026–1035.

Yazici, Y. and Kagen, L.J. (2000). The association of malignancy with myositis. *Curr. Opin. Rheumatol. 12,* 498–500.

CHAPTER

35

Thyroid Disease

ANTHONY P. WEETMAN

Clinical Sciences Centre, University of Sheffield, Northern General Hospital, Sheffield, UK

AUTOIMMUNE THYROIDITIS

Historical Background

The clinical features of myxedema, the end stage of autoimmune thyroiditis (AT), were defined in 1874 by Gull, and Murray, in Newcastle-upon-Tyne, was the first to give thyroid extract as treatment in 1891. The characteristic lymphocytic infiltration (struma lymphomatosa) was first noted by Hashimoto's in 1912. Proof that AT was due to loss of self-tolerance came from Rose and Witebsky (1956) who showed that rabbits immunized with homologous thyroid extract and adjuvant developed a thyroid lymphocytic infiltrate and thyroglobulin (TG) antibodies. The latter were detected in Hashimoto's thyroiditis during the same year by Roitt and Doniach (Roitt et al., 1956).

Clinical, Pathologic, and Epidemiologic Features

Several types of AT exist (Table 35.1). Two in particular, goitrous (Hashimoto's) thyroiditis and atrophic thyroiditis (primary myxedema), result in hypothyroidism (Pearce et al., 2003), and it is likely these represent two ends of a spectrum of thyroid destruction. The goiter in Hashimoto's thyroiditis is usually firm and painless, with an irregular surface. Patients are often euthyroid at presentation but serum thyroid-stimulating hormone (TSH) levels can be elevated even if thyroxine (T4) levels are within the reference range, representing subclinical thyroid failure. Primary myxedema is identified only when hypothyroidism is apparent clinically and biochemically.

Autoimmunity accounts for over 90% of noniatrogenic hypothyroidism in iodine-sufficient countries. Women are 5–10 times more likely to be affected, with a peak incidence at 50–60 years of age (Vanderpump et al., 1995). The prevalence of autoimmune hypothyroidism in the general white population is 1–2% in women, whereas thyroid autoantibodies can be found in up to 20%, reflecting the presence of focal thyroiditis.

Pathologic changes in AT range from mild focal thyroiditis to extensive lymphocytic infiltration and scarring (Figure 35.1). In Hashimoto's thyroiditis there is a dense infiltration by lymphocytes, plasma cells, and macrophages,

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 35.1 Types of autoimmune thyroiditis

Type	Course	Features
Goitrous (Hashimoto's) thyroiditis	Chronic: leads to hypothyroidism	Goiter: moderate to extensive lymphocytic infiltration and variable fibrosis
Atrophic thyroiditis (primary myxedema)	Chronic hypothyroidism	Atrophy: fibrosis and variable lymphocytic infiltrate
Juvenile thyroiditis	Chronic but may remit	Small goiter with moderate lymphocytic infiltrate
Postpartum thyroiditis	Transient thyrotoxicosis and/or hypothyroidism 3–6 months after delivery	Small goiter with some lymphocytic infiltrate
Silent thyroiditis	Transient thyrotoxicosis and/or hypothyroidism	Small goiter with some lymphocytic infiltrate
Focal thyroiditis	Progressive in some patients	Occurs in 20–40% thyroid specimens at autopsy; associated with thyroid carcinoma

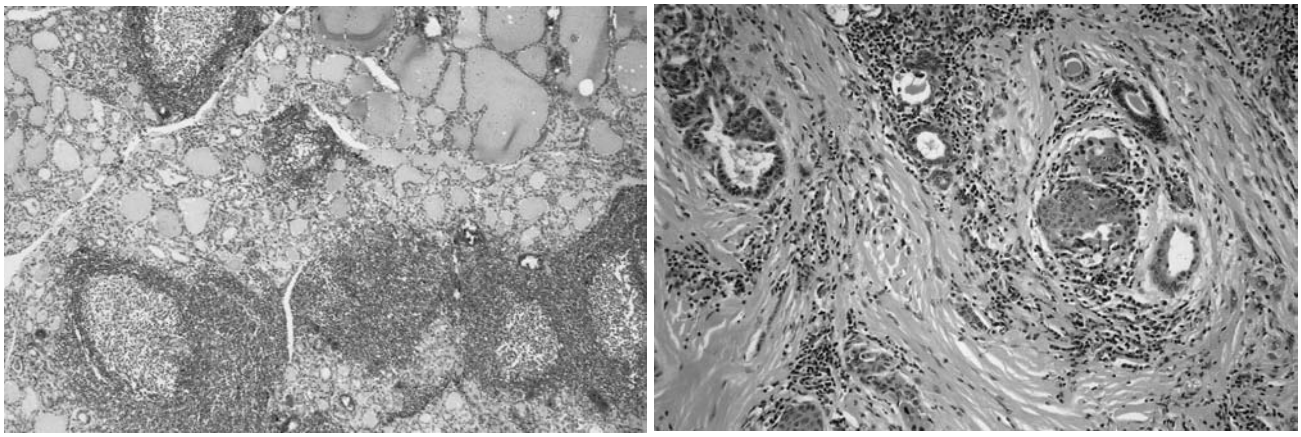


FIGURE 35.1 Pathology of autoimmune hypothyroidism. *A,* Hashimoto's thyroiditis showing germinal center formation. *B,* Primary myxedema with extensive fibrosis. (Original magnification: ×100.) See color plate section. Courtesy of Dr Judith Channer, Northern General Hospital, Sheffield.

and germinal center formation. Thyroid follicles are progressively destroyed and, in the process, the cells undergo hyperplasia and oxyphil metaplasia. In rare cases there are concurrent changes of Graves' disease, so-called hashitoxicosis. There is a variable degree of fibrosis and when this is extensive, the picture may resemble primary myxedema, in which the gland is atrophic and there is extensive fibrosis with loss of normal lobular architecture and minimal or modest lymphocytic infiltration. The histology in postpartum and silent thyroiditis resembles Hashimoto's thyroiditis.

Autoimmune Features

Autoantibodies

Circulating autoantibodies against TG and thyroid peroxidase (TPO) are found, often at very high levels, in most patients with AT. These antibodies are common, albeit at low levels, in association with focal thyroiditis.

Thyroglobulin Antibodies

Thyroglobulin is a 660 kDa homodimeric glycoprotein secreted by thyroid follicular cells (TFC) and stored in the luminal colloid. At 4–8 hormonogenic sites, iodinated tyrosine residues couple to form T4 or tri-iodothyronine (T3). The iodination state of TG alters its immunogenicity in animals and in man (Saboori et al., 1999). Oxidative stress during TG synthesis may be particularly critical in the generation of immunoreactive C-terminal fragments of the molecule (Duthoit et al., 2000). There are two major and one minor antibody epitopes on each 330 kDa subunit, and the wide spacing of these prevents IgG cross-linking and therefore complement fixation. The restriction of the response to three epitopes is only relative, as TG antibodies recognize an increasing number of determinants as their titer rises and

somatic hypermutation occurs (McIntosh et al., 1998). Antibody reactivity is predominantly in the IgG_1 and IgG_4 subclasses but not light chain restricted.

Thyroid Peroxidase Antibodies

TPO is a 100–105 kDa apical membrane protein responsible for tyrosine iodination and coupling in the formation of thyroid hormones. Molecular characterization of this autoantigen has been reviewed extensively (McLachlan and Rapoport, 1992). Antibodies to TPO have a similar IgG subclass distribution to TG, but are κ-light chain restricted (McIntosh et al., 1998). Eighty percent of TPO antibodies recognize an immunodominant region involving overlapping, conformational epitopes in two extracellular domains and specific patterns of TPO autoantibody recognition are genetically transmitted in AT families (Jaume et al., 1999). The epitopic domains have been defined (Gora et al., 2004) but full characterization will require crystallization of TPO. *In vitro*, TPO can bind C4 complement component, which may contribute to the susceptibility of TFC to destruction (Blanchin et al., 2003).

Other Autoantibodies

Thyroid stimulating hormone (TSH) receptor (TSH-R)-blocking antibodies are found in around 20% of patients with AT (Weetman and McGregor, 1994). Growth-stimulating and growth-blocking autoantibodies, operating independently of the TSH-R, have been suggested to determine thyroid size (Drexhage, 1996) but there is considerable controversy regarding the methodology used to detect these antibodies. Two thyroid-specific autoantibodies recognize a poorly characterized second (i.e., non-TG) colloid antigen and the Na^+/I^- transporter, but any importance of these in pathogenesis is unknown (Ajjan et al., 2000). Autoantibodies against T4 and T3 occur in 15–35% of patients with autoimmune thyroiditis.

T-Cell Responses

A major site of autoreactivity is within the thyroid itself, although autoimmune responses can also be detected within the draining lymph nodes and bone marrow (Weetman et al., 1984). Recruitment of lymphocytes to the thyroid requires upregulation, on endothelial cells, of various adhesion molecules and selectins, and the infiltrating lymphocytes express the reciprocal adhesion molecules, including CD11a, CD18, CD29, CD49a, and CD49e (Marazuela, 1999). The local infiltrate produces an array of chemokines which aid homing, including CXCL12, CXCL13 and CCL22, particularly when lymphoid follicles are present, and the TFC contribute to the chemokine pool, exacerbating the autoimmune process (Armengol et al., 2003; Kim et al., 2003). Most phenotyping and functional studies on T cells have used the readily sampled peripheral blood: this may reflect poorly (if at all) the responses within the autoimmune target.

Studies of T-Cell Phenotypes

The number of circulating $HLA\text{-}DR^+$ (activated) T cells is elevated and $CD8^+$ T cells are reduced, but only in active thyroiditis (Iwatani et al., 1992). $CD4^+$ T cells predominate in the thyroid infiltrate and many of these are activated (Aichinger et al., 1985). The majority of T cells express the αβ receptor and no obvious bias in T-cell receptor usage is apparent, despite initial claims (McIntosh et al., 1997). Although a pauciclonal T-cell response seems likely in early AT, by the time of clinical presentation there is spreading of the immune response to produce a polyclonal T-cell response, directed against an array of autoantigens and epitopes.

Functional Studies

Weak T-cell proliferative responses against TG and TPO are found in many patients and can be enhanced by IL-2 supplementation (Butscher et al., 2001). To identify T-cell epitopes for TPO, circulating T cells from patients with autoimmune thyroiditis have been stimulated with overlapping synthetic peptides (Tandon et al., 1991). No dominant epitope has been identified; instead there is considerable heterogeneity both within and between individual patients. Migration-inhibition factor-based assays have been used in attempts to identify a defect in thyroid antigen-specific suppressor T cells in AT (Volpe, 1988; Martin and Davies, 1992) but no consistent results have been produced and there is no evidence in man yet to implicate a defect in $CD4^+CD25^+$ T regulatory cells.

Cloned intrathyroidal T cells from patients release IFN-γ, IL-2, IL-6, and tumor necrosis factor (TNF) with no distinct Th1 or Th2 pattern (Grubeck-Loebenstein et al., 1990). Reverse transcription of cytokine mRNA and cDNA amplification has revealed a mixed Th1 and Th2 response in Hashimoto's thyroiditis (Ajjan et al., 1996). The demonstration of major histocompatibility complex (MHC) class II expression on TFC in Hashimoto's thyroiditis and Graves' disease gave rise to the concept that such "aberrant" expression could initiate or perpetuate the autoimmune response by converting the TFC into antigen-presenting cells (Bottazzo et al., 1983). However class II expression is restricted to TFC adjacent to T cells producing IFN-γ (Hamilton et al., 1991) and only this cytokine is capable of initiating class II expression *in vitro*. MHC class II expression is found on TFC in experimental autoimmune thyroiditis (EAT) induced by neonatal thymectomy (see below) but always follows the appearance of a lymphocytic infiltrate (Cohen et al., 1988). TFC do not express costimulatory molecules even after cytokine exposure (Marelli-Berg et al., 1997). Together these results indicate that TFC are unlikely to initiate the autoimmune response.

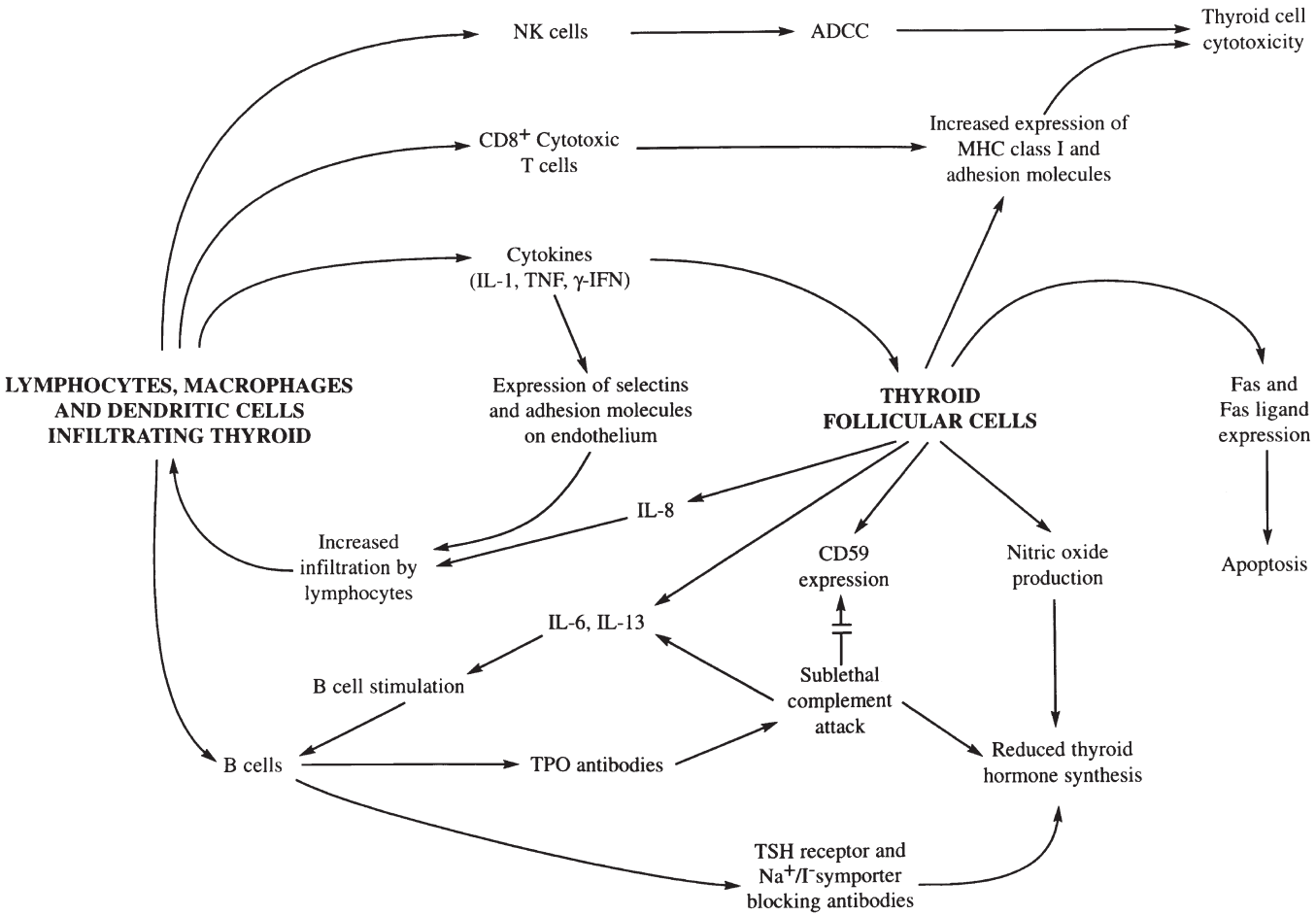


FIGURE 35.2 Role of thyroid follicular cells in autoimmune thyroiditis. From Weetman, A.P., Ajjan, R. and Watson, P.F. (1997) Cytokines and Graves' disease. *Baillière's Clin. Endocrinol. Metab. 11,* 481–497, with permission.

T-cell lines and clones derived from the thyroids of patients can be activated by class II-expressing TFC (Londei et al., 1985). Such T cells are likely to have been previously activated by classic antigen-presenting cells and are no longer dependent on B7 costimulation (Marelli-Berg et al., 1997). Anergy can be induced in CD80 (B7)-dependent, naïve T cells by class II-positive TFC and this type of peripheral tolerance may be important in regulating autoreactive T cells within the normal thyroid. In established AT, however, T cells are resistant to tolerance indication (Dayan et al., 1993) and class II-expressing TFC may then help to perpetuate the autoimmune response. TFC may participate in the autoimmune process in other ways besides MHC class II expression (Figure 35.2).

Genetic Features

The role of genetic factors in AT is suggested by the frequent presence of thyroid autoantibodies in other family members and the association of thyroiditis with other endocrinopathies as part of the type 2 autoimmune polyglandular syndrome. Twin studies show a 0.55 concordance rate in monozygotic but not dizygotic twins, and similar findings have been reported for the aggregation of thyroid autoantibodies in euthyroid twins of individuals with AT (Brix et al., 2000; 2004).

As with most autoimmune disorders, associations with HLA alleles have been extensively investigated, producing conflicting results (Tomer and Davies, 2003). Initially it appeared that primary myxedema and Hashimoto's thyroiditis in white people had distinct associations with HLA-DR3 and -DR5 respectively, but subsequent studies have shown that Hashimoto's thyroiditis is associated with HLA-DR3 and to a lesser extent -DR4. Postpartum thyroiditis has a weak association with HLA-DR5.

Another established susceptibility locus besides HLA is CTLA-4, with polymorphisms in this gene conferring a relative risk of around 2, possibly due to the increased

production of an alternative splice form of CTLA-4 with immunoregulatory consequences (Kotsa et al., 1997; Ueda et al., 2003). Genome-scanning methods, applied to relatively small series of families, have suggested a number of susceptibility loci on chromosomes 6p, 8q, 10q, and 12q (Tomer and Davies, 2003) but these have not yet been independently confirmed. Around 40% of Turner syndrome patients have TPO antibodies and 15% become hypothyroid, with a particularly high risk in women with an X-isochromosome (Elsheikh et al., 2001). Linkage and association studies have suggested that the gene-encoding TG may be a susceptibility locus, but the largest study to date in UK patients found no evidence to support this, emphasizing the replication difficulties that are posed by association studies looking at candidate loci (Collins et al., 2004).

Environmental Influences

The female preponderance of AT is in part due to the influence of sex steroids. Certainly in EAT, estrogens or progesterone exacerbate thyroiditis and this is reversed by testosterone (Okayasu et al., 1981; Ansar-Ahmed et al., 1983), while use of exogenous estrogens lowers the risk of developing TPO antibodies in genetically predisposed individuals (Strieder et al., 2003). Early nutritional factors may also have an impact, as low birth weight appears to be associated with an increased prevalence of thyroid autoantibodies (Phillips et al., 2002). Pregnancy ameliorates AT but there is an exacerbation in the year after delivery, reflected by a rise in TPO antibody levels. In some women with a previously mild thyroiditis, the enhanced autoimmune response is sufficient to cause biochemical or clinical thyroid dysfunction, and this is termed postpartum thyroiditis (Muller et al., 2001). Although usually a transient phenomenon, up to a quarter of such women develop permanent hypothyroidism within 10 years, so that pregnancy is a risk factor which precipitates clinical disease in predisposed subjects (Othman et al., 1990). The exacerbation of underlying thyroiditis is assumed to be due primarily to hormonal changes. Fetal microchimerism may play a role via intrathyroidal chimeric cells breaking immunologic tolerance (Ando and Davies, 2003).

There is no convincing evidence for a direct role of infection in etiology (Tomer and Davies, 1993). The prevalence of lymphocytic thyroiditis appears to be rising in developed countries, possibly as a result of increasing iodine intake. Certainly iodine is essential for the development of EAT in obese strain (OS) chickens, and increasing iodine administration worsens EAT in both OS chickens and BioBreeding (BB) rats (Ruwhof and Drexhage, 2001). As well as enhancing the immunogenicity of TG, iodine may be involved at an early stage in causing thyroid injury through the generation of reactive oxygen metabolites (Bagchi et al., 1995). Indirect evidence for iodine-induced injury is provided by studies of iodization programs in iodine-deficient regions showing an increase in thyroid autoantibodies and lymphocytic thyroiditis shortly after supplementation.

Anthracene derivatives and other chemicals induce EAT in the Buffalo strain rat. Administration of lithium may exacerbate autoimmune thyroiditis both immunologically and biochemically, but the adverse effect of the cytokine IFN-α on thyroid autoantibody development is more striking (Carella et al., 2001). Radioactive fallout from the Chernobyl nuclear disaster led to an almost 10-fold increase in the prevalence of thyroid autoantibodies in girls aged 9–10 at the time of the accident, presumably through release of thyroid autoantigens from the damaged thyroid (Pacini et al., 1998). Thyroid autoantibodies can appear transiently after subacute thyroiditis, supporting this mechanism (Iitaka et al., 1998).

Animal Models

AT can be induced experimentally in animals and also occurs spontaneously, with features most closely resembling Hashimoto's thyroiditis.

Immunization-Induced Thyroiditis

In mice and rats the strength of the autoimmune response to immunization with TG in adjuvant is strain dependent. Murine EAT susceptibility (as shown by the severity of lymphocytic thyroiditis) is governed by the class II I-A subregion of the H-2 major histocompatibility complex (Vladutiu and Rose, 1971). H-$2^{k,s}$ strains may even develop EAT with syngeneic TG immunization alone, demonstrating that untolerized autoreactive T cells exist in normal animals, in which they are usually under active regulation (ElRehewy et al., 1981). The influence of I-E is strain dependent but less clear. MHC class I K and D alleles also influence susceptibility, presumably by determining the strength of effector cytotoxic T-cell interaction with the thyroid cell target. Transgenic mice expressing HLA-DR3 but not HLA-DR2 develop EAT after TG immunization, confirming a role for this HLA specificity in thyroiditis (Kong et al., 1996). Genes outside the MHC have a minor role: TG antibody responses depend on both H-2 and immunoglobulin heavy chain genes. In the rat, immunization-induced thyroiditis is under partial MHC control but non-MHC genes possibly play a greater role than in mice (De Assis-Paiva et al., 1989).

Female animals have worse EAT than males and this is dependent on sex hormones, estrogen excess worsening thyroiditis and testosterone ameliorating it (Okayasu et al., 1981). Another important influence in mice is the level of circulating TG, which can induce $CD4^+$ and $CD8^+$ suppressor T cells (Kong et al., 1989). Other controlling factors are shown in Figure 35.3. The thyroiditis after immunization consists of $CD4^+$ and $CD8^+$ T cells and macrophages, with

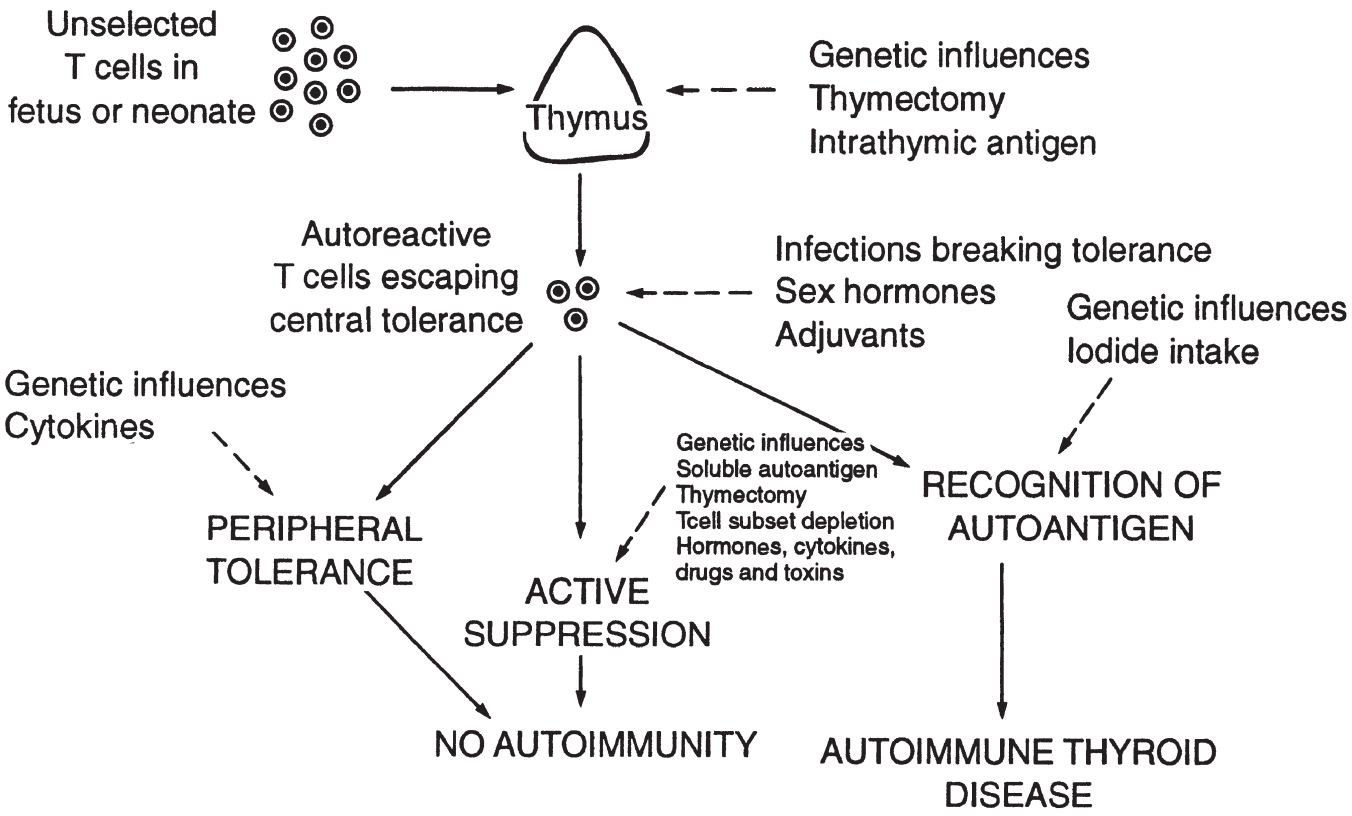


FIGURE 35.3 Sites of various controlling factors in the development of experimental autoimmune thyroiditis. From Weetman and McGregor (1994), with permission.

only a small percentage of B cells. Disease can be transferred by T cells but not by TG antibodies and the critical effector cells are $CD8^+$ cytotoxic T cells which require specific $CD4^+$ T cells for their induction (Creemers et al., 1983). TPO-based models have been difficult to establish, yet would more resemble the human counterpart. The major difficulty has been antigen availability; recombinant murine TPO will induce EAT after immunization of $H\text{-}2^b$ mice especially (Ng et al., 2004).

Experimental Autoimmune Thyroiditis Resulting from T-Cell Modulation

Neonatal thymectomy in certain strains of mice or rats, or thymectomy plus sublethal irradiation in certain strains of rats, results in severe EAT (Penhale et al., 1973; Kojima et al., 1976), as well as other autoimmune endocrinopathies. From these initial observations came the understanding of the crucial role of $CD4^+$, $CD25^+$ immunoregulatory T cells in preventing autoimmunity (Sakaguchi et al., 2001). Other maneuvers affecting T cells, such as treatment of neonatal mice with cyclosporine A or T-cell depletion and reconstitution, can induce EAT (Sakaguchi and Sakaguchi, 1989). Recent work has shown that depletion of CD7/CD28 in knock-out mice also prevents generation of $CD4^+$, $CD25^+$ regulatory cells and results in EAT (Sempowski et al., 2004). The induction of EAT by T-cell modulation elegantly demonstrates the presence of thyroid-reactive T cells in the normal newborn repertoire. These $CD4^+$ T cells may be destined for deletion after birth but there is now strong evidence for incomplete tolerance and active suppression even later.

In addition to MHC and non-MHC genes, environmental factors play an important role in susceptibility. Rats raised under specific pathogen-free conditions until weaning are resistant to EAT following thymectomy and irradiation, but transfer of normal gut microflora results in EAT in the germfree animals (Penhale and Young, 1988). It is unclear whether radiation-induced damage to the intestine is involved in this effect of gut microflora.

An extreme example of T-cell modulation has been the generation of mice transgenic for the T-cell receptor of a T cell clone specific for a cryptic epitope of TPO, derived from a patient with AT (Quaratino et al., 2004). These mice developed thyroiditis and hypothyroidism despite this being in the context of murine TPO and antigen presentation of the rel-

evant epitope by H-2 rather than HLA: the development of disease in these animals indicates that a cryptic epitope can have a significant pathogenic role in autoimmunity.

Spontaneous Autoimmune Thyroiditis

Several species develop spontaneous EAT but most is known about the BB and Buffalo strains of rat and the OS chicken. Lymphocytic thyroiditis and TG antibodies occur in around 60% of diabetic and 10% of nondiabetic BB rats but diabetes-prone sublines have a range of prevalence, from 100% in the NB line to 5% in the BE line, suggesting that diabetes is not tightly linked genetically to EAT (Crisa et al., 1992). The Buffalo strain rat has a low spontaneous incidence of thyroiditis, reaching a maximum of 25% in old, multiparous females (Noble et al., 1976).

Spontaneous autoimmune thyroiditis in the OS chicken is the closest animal model of Hashimoto's thyroiditis. The birds were originally bred from a White Leghorn flock of Cornell strain chickens for phenotypic features of hypothyroidism. Over time, the factors influencing disease development have changed: the importance of MHC genes and sex has diminished and the main genetic determinants in current OS chickens govern target organ susceptibility, T-cell hyperreactivity, and corticosteroid responses (Wick et al., 1989). Unlike other models, OS chickens develop severe hypothyroidism as well as a lymphocytic thyroiditis and TG antibodies, and require T4 supplementation to thrive. The disease is T-cell dependent as thymectomy at birth prevents disease, although later thymectomy exacerbates thyroiditis, presumably by altering the balance of T-cell-mediated suppressor activity (Pontes de Carvalho et al., 1981).

Pathogenic Mechanisms

Several antibody- and cell-mediated mechanisms contribute to thyroid injury in autoimmune hypothyroidism, and differences in the relative importance of each may determine some of the clinical and pathologic variants described above (Figure 35.4).

Antibody-Mediated Injury

Immune complexes are deposited in the basement membrane around the thyroid follicles in Hashimoto's thyroiditis (Pfaltz and Hediger, 1986) and terminal complement complexes are also present at this location, indicating the formation of membrane attack complexes (Weetman et al., 1989). TFC are relatively resistant to lysis, through enhanced expression of multiple regulators of complement activation, especially CD59, in response to cytokines derived from the infiltrating lymphocytes and macrophages (Tandon et al., 1994). After sublethal complement attack, TFC are less able to respond to TSH stimulation and also

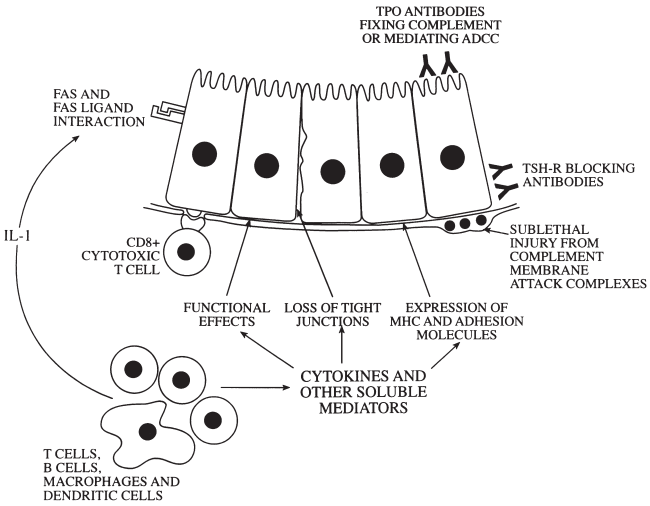


FIGURE 35.4 Mechanisms of thyroid destruction in autoimmune hypothyroidism. From Weetman, A.P. (2002) Autoimmune thyroid disease. In J.A.H. Wass and S.M. Shalet (eds), *Oxford Textbook of Endocrinology and Diabetes* (pp. 392–408). Oxford: Oxford University Press, with permission.

release cytokines (IL-1, IL-6), prostaglandin E2 and reactive oxygen metabolites, which may have proinflammatory effects (Weetman et al., 1992). TPO antibodies are generally assumed to be the major mediators of complement fixation and activation within the thyroid but unidentified autoantibodies are also involved (Chiovato et al., 1993) and additional activation via the alternative pathway probably occurs. Cytokines like IL-1 may be critical in dissociating the junctional complex and thus allowing access of autoantibodies to apically expressed TPO and other such antigens (Nilsson et al., 1998), in turn implying a secondary role for these antibodies in pathogenesis.

Autoantibodies may also provoke damage by antibody-dependent cell-mediated cytotoxicity (ADCC). A difference in the level of serum autoantibodies capable of mediating ADCC has been proposed to explain the clinical pictures of atrophic and goitrous thyroiditis, with more ADCC activity in the former (Bogner et al., 1995), but this has not proved reproducible, as others have found equally potent ADCC activity in patients with Hashimoto's thyroiditis, primary myxedema, and Graves' disease (Metcalfe et al., 1997).

Finally, autoantibodies can alter target cell function. TSH-R blocking antibodies have a clearly defined importance, as their placental transfer causes transient neonatal hypothyroidism (Matsuura et al., 1980). Although they appear to be particularly associated with atrophic thyroiditis in Asian patients (Arikawa et al., 1985), TSH-R blocking antibodies occur in both goitrous and atrophic thyroiditis in white people (Steel et al., 1984; Kraiem et al., 1987).

T-Cell Mediated Injury

Despite the strong lead provided by studies in EAT, there is only modest direct evidence that T-cell mediated injury is important in autoimmune hypothyroidism. Two groups have derived $CD8^+$ T-cell clones and lines from patients with Hashimoto's thyroiditis which lyse autologous TFC in a MHC class I-restricted fashion (MacKenzie et al., 1987; Sugihara et al., 1995). The autoantigen specificity of these T cells has not been elucidated. Indirect evidence for the importance of cytotoxic T cells in pathogenesis is provided by the demonstration of frequent perforin-containing T cells in the intrathyroidal $CD8^+$ T-cell population in Hashimoto's thyroiditis (Wu et al., 1994). In addition, TFC in Hashimoto's thyroiditis express adhesion molecules such as CD54 in response to cytokines and these increase the potential for T-cell binding, thereby enhancing cytotoxicity (Weetman et al., 1990). T cells may also provoke thyroid dysfunction by release of cytokines. Although there are only weak and inconsistent effects of IFN-γ, IL-1 and TNF on TFC survival in primary cultures (McLachlan et al., 1990), IFN-γ inhibits the response to TSH stimulation and TNF plus IFN-γ in combination have a marked inhibitory effect on TPO and TG expression in the FRTL-5 thyroid cell line (Mandrup-Poulsen et al., 1996).

Attention recently has focused on the occurrence of death receptor-mediated apoptosis as a major pathway for TFC destruction, based on the finding of both Fas (CD95) and Fas ligand (CD95L) on TFC in Hashimoto's thyroiditis (Giordano et al., 1997). Fas expression by TFC was upregulated by IL-1β, leading to the suggestion that cytokines could induce cytotoxicity through this pathway by suicide or fratricide as Fas interacted with FasL. Moreover, it has been proposed that FasL on TFC induces apoptosis in the infiltrating lymphocytes, suggesting that a T-cell mediated cytotoxic mechanism for thyroid destruction is less important than autocrine/paracrine Fas–FasL interaction (Stassi et al., 1999). Other interpretations have been put on the role of such apoptosis, not least because of the technical difficulty of quantitating FasL expression and the possible role of decoy death receptors and regulators of apoptosis signalling. An alternative proposal is that the infiltrating lymphocytes cause TFC apoptosis through their expression of TNF-related apoptosis-inducing ligand (TRAIL) engaging with various death receptors, induced by cytokines (Bretz and Baker, 2001).

Immunologic Markers in Diagnosis

The diagnosis of autoimmune hypothyroidism is usually straightforward, patients having biochemical evidence of hypothyroidism plus TG and/or TPO antibodies which can easily be measured by passive hemagglutination or ELISA (enzyme-linked immunosorbent assay). An abnormal thyroid ultrasound pattern is also highly predictive of AT (Raber et al., 2002). Fine needle aspiration biopsy is used in difficult cases, especially to exclude an associated lymphoma.

Treatment and Outcome

It is difficult to imagine a more straightforward treatment than T4 replacement and future attempts at immunomodulation seem unlikely (Roberts and Ladenson, 2004). In around 10% of patients there may be a spontaneous remission 4–8 years after starting T4, and this is associated with the disappearance of TSH-R blocking antibodies (Takasu et al., 1992). However, the permanence of such remissions has not been established and remission in other patients has not been associated with changes in TSH-R blocking antibody levels (Cho et al., 1995).

Concluding Remarks—Future Prospects

Considerable progress in understanding the pathogenesis of AT has been made in the half century since the first demonstration of EAT and the realization that a similar process accounts for Hashimoto's thyroiditis. A major future goal will therefore be to identify the earliest T-cell responses, as these may be restricted to single T-cell clones, recognizing restricted epitopes on a single inciting autoantigen, and have a particular Th1 or Th2 bias. Additional work is also needed to determine the exact genetic basis for AT and to clarify the relationships between primary myxedema, Hashimoto's thyroiditis, and postpartum thyroiditis. Finally, despite two decades of research, the role of T-cell mediated, thyroid-specific immunoregulation in man is still unclear. There is no doubt that T regulatory cells are important in EAT, but so far the mechanisms which control thyroid-specific T cells that have escaped central tolerance in human AT are elusive.

GRAVES' DISEASE

Historical Background

This disorder was first described by Caleb Parry in 1825 but it was Robert Graves' whose name became attached to the disease through his report of four cases published in 1835. Basedow was the first to highlight the association with exophthalmos in 1840. Originally believed to have a cardiac and then neurologic origin, the role of the thyroid in Graves' disease became established in the 1890s as thyroidectomy for apparently coincidental goiter improved the other disease manifestations. The cause remained obscure until Adams and Purves (1956) showed that the serum from Graves' patients contained a long-acting thyroid stimulator

which was distinct from TSH; working separately, Kriss and McKenzie went on to show that this stimulator was an IgG.

Clinical, Pathologic, and Epidemiologic Features

Although sharing many immunologic features with AT, it is the production of TSH-R stimulating antibodies which characterizes Graves' disease (Weetman, 2000). It is the most common cause of hyperthyroidism, accounting for 60–80% of cases. The prevalence is around 1% in women aged 35–60 years, with a 5–10-fold lower frequency in men (Vanderpump et al., 1995). Over 90% of patients with Graves' disease have thyroid-associated ophthalmopathy (TAO), which can be revealed by scanning techniques showing enlarged extraocular muscles (Bartalena et al., 2000). Clinically obvious eye disease is apparent in around 50% of patients (Figure 35.5). TAO is not exclusive to Graves' disease, as around 5% of patients have AT and another 5% have little evidence of thyroid dysfunction. Thyroid dermopathy (or pretibial myxedema, reflecting the usual site for this complication) occurs in only 1–5% of patients, who usually also have marked TAO (Schwartz et al., 2002).

In the untreated state there is both hypertrophy and hyperplasia of the thyroid follicles; the epithelium becomes columnar and folded into the follicular lumen, new small follicles form, and there is little colloid (Figure 35.6). A variable degree of lymphocytic infiltration occurs and germinal centers may form. Antithyroid drugs diminish the lymphocytic infiltrate and the epithelium reverts to a normal appearance. Lymphoid hyperplasia may occur in the lymph nodes, thymus, and spleen but reverses with antithyroid drugs.

Autoimmune Features

Autoantibodies

Thyroglobulin and TPO autoantibodies occur in 80% of patients. TSH-R stimulating antibodies (TSAb) can be detected in over 95% of patients with current assays. The TSH-R consists of a 398 amino acid extracellular domain, a 266 amino acid transmembrane spanning domain with seven hydrophobic regions, and an 83 amino acid intracellular domain (Rapoport et al., 1998). The receptor undergoes complex post-translational processing to form two subunits. TFC shed the A subunit, which binds TSAb more strongly than the holoreceptor, and this may be critical in initiating or amplifying the autoimmune response (Chen et al., 2003). TSH-induced activation of the TSH-R causes a rise in cAMP and, at high concentrations of the hormone, activation of the phosphatidyl-inositol intracellular signaling pathway.

The original bioassay method measured release of radioiodine from preloaded thyroid glands after injection of serum or IgG into intact animals and this activity was termed long-acting thyroid stimulator or LATS (Adams and Purves, 1956). Current assays measure cAMP release from primary TFC cultures, thyroid cell lines (such as FRTL-5), and most recently from TSH-R transfected eukaryotic cells (Ludgate and Vassart, 1995). A separate type of assay measures displacement of radiolabeled TSH from solubilized TSH-R derived from thyroid membranes by TSH-R antibodies; these antibodies are called TSH-binding inhibiting immunoglobulins or TBII. Although the TBII assay is robust and commercially available, both TSAb and TSH-R blocking antibodies (which inhibit TSH-induced cAMP release) are measured by it and therefore the level of TBII gives no direct information on functional activity. However, second-

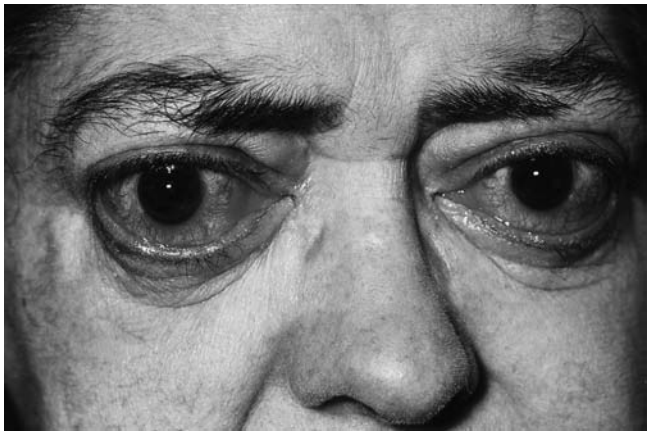


FIGURE 35.5 Eye signs in a patient with thyroid-associated ophthalmopathy showing exophthalmos, scleral injection, and periorbital edema. See color plate section.

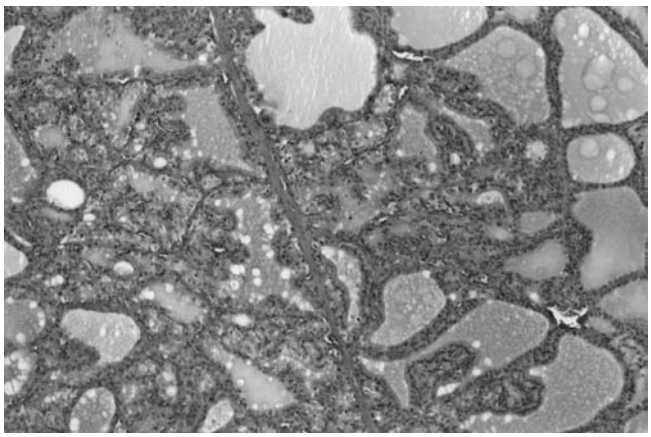


FIGURE 35.6 Pathology of Graves' disease showing columnar and folded thyroid epithelium, small new follicles, and active colloid resorption, with "scalloping" of the colloid. A lymphocytic infiltrate is not prominent in this specimen. (Original magnification: ×100.) See color plate section. Courtesy of Dr Judith Channer, Northern General Hospital, Sheffield.

generation TBII assays have a remarkable sensitivity and specificity when the clinical setting is taken into account (Costagliola et al., 1999). Monoclonal antibodies derived from animals have demonstrated the existence of a third group of neutral TSH-R antibodies, that recognize linear epitopes within the cleaved region of the receptor and neither stimulate nor block the receptor (Ando et al., 2004).

The majority of TSH-R B-cell epitopes appear to be conformational (Ludgate and Vassart, 1995). Heterogeneity between patients in their antibody-binding sites is apparent using chimeric TSH/LH hormone receptors, and mutated receptors have revealed that TSH-R blocking antibodies bind to the C terminus preferentially and recognize the holoreceptor more efficiently than TSAb, thereby having their inhibitory affect on TSH binding (Schwarz-Lauer et al., 2002). Further epitope mapping should be possible when panels of human monoclonal TSH-R antibodies are further developed (Sanders et al., 2003). A recent example of this approach has been the comparative modeling of the TSH-R interaction with a monoclonal TSAb, which indicates the possible importance of the leucine-rich repeat region of the receptor in activation by antibody (Miguel et al., 2004).

Unlike TG and TPO autoantibodies, TSAb are IgG_1 subclass restricted (Weetman et al., 1990) and are often λ-light chain restricted (Zakarija, 1983; Williams et al., 1988), suggesting a pauciclonal origin for TSAb in some patients. There is, however, heterogeneity in the activity of TSAb. Cluster analysis has grouped TSAb into four subsets based on the relative activity of their TSH-R antibodies in stimulating cAMP production and the phospholipase A_2–arachidonic acid cascade (Di Cerbo et al., 1995). Antibodies activating both pathways were found in patients with the most severe disease and largest goiters. In contrast, patients who produce both TSH-R blocking antibodies and TSAb can remain euthyroid.

T-Cell Responses

Many studies have found a reduction in circulating $CD8^+$ T cells and an increase in HLA-DR^+ T cells in active Graves' disease (Weetman and McGregor, 1994). Intrathyroidal T cells are $CD4^+$ and $CD8^+$ in varying proportions (Jansson et al., 1984). Similar homing mechanisms to AT account for this localization (Marazuela, 1999). Using overlapping TSH-R peptides covering the extracellular domain, a heterogeneous response was obtained using circulating or intrathyroidal T cells in proliferation assays, with multiple peptides stimulating different patients' cells (Tandon et al., 1992). IL-4 is not always detectable in Graves' thyroids and there is no distinct Th1 or Th2 pattern of response (Watson et al., 1994; Okumura et al.,1999). IL-1, IL-6, IL-8, and IL-10 are strongly expressed, the first two being in part derived from the TFC themselves.

Genetic Features

Monozygotic twins are around 20–30% concordant for Graves' disease, at least 10-fold higher than for dizygotic twins (Brix et al., 1998). A consistent but weak association exists between the serologically defined specificity HLA-DR3 and Graves' disease in white people, with a relative risk of around 2–3 (Tomer and Davies, 2003). The relative weakness of the contribution of HLA genes is underlined by the low concordance (7%) for Graves' disease in HLA-identical siblings (Stenszky et al., 1985). Polymorphisms in two genes expressed in T cells confer a relative risk of around 2: CTLA-4 (Yanagawa et al., 1995; Ueda et al., 2003) and PTPN22 (Velaga et al., 2004), in common with several other autoimmune disorders (see Chapter 21). As with AT, a number of polymorphisms in non-MHC candidate genes (encoding T-cell receptors, immunoglobulins, cytokines, and TSH-R) have been tested for associations with Graves' disease, with inconsistent results. The most recent candidate genes to be associated with Graves' disease are those encoding ICAM-1 and CD40 but it remains to be seen whether these will hold up after replication (Kim et al., 2003; Kretowski et al., 2003). Genome scanning methods have revealed other possible loci linked to Graves' disease (Tomer and Davies, 2003) but these have not yet been confirmed.

Environmental Influences

Women are predisposed to Graves' disease with an additional risk of developing the disease in the postpartum period: two-thirds of women with Graves' disease who had been pregnant noted the onset of their disease post partum (Jansson et al., 1987). Retrospective studies have shown a significantly higher number of adverse, major life events during the year preceding the recognition of Graves' disease when patients are compared to matched controls (Winsa et al., 1991; Matos-Santos et al., 2001). Presumably this effect of stress operates through the interactions between the nervous, endocrine, and immune systems. A high iodine intake increases the risk of developing an autoimmune response against the thyroid, the type being determined by genetic factors. For instance, within Europe the prevalence of Graves' disease increases with national iodine intake (Reinwein et al., 1986).

Evidence for a role of infections is circumstantial (Tomer and Davies, 1993). It remains possible that a variety of infections could precipitate Graves' disease, either specifically, by molecular mimicry or modulation of TFC behavior, or nonspecifically, by enhancing any ongoing immune responses. Such nonspecific enhancement, presumably mediated by cytokines, would explain the association between attacks of allergic rhinitis and recurrence of Graves' disease (Hidaka et al., 1993).

Smoking is strongly associated with the development of ophthalmopathy and weakly with the development of Graves' disease (Bartalena et al., 1995). Perhaps the most striking example of Graves' disease caused by an obvious external agent is its appearance several months after administration of a lymphocyte-depleting monoclonal antibody in a third of patients with multiple sclerosis (Coles et al., 1999). This type of response may be the result of Th2 deviation or alteration of T regulatory cells as the lymphocyte population is recovering, and such a phenomenon may underlie the similar appearance of Graves' disease occasionally after immune restoration of AIDS patients with highly active antiretroviral therapy (Gilquin et al., 1998).

Animal Models

There is still no entirely satisfactory animal model of Graves' disease. Immunization of BALB/c mice with the extracellular domain of TSH-R and adjuvant containing *Bordetella pertussis* induced TSH-R blocking but not stimulating antibodies and a severe thyroiditis (Costagliola et al., 1994), while genetic immunization with TSH-R cDNA produced thyroiditis but without TSAb production (Costagliola et al., 1998). Immunizing AKR/N mice with fibroblasts double transfected with human TSH-R and haploidentical MHC class II genes led to hyperthyroidism caused by TSAb, although without thyroiditis (Yamaguchi et al., 1997). The model appears to be successful because the murine fibroblasts used express the costimulatory molecule CD80 (B7), allowing them to stimulate naïve TSH-R specific T cells. A recent variant of this model has used the hamster and CHO cells expressing TSH-R, resulting in TSAb production and a focal thyroiditis (Ando et al., 2003).

Reproducing ophthalmopathy has proved even more challenging. Priming of T cells, derived from donor mice immunized with TSH-R, with TSH-R *in vitro* followed by transfer to naïve recipients elicited orbital changes that resemble ophthalmopathy pathologically, but there has been no replication as yet (Many et al., 1999).

Pathogenic Mechanisms

Clearly the main effector mechanism in Graves' disease is the production of TSAb but the circulating levels of these do not correlate closely with the level of thyroid hormones or the clinical severity of disease, because of a variable, concurrent response against other autoantigens and, in some patients, production of TSH-R blocking antibodies, which reduce the effects of TSAb. Women with the highest levels of TSAb who become pregnant give birth to babies with transient neonatal thyrotoxicosis.

Immunologic Markers in Diagnosis

TG and TPO antibodies provide readily available evidence for Graves' disease; although not specific, their presence in a patient with hyperthyroidism strongly suggests the diagnosis. TSAb are not generally measured because present bioassays are laborious. TBII estimation is a commercially available surrogate but these antibodies are absent in some newly diagnosed patients and present in AT patients who have TSH-R blocking antibodies. TSAb or TBII levels have been investigated as predictive markers for the success of antithyroid drug treatment in Graves' disease. Although patients with the highest levels tend to relapse the most, the results are insufficiently accurate for clinical use (Davies et al., 1998). The most important indication for TSAb assay in Graves' disease is during pregnancy to predict the risk of neonatal thyrotoxicosis (Laurberg et al., 1998).

Treatment and Outcome

Treatment consists of antithyroid drugs (carbimazole, methimazole or propylthiouracil), radio-iodine or thyroidectomy. Around 40% of patients treated with antithyroid drugs achieve a permanent remission. These drugs ameliorate EAT (Rennie et al., 1983) and TSAb levels fall during treatment due to a decrease in the expression of proinflammatory molecules by TFC (Weetman et al., 1992). Relapse after antithyroid drugs is particularly likely in those patients with evidence of a strong Th2 response (Komiya et al., 2001). Radio-iodine treatment is followed by a striking rise in TSH-R and other thyroid autoantibodies at 3–6 months. TSH-R antibody levels gradually fall in most but not all patients over the year following subtotal thyroidectomy (De Bruin et al., 1988). Complete ablation of thyroid tissue results in disappearance of all thyroid autoantibodies, confirming the need for these autoantigens to maintain antibody production (Chiovato et al., 2003).

Thyroid-Associated Ophthalmopathy and Dermopathy

These complications of Graves' disease are most likely due to an autoimmune response which stimulates fibroblasts localized within the extraocular muscle or dermis, causing glycosaminoglycan release and edema (Ludgate and Baker, 2002; Smith, 2005). Supporting this, the extraocular muscles and dermis are infiltrated by activated T cells and local cytokine production (IFN-γ, IL-1, and TNF) can be demonstrated; these cytokines stimulate glycosaminoglycan synthesis by fibroblasts *in vitro* (Figures 35.7 and 35.8). Orbital antibodies have been repeatedly sought in TAO with no consensus and the nature of the T-cell autoantigen is unknown although the TSH-R is a strong candidate. Treatment of TAO usually consists of supportive measures or corticosteroids

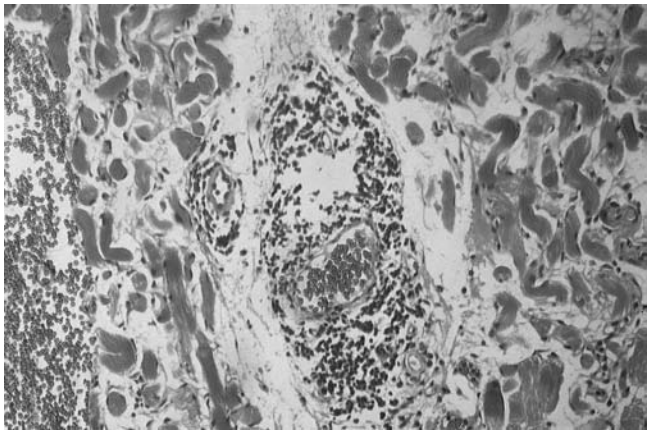


FIGURE 35.7 Photomicrograph of an extraocular muscle from a patient with thyroid-associated ophthalmopathy. There is an extensive lymphocytic infiltrate and edema; the muscle fibers are intact. (Original magnification: ×200.) See color plate section.

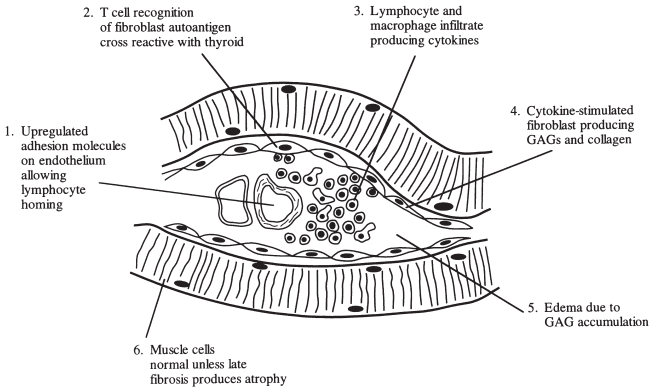


FIGURE 35.8 Pathogenic mechanisms in thyroid-associated ophthalmopathy. From Weetman, A.P. (1999) Thyroid associated ophthalmopathy and dermopathy. In R.Volpe (ed.), *Autoimmune Endocrinopathies* (pp. 245–270). New Jersey: Humana Press Inc., with permission.

and in many the condition spontaneously becomes stable or regresses (Bartalena et al., 2000).

CONCLUDING REMARKS—FUTURE PROSPECTS

Graves' disease shares many features with AT and what determines the type of disorder is a critical issue. So far genetic susceptibility factors have proven nonspecific but more detailed analysis may reveal specific genotypes for Graves' disease. Future studies will clarify the relative importance of stress, iodine intake, infection, and hormonal alterations. It will be particularly important to study mechanisms at the earliest possible stage of disease so that an initiating rather than an enhancing role for these factors can be assessed. Experiments of nature, such as the appearance of Graves' disease after immunologic treatments, may provide fresh insights into regulatory mechanisms and the early phase of disease.

The development of a robust animal model with which the initiation and progression of disease can be analyzed would be a major step forward. Such a model would be especially welcome if TAO also occurred simultaneously and would help clarify the role of TSH-R as an autoantigen in TAO. Our current therapies for Graves' disease are not ideal, with many patients having recurrences or exchanging hyperthyroidism for permanent hypothyroidism. Immunologically directed treatment offers considerable potential advantages, but will need to be specific and innocuous. Such a strategy is even more appealing in TAO for which current treatment is unsatisfactory.

References

Adams, D.D. and Purves, H.D. (1956). Abnormal responses in the assay of thyrotrophin. *Proc. Univ. Otago Med. School 34,* 11–12.

Aichinger, G., Fill, H. and Wick, G. (1985). *In situ* immune complexes, lymphocyte subpopulations, and HLA-DR-positive epithelial cells in Hashimoto's thyroiditis. *Lab. Invest. 52*, 132–140.

Ajjan, R.A., Watson, P.F., McIntosh, R.S. and Weetman, A.P. (1996). Intrathyroidal cytokine gene expression in Hashimoto's thyroiditis. *Clin. Exper. Immunol. 105*, 523–528.

Ajjan, R.A., Kemp, E.H., Waterman, E.A., Watson, P.F., Endo, T., Onaya, T. and Weetman, A.P. (2000). Detection of binding and blocking autoantibodies to the human sodium-iodide symporter in patients with autoimmune thyroid disease. *J. Clin. Endocrinol. Metab.* 85, 2020–2027.

Ando, T. and Davies, T.F. (2003). Postpartum autoimmune thyroid disease: the potential role of fetal microchimerism. *J. Clin. Endocrinol. Metab. 88*, 2965–2971.

Ando, T., Imaizumi, M., Graves, P., Unger, P. and Davies, T.F. (2003). Induction of thyroid-stimulating hormone receptor autoimmunity in hamsters. *Endocrinology 144*, 671–680.

Ando, T., Latif, R., Daniel, S., Eguchi, K and Davies, T.F. (2004). Dissecting linear and conformational epitopes on the native thyrotropin receptor. *Endocrinology 145*, 5185–5193.

Ansar-Ahmed, S., Young, P.R. and Penhale, W.J. (1983). The effects of female sex steroids on the development of autoimmune thyroiditis in thymectomized and irradiated rats. *Clin. Exper. Immunol. 54*, 351–358.

Arikawa, K., Ichikawa, Y., Yoshida, T., Shinozawa, T., Homma, M., Momotani, N. and Ito, K. (1985). Blocking type antithyrotropin receptor antibody in patients with nongoitrous hypothyroidism: its incidence and characteristics of action. *J. Clin. Endocrinol. Metab. 60,* 953–959.

Armengol, M.P., Cardoso-Schmidt, C.B., Fernandez, M., Ferrer, X., Pujol-Borrell, R. and Juan, M. (2003). Chemokines determine local lymphoneogenesis and a reduction of circulating CXCR4+ T and CCR7 B and T lymphocytes in thyroid autoimmune diseases. *J. Immunol. 170*, 6320–6328.

Bagchi, N., Brown, T.R. and Sundick, R.S. (1995). Thyroid cell injury is an initial event in the induction of autoimmune thyroiditis by iodine in obese strain chickens. *Endocrinology 136,* 5054–5060.

Bartalena, L., Bogazzi, F., Tanda, M.L., Manetti, L., Dell-Unto, E. and Martino, E. (1995). Cigarette smoking and the thyroid. *Eur. J. Endocrinol. 133*, 507–512.

Bartalena, L., Pinchera, A. and Marcocci, C. (2000). Management of Graves' ophthalmopathy: reality and perspectives. *Endocr. Rev. 21*, 168–199.

Blanchin, S., Estienne, V., Durand-Gorde, J.M., Carayon, P. and Ruf, J. (2003). Complement activation by direct C4 binding to thyroperoxidase in Hashimoto's thyroiditis. *Endocrinology 144*, 5422–5429.

Bogner, U., Hegedus, L., Hansen, J.M., Finke, R. and Schleusener, H. (1995). Thyroid cytotoxic antibodies in atrophic and goitrous autoimmune thyroiditis. *Eur. J. Endocrinol. 132*, 69–74.

Bottazzo, G.F., Pujol-Borrell, R., Hanafusa, T. and Feldmann, M. (1983). Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet 2*, 1115–1119.

Bretz, J.D. and Baker, J.R. (2001). Apoptosis and autoimmune thyroid disease: following a TRAIL to thyroid destruction? *Clin. Endocrinol. 55*, 1–11.

Brix, T.H., Kyvik, K.O. and Hegedus, L. (1998). What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid 8*, 727–734.

Brix, T.H., Kyvik, K.O. and Hegedus, L. (2000). A population-based study of chronic autoimmune hypothyroidism in Danish twins. *J. Clin. Endocrinol. Metab. 85*, 536–539.

Brix, T.H., Hansen, P.S., Kyvik, K.O. and Hegedus, L. (2004). Aggregation of thyroid autoantibodies in first-degree relatives of patients with autoimmune thyroid disease is mainly due to genes: a twin study. *Clin. Endocrinol. 60*, 329–334.

Butscher, W.G., Ladenson, P.W. and Burek, C.L. (2001). Whole-blood proliferation assay for autoimmune thyroid disease: comparison to density-gradient separated-peripheral blood lymphocytes. *Thyroid 11*, 531–537.

Carella, C., Mazziotti, G., Morisco, F., Manganella, G., Rotondi, M., Tuccillo, C., Sorvillo, F., Caporaso, N. and Amato, G. (2001). Long-term outcome of interferon-alpha-induced thyroid autoimmunity and prognostic influence of thyroid autoantibody pattern at the end of treatment. *J. Clin. Endocrinol. Metab. 86*, 1925–1929.

Chen, C.R., Pichurin, P., Nagayama, Y., Latrofa, F., Rapoport, B. and McLachlan, S.M. (2003). The thyrotropin receptor autoantigen in Graves' disease is the culprit as well as the victim. *J. Clin. Invest. 111*, 1897–1904.

Chiovato, L., Bassi, P., Santini, F., Mammoli, C., Lapi, P., Carayon, P. and Pinchera, A. (1993). Antibodies producing complement-mediated thyroid cytotoxicity in patients with atrophic or goitrous autoimmune thyroiditis. *J. Clin. Endocrinol. Metab. 77*, 1700–1705.

Chiovato, L., Latrofa, F., Braverman, L.E., Pacini, F., Capezzone, M., Masserini, L., Grasso, L. and Pinchera, A. (2003). Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann. Intern. Med. 139*, 346–351.

Cho, B.Y., Kim, W.B., Chung, J.H., Yi, K.H., Shong, Y.K., Lee, H.K. and Koh, C.S. (1995). High prevalence and little change in TSH receptor blocking antibody titres with thyroxine and antithyroid drug therapy in patients with non-goitrous autoimmune thyroiditis. *Clin. Endocrinol. 43*, 465–471.

Cohen, S.B., Dijkstra, C.D. and Weetman, A.P. (1988). Sequential analysis of experimental autoimmune thyroiditis induced by neonatal thymectomy in the Buffalo strain rat. *Cell. Immunol. 114,* 126–136.

Coles, A.J., Wing, M., Smith, S., Coraddu, F., Greer, S., Taylor, C., Weetman, A., Hale, G., Chatterjee, V.K., Waldmann, H. and Compston, A. (1999). Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet 354*, 1691–1695.

Collins, J.E., Heward, J.E., Howson, J.M.M., Foxall, H., Carr-Smith, J., Franklyn, J.A. and Gough, S.C.L. (2004). Common allelic variants of exons 10, 12, and 33 of the thyroglobulin gene are not associated with autoimmune thyroid disease in the United Kingdom. *J. Clin. Endocrinol. Metab. 89*, 6336–6339.

Costagliola, S., Many, M.C., Stalmans-Falys, M., Tonacchera, M., Vassart, G. and Ludgate, M. (1994). Recombinant thyrotropin receptor and the induction of autoimmune thyroid disease in BALB/c mice: a new animal model. *Endocrinology 135*, 2150–2159.

Costagliola, S., Rodien, P., Many, M.C., Ludgate, M. and Vassart, G. (1998). Genetic immunization against the human thyrotropin receptor causes thyroiditis and allows production of monoclonal antibodies recognizing the native receptor. *J. Immunol. 160*, 1458–1465.

Costagliola, S., Morgenthaler, N.G., Hoermann, R., Badenhoop, K., Struck, J., Freitag, D., Poertl, S., Weglohner, W., Hollidt, J.M., Quadbeck, B., Dumont, J.E., Schumm-Draeger, P.M., Bergmann, A., Mann, K., Vassart, G. and Usadel, K.H. (1999). Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. *J. Clin. Endocrinol. Metab. 84*, 90–97.

Creemers, P., Rose, N.R. and Kong, Y.M. (1983). Experimental autoimmune thyroiditis. *In vitro* cytotoxic effects of T lymphocytes on thyroid monolayers. *J. Exper. Med. 157*, 559–571.

Crisa, L., Mordes, J.P. and Rossini, A.A. (1992). Autoimmune diabetes mellitus in the BB rat. *Diabetes/Metab. Rev. 8*, 4–37.

Davies, T.F., Roti, E., Braverman, L.E. and DeGroot, L.J. (1998). Thyroid controversy – stimulating antibodies. *J. Clin. Endocrinol. Metab. 83*, 3777–3785.

Dayan, C.M., Chu, N.R., Londei, M., Rapoport, B. and Feldmann, M. (1993). T-cells involved in human autoimmune disease are resistant to tolerance induction. *J. Immunol. 151*, 1606–1613.

De Assis-Paiva, H.J., Rayner, D.C., Roitt, I.M. and Cooke, A. (1989). Cellular infiltration in induced rat thyroiditis: phenotypic analysis and relationship to genetic restriction. *Clin. Exper. Immunol. 75,* 106–112.

De Bruin, T.W., Patwardhan, N.A., Brown, R.S. and Braverman, L.E. (1988). Graves' disease: changes in TSH receptor and anti-microsomal antibodies after thyroidectomy. *Clin. Exper. Immunol. 72*, 481–485.

Di Cerbo, A., Di Paola, R., Bonati, M., Zingrillo, M., De Filippis, V. and Corda, D. (1995). Subgroups of Graves' patients identified on the basis of the biochemical activities of their immunoglobulins. *J. Clin. Endocrinol. Metab. 80*, 2785–2790.

Drexhage, H.A. (1996). Autoimmunity and thyroid growth. Where do we stand? *Eur. J. Endocrinol. 135*, 39–45.

Duthoit, C., Estienne, V., Delom, F., Durand-Gorde, J.M., Mallet, B., Carayon, P. and Ruf, J. (2000). Production of immunoreactive thyroglobulin C-terminal fragments during thyroid hormone synthesis. *Endocrinology 141*, 2518–2525.

ElRehewy, M., Kong, Y.M., Giraldo, A.A. and Rose, N.R. (1981). Syngeneic thyroglobulin is immunogenic in good responder mice. *Eur. J. Immunol. 11*, 146–151.

Elsheikh, M., Wass, J.A. and Conway, G.S. (2001). Autoimmune thyroid syndrome in women with Turner's syndrome – the association with karyotype. *Clin. Endocrinol. 55*, 223–226.

Gilquin, J., Viard, J.P., Jubault, V., Sert, C. and Kazatchkine, M.D. (1998). Delayed occurrence of Graves' disease after immune restoration with HAART. Highly active antiretroviral therapy. *Lancet 352*, 1907–1908.

Giordano, C., Stassi, G., De-Maria, R., Todaro, M., Richiusa, P., Papoff, G., Ruberti, G., Bagnasco, M., Testi, R. and Galluzzo, A. (1997). Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science 275*, 960–963.

Gora, M., Gardas, A., Wiktorowicz, W., Hobby, P., Watson, P.F., Weetman, A.P., Sutton, B.J. and Banga, J.P. (2004). Evaluation of conformational epitopes on thyroid peroxidase by antipeptide antibody binding and mutagenesis. *Clin. Exper. Immunol. 136*, 137–144.

Grubeck-Loebenstein, B., Turner, M., Pirich, K., Kassal, H., Londei, M., Waldhausl, W. and Feldmann, M. (1990). CD4+ T-cell clones from autoimmune thyroid tissue cannot be classified according to their lymphokine production. *Scand. J. Immunol. 32*, 433–440.

Hamilton, F., Black, M., Farquharson, M.A., Stewart, C. and Foulis, A.K. (1991). Spatial correlation between thyroid epithelial cells expressing class II MHC molecules and interferon-gamma-containing lymphocytes in human thyroid autoimmune disease. *Clin. Exper. Immunol. 83*, 64–68.

Hidaka, Y., Amino, N., Iwatani, Y., Itoh, E., Matsunaga, M. and Tamaki, H. (1993). Recurrence of thyrotoxicosis after attack of allergic rhinitis in patients with Graves' disease. *J. Clin. Endocrinol. Metab. 77,* 1667–1670.

Iitaka, M., Momotani, N., Hisaoka, T., Noh, J.Y., Ishikawa, N., Ishii, J., Katayama, S. and Ito, K. (1998). TSH receptor antoibody-associayed thyroid dysfunction following subacute thyroiditis. *Clin. Endocrinol. 48*, 445–453.

Iwatani, Y., Amino, N., Hidaka, Y., Kaneda, T., Ichihara, K., Tamaki, H., Matsuzuka, F., Fukata, S., Kuma, K. and Miyai, K. (1992). Decreases in alpha beta T-cell receptor negative T-cells and CD8 cells, and an increase in CD4+ CD8+ cells in active Hashimoto's disease and subacute thyroiditis. *Clin. Exper. Immunol. 87*, 444–449.

Jansson, R., Karlsson, A. and Forsum, U. (1984). Intrathyroidal HLA-DR expression and T lymphocyte phenotypes in Graves' thyrotoxicosis, Hashimoto's thyroiditis and nodular colloid goitre. *Clin. Exper. Immunol. 58*, 264–272.

Jansson, R., Dahlberg, P.A., Winsa, B., Meirik, O., Safwenberg, J. and Karlsson, A. (1987). The postpartum period constitutes an important risk for the development of clinical Graves' disease in young women. *Acta Endocrinol. 116*, 321–325.

Jaume, J.C., Guo, J., Pauls, D.L., Zakarija, M., McKenzie, J.M., Egeland, J.A., Burek, C.L., Rose, N.R., Hoffman, W.H., Rapoport, B. and McLachlan, S.M. (1999). Evidence for genetic transmission of thyroid peroxidase autoantibody epitopic "fingerprints." *J. Clin. Endocrinol. Metab. 84*, 1424–1431.

Kim, T.Y., Park, Y.J., Hwang, J.K., Song, J.Y., Park, K.S., Cho, B.Y. and Park, D.O.J. (2003). A C/T polymorphism in the 5′-untranslated region of the CD40 gene is associated with Graves' disease in Koreans. *Thyroid 13*, 919–925.

Kojima, A., Tanaka-Kojima, Y., Sakakura, T. and Nishizuka, Y. (1976). Spontaneous development of autoimmune thyroiditis in neonatally thymectomized mice. *Lab. Invest. 34*, 550–557.

Komiya, I., Yamada, T., Sato, A., Kouki, T., Nishimori, T. and Takasu, N. (2001). Remission and recurrence of hyperthyroid Graves' disease during and after methimazole treatment when assessed by IgE and interleukin 13. *J. Clin. Endocrinol. Metab. 86*, 3540–3544.

Kong, Y.C., Lomo, L.C., Motte, R.W., Giraldo, A.A., Baisch, J., Strauss, G., Hammerling, G.J. and David, C.S. (1996). HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis in transgenic mice: definitive association with HLA-DRB1*0301 (DR3) gene. *J. Exper. Med. 184*, 1167–1172.

Kong, Y.M., Giraldo, A.A., Waldmann, H., Cobbold, S.P. and Fuller, B.E. (1989). Resistance to experimental autoimmune thyroiditis: L3T4+ cells as mediators of both thyroglobulin-activated and TSH-induced suppression. *Clin. Immunol. Immunopathol. 51*, 38–54.

Kotsa, K., Watson, P.F. and Weetman, A.P. (1997). A CTLA-4 gene polymorphism is associated with both Graves' disease and autoimmune hypothyroidism. *Clin. Endocrinol. 46*, 551–554.

Kraiem, Z., Lahat, N., Glaser, B., Baron, E., Sadeh, O. and Sheinfeld, M. (1987). Thyrotrophin receptor blocking antibodies: incidence, characterization and in-vitro synthesis. *Clin. Endocrinol. 27*, 409–421.

Kretowski, A., Wawrusiewicz, N., Mironczuk, K., Mysliwiec, J., Kretowska, M. and Kinalska, I. (2003). Intercellular adhesion molecule 1 gene polymorphisms in Graves' disease. *J. Clin. Endocrinol. Metab. 88*, 4945–4949.

Laurberg, P., Nygaard, B., Glinoer, D., Grussendorf, M. and Orgiazzi, J. (1998). Guidelines for TSH-receptor antibody measurements in pregnancy: results of an evidence-based symposium organized by the European Thyroid Association. *Eur. J. Endocrinol. 139*, 584–586.

Londei, M., Bottazzo, G.F. and Feldmann, M. (1985). Human T-cell clones from autoimmune thyroid glands: specific recognition of autologous thyroid cells. *Science 228*, 85–89.

Ludgate, M. and Baker, G. (2002). Unlocking the immunological mechanisms of orbital inflammation in thyroid eye disease. *Clin. Exper. Immunol. 127*, 193–198.

Ludgate, M.E. and Vassart, G. (1995). The thyrotropin receptor as a model to illustrate receptor and receptor antibody diseases. *Baillière's Clin. Endocrinol. Metab. 9*, 95–113.

MacKenzie, W.A., Schwartz, A.E., Friedman, E.W. and Davies, T.F. (1987). Intrathyroidal T-cell clones from patients with autoimmune thyroid disease. *J. Clin. Endocrinol. Metab. 64*, 818–824.

Mandrup-Poulsen, T., Nerup, J., Reimers, J.I., Pociot, F., Andersen, H.U., Karlsen, A., Bjerre, U. and Bergholdt, R. (1996). Cytokines and the endocrine system. II. Roles in substrate metabolism, modulation of thyroidal and pancreatic endocrine cell functions and autoimmune endocrine diseases. *Eur. J. Endocrinol. 134*, 21–30.

Many, M.C., Costagliola, S., Detrait, M., Denef, F., Vassart, G. and Ludgate, M.C. (1999). Development of an animal model of autoimmune thyroid eye disease. *J. Immunol. 162*, 4966–4974.

Marazuela, M. (1999). Lymphocyte traffic and homing in autoimmune thyroid disorders. *Eur. J. Endocrinol. 140,* 287–290.

Marelli-Berg, F.M., Weetman, A., Frasca, L., Deacock, S.J., Imami, N., Lombardi, G. and Lechler, R.I. (1997). Antigen presentation by epithelial cells induces anergic immunoregulatory CD45RO+ T-cells and deletion of CD45RA+ T-cells. *J. Immunol. 159*, 5853–5861.

Martin, A. and Davies, T.F. (1992). T-cells and human autoimmune thyroid disease: emerging data show lack of need to invoke suppressor T-cell problems. *Thyroid 2,* 247–261.

Matos-Santos, A., Nobre, E.L., Costa, J.G., Nogueira, P.J., Macedo, A., Galvao-Teles, A. and de Castro, J.J. (2001). Relationship between the number and impact of stressful life events and the onset of Graves' disease and toxic nodular goitre. *Clin. Endocrinol. 55*, 15–19.

Matsuura, N., Yamada, Y., Nohara, Y., Konishi, J., Kasagi, K., Endo, K., Kojima, H. and Wataya, K. (1980). Familial neonatal transient hypothyroidism due to maternal TSH-binding inhibitor immunoglobulins. *N. Engl. J. Med. 303*, 738–741.

McIntosh, R.S., Watson, P.F. and Weetman, A.P. (1997). Analysis of the T-cell receptor V alpha repertoire in Hashimoto's thyroiditis: evidence for the restricted accumulation of CD8+ T-cells in the absence of CD4+ T-cell restriction. *J. Clin. Endocrinol. Metab. 82*, 1140–1146.

McIntosh, R., Watson, P. and Weetman, A. (1998). Somatic hypermutation in autoimmune thyroid disease. *Immunol. Rev. 162*, 219–231.

McLachlan, S.M. and Rapoport, B. (1992). The molecular biology of thyroid peroxidase: cloning, expression and role as autoantigen in autoimmune thyroid disease. *Endocr. Rev. 13*, 192–206.

McLachlan, S.M., Taverne, J., Atherton, M.C., Cooke, A., Middleton, S., Pegg, C.A., Clark, F. and Rees Smith, B. (1990). Cytokines, thyroid autoantibody synthesis and thyroid cell survival in culture. *Clin. Exper. Immunol. 79*, 175–181.

Metcalfe, R.A., Oh, Y.S., Stroud, C., Arnold, K. and Weetman, A.P. (1997). Analysis of antibody-dependent cell-mediated cytotoxicity in autoimmune thyroid disease. *Autoimmunity 25*, 65–72.

Miguel, R.N., Sanders, J., Jeffreys, J., Delpraetere, H., Evans, M., Richards, T., Blundell, T.L., Rees Smith, B. and Furmaniak, J. (2004). Analysis of the thyrotropin receptor-thyrotropin interaction by comparative modelling. *Thyroid 14*, 991–1011.

Muller, A.F., Drexhage, H.A. and Berghout, A. (2001). Postpartum thyroiditis and autoimmune thyroiditis in women of childbearing age: recent insights and consequences for antenatal and postnatal care. *Endocr. Rev. 22*, 605–630.

Ng, H.P., Banga, J.P. and Kung, A.W. (2004). Development of a murine model of autoimmune thyroiditis induced with homologous mouse thyroid peroxidase. *Endocrinology 145*, 809–816.

Nilsson, M., Husmark, J., Bjorkman, U. and Ericson, L.E. (1998). Cytokines and thyroid epithelial integrity: interleukin-1alpha induces

dissociation of the junctional complex and paracellular leakage in filter-cultured human thyrocytes. *J. Clin. Endocrinol. Metab. 83*, 945–952.

Noble, B., Yoshida, T., Rose, N.R. and Bigazzi, P.E. (1976). Thyroid antibodies in spontaneous autoimmune thyroiditis in the Buffalo rat. *J. Immunol. 117*, 1447–1455.

Okayasu, I., Kong, Y.M. and Rose, N.R. (1981). Effect of castration and sex hormones on experimental autoimmune thyroiditis. *Clin. Immunol. Immunopathol. 20*, 240–245.

Okumura, M., Hidaka, Y., Matsuzuka, F., Takeoka, K., Tada, H., Kuma, K. and Amino, N. (1999). CD30 expression and interleukin-4 and interferon-gamma production of intrathyroidal lymphocytes in Graves' disease. *Thyroid 9*, 333–339.

Othman, S., Phillips, D.I., Parkes, A.B., Richards, C.J., Harris, B., Fung, H., Darke, C., John, R., Hall, R. and Lazarus, J.H. (1990). A long-term follow-up of postpartum thyroiditis. *Clin. Endocrinol. 32*, 559–564.

Pacini, F., Vorontsova, T., Molinaro, E., Kuchinskaya, E., Agate, L., Shavrova, E., Astachova, L., Chiovato, L. and Pinchera, A. (1998). Prevalence of thyroid autoantibodies in children and adolescents from Belarus exposed to the Chernobyl radioactive fallout. *Lancet 352*, 763–766.

Pearce, E.N., Farwell, A.P. and Braverman, L.E. (2003). Thyroiditis. *N. Engl. J. Med. 348*, 2646–2655.

Penhale, W.J. and Young, P.R. (1988). The influence of the normal microbial flora on the susceptibility of rats to experimental autoimmune thyroiditis. *Clin. Exper. Immunol. 72*, 288–292.

Penhale, W.J., Farmer, A., McKenna, R.P. and Irvine, W.J. (1973). Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. *Clin. Exper. Immunol. 15*, 225–236.

Pfaltz, M. and Hedinger, C.E. (1986). Abnormal basement membrane structures in autoimmune thyroid disease. *Lab. Invest. 55*, 531–539.

Phillips, D.I., Osmond, C., Baird, J., Huckle, A. and Rees Smith, B. (2002). Is birthweight associated with thyroid autoimmunity? A study in twins. *Thyroid 12*, 377–380.

Pontes de Carvalho, L.C., Wick, G. and Roitt, I.M. (1981). Requirement of T-cells for the development of spontaneous autoimmune thyroiditis in obese strain chickens. *J. Immunol. 126*, 750–753.

Quaratino, S., Badami, E., Pang, Y.Y., Bartok, I., Dyson, J., Kioussis, D., Londei, M. and Miauri, L. (2004). Degenerate self-reactive human T-cell receptor causes spontaneous autoimmune disease in mice. *Nat. Med. 10*, 920–926.

Raber, W., Gessl, A., Nowotny, P. and Vierhapper, H. (2002). Thyroid ultrasound versus antithyroid peroxidase antibody determination: a cohort study of four hundred fifty-one subjects. *Thyroid 12*, 725–731.

Rapoport, B., Chazenbalk, G.D., Jaume, J.C. and McLachlan, S.M. (1998). The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. *Endocr. Rev. 19*, 673–716.

Reinwein, D., Benker, G., Konig, M.P., Pinchera, A., Schatz, H. and Schleusener, H. (1986). Hyperthyroidism in Europe: clinical and laboratory data of a prospective multicentric survey. *J. Endocrinol. Invest. 9* (Suppl 2), 1–36.

Rennie, D.P., McGregor, A.M., Keast, D., Weetman, A.P., Foord, S.M., Dieguez, C., Williams, E.D. and Hall, R. (1983). The influence of methimazole on thyroglobulin-induced autoimmune thyroiditis in the rat. *Endocrinology 112*, 326–330.

Roberts, C.G. and Ladenson, P.W. (2004). Hypothyroidism. *Lancet 363*, 793–803.

Roitt, I.M., Doniach, D., Campbell, P.N., and Vaughn Hudson, R. (1956). Autoantibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet*, *ii*, 820–821.

Rose, N.R. and Witebsky, E. (1956). Studies in organ specificity. V changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *J. Immunol. 76*, 417–427.

Ruwhof, C. and Drexhage, H.A. (2001). Iodine and thyroid autoimmune disease in animal models. *Thyroid 11*, 427–436.

Saboori, A.M., Rose, N.R., Yuhasz, S.C., Amzel, L.M. and Burek, C.L. (1999). Peptides of human thyroglobulin reactive with sera of patients with autoimmune thyroid disease. *J. Immunol. 163*, 6244–6250.

Sakaguchi, S. and Sakaguchi, N. (1989). Organ-specific autoimmune disease induced in mice by elimination of T-cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. *J. Immunol. 142*, 471–480.

Sakaguchi, S., Sakaguchi, N., Shimizu, J., Yamazaki, S., Sakihama, T., Itoh, M., Kuniyasu, Y., Nomura, T., Toda, M. and Takahashi, T. (2001). Immunologic tolerance maintained by CD25+ CD4+ regulatory T-cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev. 182*, 18–32.

Sanders, J., Evans, M., Premawardhana, L.D., Depraetere, H., Jeffreys, J., Richards, T., Furmaniak, J. and Rees-Smith, B. (2003). Human monoclonal thyroid stimulating autoantibody. *Lancet 362*, 126–128.

Schwartz, K.M., Fatourechi, V., Ahmed, D.D. and Pond, G.R. (2002). Dermopathy of Graves' disease (pretibial myxedema): long-term outcome. *J. Clin. Endocrinol. Metab. 87*, 438–446.

Schwarz-Lauer, L., Chazenbalk, G.D., McLachlan, S.M., Ochi, Y., Nagayama, Y. and Rapoport, B. (2002). Evidence for a simplified view of autoantibody interactions with the thyrotropin receptor. *Thyroid 12*, 115–120.

Sempowski, G.D., Cross, S.J., Heinly, C.S., Scearce, R.M. and Haynes, B.F. (2004). CD7 and CD28 are required for murine CD4+CD25+ regulatory T-cell homeostasis and prevention of thyroiditis. *J. Immunol. 172*, 787–794.

Smith, T.J. (2005). Insights into the role of fibroblasts in human autoimmune diseases. *Clin. Exper. Immunol. 141*, 388–397.

Stassi, G., Todaro, M., Bucchieri, F., Stoppacciaro, A., Farina, F., Zummo, G., Testi, R. and De Maria, R. (1999). Fas/Fas ligand-driven T-cell apoptosis as a consequence of ineffective thyroid immunoprivilege in Hashimoto's thyroiditis. *J. Immunol. 162*, 263–267.

Steel, N.R., Weightman, D.R., Taylor, J.J. and Kendall-Taylor, P. (1984). Blocking activity to action of thyroid stimulating hormone in serum from patients with primary hypothyroidism. *Br. Med. J. 288,* 1559–1562.

Stenszky, V., Kozma, L., Balazs, C., Rochlitz, S., Bear, J.C. and Farid, N.R. (1985). The genetics of Graves' disease: HLA and disease susceptibility. *J. Clin. Endocrinol. Metab. 61*, 735–740.

Strieder, T.G., Prummel, M.F., Tijssen, J.G., Endert, E. and Wiersinga, W.M. (2003). Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. *Clin. Endocrinol. 59*, 396–401.

Sugihara, S., Fujiwara, H., Niimi, H. and Shearer, G.M. (1995). Self-thyroid epithelial cell (TEC)-reactive CD8+ T-cell lines/clones derived from autoimmune thyroiditis lesions. They recognize self-thyroid antigens directly on TEC to exhibit T helper cell 1-type lymphokine production and cytotoxicity against TEC. *J. Immunol. 155*, 1619–1628.

Takasu, N., Yamada, T., Takasu, M., Komiya, I., Nagasawa, Y., Asawa, T., Shinoda, T., Aizawa, T. and Koizumi, Y. (1992). Disappearance of thyrotropin-blocking antibodies and spontaneous recovery from hypothyroidism in autoimmune thyroiditis. *N. Engl. J. Med. 326*, 513–518.

Tandon, N., Freeman, M. and Weetman, A.P. (1991). T-cell responses to synthetic thyroid peroxidase peptides in autoimmune thyroid disease. *Clin. Exper. Immunol. 86*, 56–60.

Tandon, N., Freeman, M.A. and Weetman, A.P. (1992). T-cell responses to synthetic TSH receptor peptides in Graves' disease. *Clin. Exper. Immunol. 89*, 468–473.

Tandon, N., Yan, S.L., Morgan, B.P. and Weetman, A.P. (1994). Expression and function of multiple regulators of complement activation in autoimmune thyroid disease. *Immunology 81*, 643–647.

Tomer, Y. and Davies, T.F. (1993). Infection, thyroid disease, and autoimmunity. *Endocr. Rev. 14*, 107–120.

Tomer, Y. and Davies, T.F. (2003). Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocr. Rev. 24*, 694–717.

Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., Rainbow, D.B., Hunter, K.M., Smith, A.N., Di Genova, G., Herr, M.H., Dahlman, I., Payne, F., Smyth, D., Lowe, C., Twells, R.C., Howlett, S., Healy, B., Nutland, S., Rance, H.E., Everett, V., Smink, L.J., Lam, A.C., Cordell, H.J., Walker, N.M., Bordin, C., Hulme, J., Motzo, C., Cucca, F., Hess, J.F., Metzker, M.L., Rogers, J., Gregory, S., Allahabadia, A., Nithiyananthan, R., Tuomilehto-Wolf, E., Tuomilehto, J., Bingley, P., Gillespie, K.M., Undlien, D.E., Ronningen, K.S., Guja, C., Ionescu-TirgoviŸte, C., Savage, D.A., Maxwell, A.P., Carson, D.J., Patterson, C.C., Franklyn, J.A., Clayton, D.G., Peterson, L.B., Wicker, L.S., Todd, J.A. and Gough, S.C. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature 423*, 506–511.

Vanderpump, M.P., Tunbridge, W.M., French, J.M., Appleton, D., Bates, D., Clark, F., Grimley-Evans, J., Hasan, D.M., Rodgers, H. and Tunbridge, F. (1995). The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin. Endocrinol. 43*, 55–68.

Velaga, M.R., Wilson, V., Jennings, C.E., Owen, C.J., Herington,S., Donaldson, P.T., Ball, S.G., James, R.A., Quinton, R., Perros, P., and Pearce, S.H.S. (2004). The codon 620 trytophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. *J. Clin. Endocrinol. Metab. 89*, 5862–5865.

Vladutiu, A.O. and Rose, N.R. (1971). Autoimmune murine thyroiditis relation to histocompatibility (H–2) type. *Science 174*, 1137–1139.

Volpe, R. (1988). The immunoregulatory disturbance in autoimmune thyroid disease. *Autoimmunity 2*, 55–72.

Watson, P.F., Pickerill, A.P., Davies, R. and Weetman, A.P. (1994). Analysis of cytokine gene expression in Graves' disease and multinodular goiter. *J. Clin. Endocrinol. Metab. 79*, 355–360.

Weetman, A.P. (2000). Graves' disease. *N. Engl. J. Med. 343*, 1236–1248.

Weetman, A.P. and McGregor, A.M. (1994). Autoimmune thyroid disease: further developments in our understanding. *Endocr. Rev. 15*, 788–830.

Weetman, A.P., McGregor, A.M., Wheeler, M.H. and Hall, R. (1984). Extrathyroidal sites of autoantibody synthesis in Graves' disease. *Clin. Exper. Immunol. 56*, 330–336.

Weetman, A.P., Cohen, S.B., Oleesky, D.A. and Morgan, B.P. (1989). Terminal complement complexes and C1/C1 inhibitor complexes in autoimmune thyroid disease. *Clin. Exper. Immunol. 77*, 25–30.

Weetman, A.P., Yateman, M.E., Ealey, P.A., Black, C.M., Reimer, C.B., Williams, R.C., Shine, B. and Marshall, N.J. (1990). Thyroid-stimulating antibody activity between different immunoglobulin G subclasses. *J. Clin. Invest. 86*, 723–727.

Weetman, A.P., Tandon, N. and Morgan, B.P. (1992). Antithyroid drugs and release of inflammatory mediators by complement-attacked thyroid cells. *Lancet 340*, 633–636.

Wick, G., Brezinschek, H.P., Hala, K., Dietrich, H., Wolf, H. and Kroemer, G. (1989). The obese strain of chickens: an animal model with spontaneous autoimmune thyroiditis. *Adv. Immunol. 47*, 433–500.

Williams, R.C., Marshall, N.J., Kilpatrick, K., Montano, J., Brickell, P.M., Goodall, M., Ealey, P.A., Shine, B., Weetman, A.P. and Craig, R.K. (1988). Kappa/lambda immunoglobulin distribution in Graves' thyroid-stimulating antibodies. Simultaneous analysis of C lambda gene polymorphisms. *J. Clin. Invest. 82*, 1306–1312.

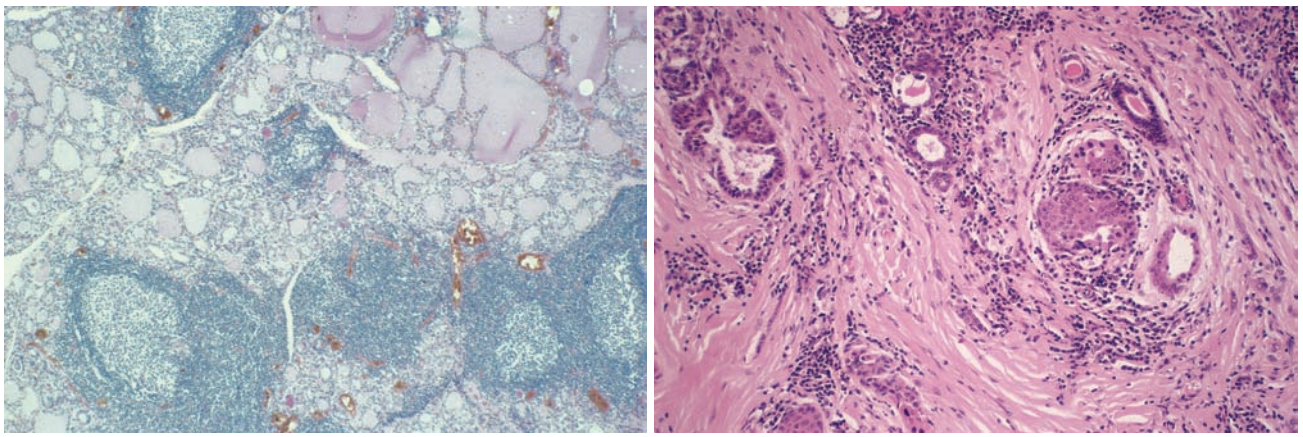
Winsa, B., Adami, H.O., Bergstrom, R., Gamstedt, A., Dahlberg, P.A., Adamson, U., Jansson, R. and Karlsson, A. (1991). Stressful life events and Graves' disease. *Lancet 338*, 1475–1479.

Wu, Z., Podack, E.R., McKenzie, J.M., Olsen, K.J. and Zakarija, M. (1994). Perforin expression by thyroid-infiltrating T-cells in autoimmune thyroid disease. *Clin. Exper. Immunol. 98,* 470–477.

Yamaguchi, K., Shimojo, N., Kikuoka, S., Hoshioka, A., Hirai, A., Tahara, K., Kohn, L.D., Kohno, Y. and Niimi, H. (1997). Genetic control of anti-thyrotropin receptor antibody generation in H-2K mice immunized with thyrotropin receptor-transfected fibroblasts. *J. Clin. Endocrinol. Metab. 82*, 4266–4269.

Yanagawa, T., Hidaka, Y., Guimaraes, V., Soliman, M. and DeGroot, L.J. (1995). CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J. Clin. Endocrinol. Metab. 80*, 41–45.

Zakarija, M. (1983). Immunochemical characterization of the thyroid-stimulating antibody (TSAb) of Graves' disease: evidence for restricted heterogeneity. *J. Clin. Lab. Immunol. 10*, 77–85.



A B

FIGURE 35.1 Pathology of autoimmune hypothyroidism. *A,* Hashimoto's thyroiditis showing germinal center formation. *B,* Primary myxedema with extensive fibrosis. (Original magnification: ×100.) Courtesy of Dr Judith Channer, Northern General Hospital, Sheffield.

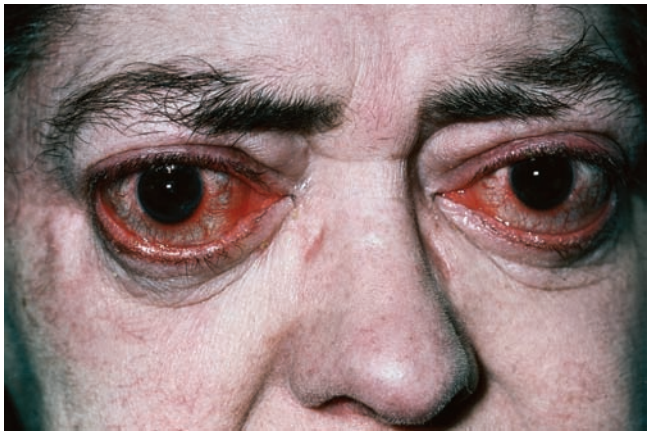


FIGURE 35.5 Eye signs in a patient with thyroid-associated ophthalmopathy showing exophthalmos, scleral injection, and periorbital edema.

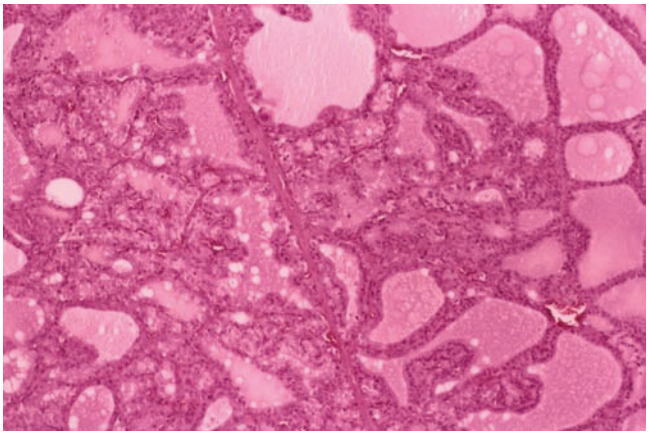


FIGURE 35.6 Pathology of Graves' disease showing columnar and folded thyroid epithelium, small new follicles, and active colloid resorption, with "scalloping" of the colloid. A lymphocytic infiltrate is not prominent in this specimen. (Original magnification: ×100.) Courtesy of Dr Judith Channer, Northern General Hospital, Sheffield.

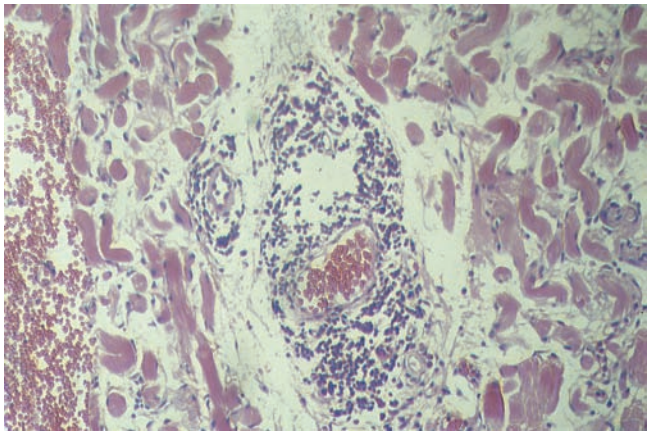


FIGURE 35.7 Photomicrograph of an extraocular muscle from a patient with thyroid-associated ophthalmopathy. There is an extensive lymphocytic infiltrate and edema; the muscle fibers are intact. (Original magnification: ×200.)

CHAPTER

36

Type 1 Diabetes

JEAN-FRANÇOIS BACH

Immunologie Clinique Hôpital Necker, France

It was only in the 1950s that a clear distinction was made between insulin-dependent diabetes mellitus (IDDM), now called type 1 diabetes (T1D), and non-insulin dependent diabetes mellitus (NIDDM), now called type 2 diabetes (T2D).

The major discovery of the autoimmune origin of T1D was derived in large part from the availability of two spontaneous animal models of the disease, the nonobese diabetic (NOD) mouse and the Bio-Breeding (BB) rat.

In the years which have followed these major milestones, a considerable number of immunologic, genetic, and clinical studies have been performed which allow us to provide a comprehensive description of disease pathogenesis and to a lesser extent its etiology. This knowledge is central for the understanding of the natural history of the disease. It is also crucial to devise immune-based strategies which could substitute immunotherapy for the presently used palliative insulin therapy with its limitations.

The aim of this chapter is to review the main questions mentioned above, without going into the details of each aspect which can be found in more extensive or more specialized texts (Bach, 1994; Yang and Santamaria, 2003).

CLINICAL SPECTRUM AND EPIDEMIOLOGY

Disease Presentation

In most cases, T1D appears suddenly in previously apparently healthy individuals. The first clinical manifestations include polyuria, polydipsia and, if the disease is not recognized early on, weight loss. In some patients, in the absence of insulin therapy, ketosis appears with nausea, vomiting, and dehydration, rapidly leading to coma. When insulin

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

treatment is initiated, all these symptoms rapidly disappear. They may reappear, though, in the case of insufficient insulin dosage. Excessive dosage leads to hypoglycemia with sweating, malaise, and eventually coma. Once insulin dependency appears, it is usually definitive, even if in a few cases it may regress or disappear for a few months ("honeymoon"). This clinical remission is explained by the β cell rest afforded by intensive insulin therapy (Shah et al., 1989).

After a few years of disease, degenerative complications may appear with a risk directly related to the quality of metabolic control which in turn depends on the rigor of insulin therapy. Aggressive insulin therapy to the limit of hypoglycemia has been clearly shown to reduce the incidence and the severity of degenerative complications (DCCT, 1993). These complications include nephropathy leading to renal failure, retinopathy with possible blindness, neuropathy, and trophic complications (gangrene) which may be sufficiently severe to require amputation. Diabetic patients are also exposed to an increased risk of atherosclerosis-linked complications, heart involvement, and vascular cerebral accidents.

T1D may develop more progressively, preceded by a long phase of diabetes initially classified as T2D. This presentation, named latent autoimmune diabetes of the adult (LADA), is most likely a slowly progressing form of T1D (Pozzilli and Di Mario, 2001). It usually affects adults who present with T2D without excess body weight and who have a family history of T2D. The disease can be recognized by the presence of β cell-specific autoantibodies (Falorni and Calcinaro, 2002). LADA often, although not always, converts to T1D, justifying early insulin treatment when needed (Turner et al., 1997), and perhaps immunotherapy when the strategy becomes available. T1D includes a strong hereditary component. As we shall see, genetic inheritance is usually polygenic. There are exceptions to this rule which include a set of familial (highly hereditary) forms of the disease linked to a single mutation (APECED and IPEX) or to a limited number of predisposing genes.

APECED (Autoimmune Polyendocrinopathy, Candidiasis, Ectodermal Dysplasia) is a rare sex-linked hereditary syndrome including T1D, thyroiditis, parathyroiditis, and mucocutaneous candidiasis. The disease is due to a mutation in a gene coding for a transcription factor called AIRE (Halonen et al., 2004; Peterson et al., 2004) (see Chapter 38). IPEX is a severe autoimmune syndrome associating severe enterocolitis, T1D, and thyroiditis. It affects infants and is lethal without immunosuppressive treatment or bone marrow transplantation, essentially due to the severity of enterocolitis. The disease has recently been associated with a mutation in a gene coding for a transcription factor called FoxP3 involved in the differentiation of $CD4^+$ $CD25^+$ regulatory T cells (Bennett et al., 2001). There is an animal model of this syndrome, the scurfy mouse, with the mutation on the same gene (Wildin et al., 2001) (see Chapter 26). A familial form of T1D has been observed in Bedouins (Verge et al., 1998). Finally, there are the rare forms of T1D that are resistant to insulin therapy and are linked to autoantibodies specific to the insulin receptor (Flier et al., 1975).

Prediabetes

The onset of T1D is preceded by several years of a prediabetic phase where islet-specific autoimmunity has already developed but with insufficient intensity to destroy islet β-cells to a degree which would generate clinically detectable insulin dependency (Gottlieb and Eisenbarth, 1998). There is still debate about the percentage of persistent β cells at the time of clinical onset, a crucial issue when discussing immunotherapy in recently diagnosed diabetes. The autoimmunity-associated prediabetic phase can be defined in animal models by histopathology. In man, this definition relies on the detection of β cell-specific autoantibodies, a definition which is not really satisfactory since such autoantibodies are probably not directly pathogenic, at variance with T cells. Importantly, there is a short period of time during the few months preceding clinical onset when metabolic dysfunction can be detected by decreased C-peptide levels following glucose infusion.

Epidemiology

T1D most commonly starts during childhood but may appear in adulthood in a significant proportion (30–40%) of cases (Karvonen et al., 2001) and even more if one includes LADA. There is an equal sex frequency, except in cases associated with extrapancreatic autoimmunity (Umpierrez et al., 2003) in which females predominate.

The incidence and prevalence of T1D vary according to country. It is increasing in every country with more of a tendency toward earlier onset than in the past, and now the disease affects significant numbers of very young children (Gale, 2002). In the US, T1D patients are probably more than 10^6 and even more again if one includes LADA, the frequency of which is ill defined.

THE AUTOIMMUNE ORIGIN OF TYPE 1 DIABETES

The first suggestions of an autoimmune origin for T1D arose in the 1960s from the clustering of diabetes with other diseases of known autoimmune origin, notably thyroiditis, but the definitive evidence was the discovery of islet β-cell autoantibodies (ICA) by Bottazzo and Doniach (Bottazzo et al., 1974). Sera from T1D patients were shown by indirect immunofluorescence to bind to islets on sections of human

pancreas. Ultimately, several β-cell autoantigens were chemically characterized, notably insulin (Srikanta et al., 1986), glutamic acid decarboxylase (GAD) (Baekkeskov et al., 1990), and IA-2, a tyrosine phosphatase-like molecule (Lan et al., 1996). The presence of islet-specific antibodies in T1D is not constant, even at the onset of overt diabetes, but more than 90% of patients are positive by the ICA assay or one of the assays using chemically defined antigens. Normal sera are usually negative, though a very small percentage (<1%) may show low levels of antibodies.

Intensive research for islet-specific T cells has not provided clear-cut results. There have been multiple reports of islet antigen-induced proliferation of lymphocytes from T1D patients (Scheinin, 1988; Atkinson et al., 1992; Durinovic-Bello et al., 1996). Initially these reports proved difficult to reproduce and the results of international workshops attempting to demonstrate islet-specific T-cell reactivity were disappointing (Roep et al., 1999; Schloot et al., 2003), although the latest workshops have been more encouraging. Insulin- or GAD-specific T-cell lines and T-cell clones have been derived from peripheral blood lymphocytes (PBL) of T1D patients, but not more efficiently than from PBL of normal subjects (Endl et al., 1997; Bach et al., 1997). Interest in this central topic has been recently renewed by studies using HLA DR4 eluted peptides (Peakman et al., 1999) and from the usage of GAD-specific class II tetramers (Liu et al., 2000; Reijonen et al., 2002). In these two systems, a low number of GAD-reactive T cells could be shown after T-cell *in vitro* activation in conditions in which healthy controls remained negative.

More convincing are the limited data demonstrating the major T-cell infiltration of pancreata from recently diagnosed T1D patients (Bottazzo et al., 1985; Imagawa et al., 2001).

Collectively, these data provide good evidence for islet-specific autoimmunity in T1D patients. They do not demonstrate, however, the causal relationship between this autoimmunity and disease pathogenesis. This is inferential and is based on the following evidence:

- the inhibition of disease progression after T-cell blockade as achieved with cyclosporin-A (Feutren et al., 1986; CERCTG, 1988), or an anti-CD3 antibody (Herold et al., 2002);
- T1D association with well-defined HLA alleles, best explained by the presentation of selected autoantigenic peptides to T cells by T1D-predisposing HLA molecules (Nerup et al., 1974);
- the autoimmune origin of T1D in NOD mice and BB rats (see next section), assuming that the T1D observed in these two animal models does have a pathogenesis identical or close to that of human T1D.

The capacity to transfer the disease by administration of T cells from diabetic animals to healthy syngeneic recipients is highly convincing (Bendelac et al., 1987) as well as disease prevention by a number of T-cell-specific interventions (Like et al., 1979; Chatenoud et al., 1994). Disease transfer has been obtained with CD4 (Haskins and McDuffie, 1990) and CD8 (Wong et al., 1996) T-cell clones after infusion in immunoincompetent NOD recipients.

However, it has not proven possible to meet all the criteria for an autoimmune disease proposed by Rose and Bona (1993). Surprisingly, there has been no success with diabetes induction by immunization with islet extracts or chemically defined β cell antigens, except for the induction of severe insulitis without diabetes after immunization with an insulin peptide concomitant with administration of PolyIC (Moriyama et al., 2002). Tolerance induction to some beta cell antigens induces disease protection (Kaufman et al., 1993; Tisch et al., 1993; Muir et al., 1995) but to a large extent through a nonspecific bystander suppressive effect (Zhang et al., 1991).

A DIVERSITY OF ANIMAL MODELS

Spontaneous Models

The nonobese diabetic (NOD) mouse was first proposed as an animal model of T1D by Japanese investigators (Makino et al., 1980). The NOD mouse develops insulitis starting at 3 weeks of age. Overt diabetes appears at 4–6 months of age. The disease is consistently more common in females than in males. Its incidence varies dramatically in both genders according to the sanitary conditions of the animal facility. NOD mice show other clinical and biologic manifestations of autoimmunity, including thyroiditis (at a late age), sialadenitis, and mild colitis (Bach, 1994; Bach and Mathis, 1997). NOD mice may also produce antinuclear antibodies (Baxter et al., 1994) and anti-erythrocyte antibodies (Baxter and Mandel, 1991).

Bio-Breeding (BB) rats were initially discovered as a model of T1D in Canada (Nakhooda et al., 1977). These rats develop diabetes in a high percentage of cases at variance with genetically close but distinct (BB-R rat) strains that are diabetes resistant (Crisa et al., 1992). The main immunologic feature of BB rats is lymphopenia (Poussier et al., 1982) that depends on a single gene (Hornum et al., 1995). Like NOD mice, BB rats also develop thyroiditis at a high incidence.

Thymectomy-Induced Diabetes

In the rat, thymectomy followed by sublethal irradiation induces diabetes onset in some strains but not in others in which, however, thyroiditis is observed (Fowell and Mason, 1993). In BALB/c mice, thymectomy at 3 days of age, which induces selective depletion of $CD4^+CD25^+$ T cells, induces

insulitis in the context of a polyautoimmune syndrome, but diabetes is rarely observed (Asano et al., 1996). These effects of thymectomy are reminiscent of the acceleration of diabetes onset noted in NOD mice when thymectomy is performed at weaning (3 weeks of age) (Dardenne et al., 1989) and of diabetes induction in the BB-R rat after adult thymectomy and irradiation (Ramanathan et al., 2002).

The Low-Dose Streptozotocin Model

High doses of the alkylating agent streptozotocin (STZ), which is a selective toxin for β cells, produces irreversible T1D secondary to β-cell apoptosis, whereas multiple low doses of STZ insufficient to destroy enough β cells to directly cause T1D induce an autoimmune-related insulitis and T1D (Paik et al., 1980). Low-dose STZ T1D is inhibited by anti-T-cell antibodies (Herold et al., 1987) and can be transferred into normal animals by T cells from STZ-treated mice (Buschard and Rygaard, 1978). The mechanisms of disease induction by low-dose STZ are not clear, but probably involve enhancement of β-cell antigen presentation secondary to limited β-cell destruction and perhaps aberrant expression of MHC or costimulatory molecules under the local STZ effect (Campbell et al., 1988). The model is complicated by the overlap between STZ doses leading to toxic or autoimmune diabetes. Doses that induce autoimmune type T1D in some strains may induce diabetes in NOD SCID mice which are deprived of T cells (Gerling et al., 1994), an observation possibly related to the unique and intriguing sensitivity of NOD mouse β-cells to STZ-induced apoptosis (Orlow et al., 1987; Gonzalez et al., 2002).

TCR Transgenic Models

$CD4^+$ and $CD8^+$ diabetogenic T-cell clones can be derived from the islets of NOD mice. The TCR genes of such clones were used to generate TCR transgenic mice. The first reported model, the BDC2.5 mouse, used a TCR derived from a $CD4^+$ T-cell clone shown to be diabetogenic by transfer into young or SCID NOD mice. In the initial report, transgenic mice (backcross 6) showed accelerated onset of diabetes when compared to nontransgenic littermates (Katz et al., 1993). Ultimately, after backcross 12, transgenic mice never became diabetic (You et al., 2004). Fulminant diabetes could be induced, however, in such mice by backcrossing to $RAG^{-/-}$, SCID or TCR $Ca^{-/-}$ mice which cannot generate their own TCR gene rearrangements (Kurrer et al., 1997; You et al., 2004) (see page 489). Similar data were obtained in transgenic mice expressing a TCR derived from $CD8^+$ T-cell clones (Amrani et al., 2000). In both systems, it is important to realize that the antigenic specificity of the T-cell clones was not known. Synthetic peptide mimetopes were obtained. More recently, GAD-specific TCR transgenic mice were produced which, intriguingly, did not develop diabetes even when they were backcrossed to NOD mice (Tarbell et al., 2002).

RIP Transgenic Models

When transgenes are coupled to the rat insulin promoter (RIP), the product of the transgene is selectively expressed in the β cells where insulin is produced. Several models of experimental diabetes have been developed in transgenic mice expressing various antigens in β cells. The first model was described by Hanahan wherein the SV40T antigen was expressed in β cells under conditions in which mice were not tolerant and mounted an SV40T-specific T-cell response leading to severe insulitis (Adams et al., 1987).

Numerous studies have been performed with lymphochoriomeningitis virus (LCMV) in which LCMV proteins (glycoprotein, GP, or nuclear protein, NP) were selectively expressed in β cells (see Chapter 26). Expression of these viral antigens alone was insufficient to induce diabetes since the mice were tolerant to LCMV antigens, treating these as bona fide autoantigens. Double transgenic mice were obtained when RIP-LCMV mice were crossed to mice expressing a TCR specific to the LCMV β cell antigen. In both the single and the double RIP-LCMV transgenic mice, diabetes consistently appeared only when the animals were infected with LCMV which induces a major activation of LCMV-specific T cells (Ohashi et al., 1991; Von Herrath et al., 1997; Von Herrath, 2002). Similarly double transgenic mice have been produced that selectively express influenza virus hemagglutinin in β cells (RIP-HA) but, at variance with the LCMV model, RIP-HA × HA-TCR transgenic mice did develop diabetes in some cases, but not in others (Scott et al., 1994).

Other RIP transgenic models of diabetes have been described using RIP-driven β cell-specific expression of the T-cell costimulatory molecule CD80 (B7) (Wong et al., 1995), singly or in association with transgenic expression of the T1D-associated HLA allele HLA DR4 (Wen et al., 2001).

Conclusions

The wide diversity of animal models of T1D represents a major aid in the dissection of T1D mechanisms. The spontaneous models, the BB rat and especially the NOD mouse represent by far the closest models to human T1D, although others may be very interesting insofar as they simplify the analysis of ongoing autoimmune responses. There is debate on the significance of comparing NOD mice to human T1D. One major concern is the large number of treatments that prevent disease in NOD mice (see pages 494 and 495), as compared to the very few treatments that are effective at the

time of disease onset (Bach, 2002a). Other more minor concerns include the female predominance in NOD mice, which is not observed in humans, and the production of islet cell-specific antibodies which is much lower in animal models than in humans. In any event, NOD mice and BB rats, as well as the other models described above, represent multiple copies of just a single individual, whereas human T1D is a heterogeneous disease among affected individuals. One may assume that most patients share common disease mechanisms which are operative in NOD mice. A major difference contributing to the interest of these models is that NOD mice and BB rats can be monitored during their whole lifespan, whereas patients are usually only seen at the time of overt T1D.

IMMUNOPATHOGENESIS

The Nature of the Effector Mechanisms

Data supporting a role for β cell-specific autoantibodies in the pathogenesis of T1D are weak, if not lacking. Disease cannot be transferred to healthy recipients by antibody-positive immunoglobulins. Suggestive, albeit indirect data indicate that offspring of antibody-positive diabetic NOD females present diabetes more rapidly than do offspring of nondiabetic antibody-negative mothers (Greeley et al., 2002). However, data from humans are not in accord with this. Additionally, T cells from NOD mice can transfer diabetes to NOD mice that have been rendered unable to produce antibodies by postnatal anti-μ treatment (Bendelac et al., 1988). However, there is a possible role for B cells in disease pathogenesis as indicated by the absence of diabetes in B cell-less NOD mice. B cells could act in this setting ($Ig^{-/-}$) (Serreze et al., 1998) as antigen-presenting cells for β-cell antigens.

Referring to T cells in T1D, the T-cell infiltration of the pancreas in human and murine autoimmune diabetes, as well as the arrest of disease progression by selective T-cell blockade, is persuasive. Finally, for mice at least, the disease can be transferred into healthy nondiabetic syngeneic NOD recipients by pure populations of T cells or T-cell clones (Bendelac et al., 1987; Haskins and McDuffie, 1990; Wong et al., 1996).

The respective roles of $CD4^+$ and $CD8^+$ T cells in diabetes pathogenesis have long been debated. The observation that $CD4^+$ T-cell clones could transfer the disease alone in the absence of $CD8^+$ T cells, these having been depleted by anti-CD8 antibody treatment (Bradley et al., 1992), suggested that the disease was mediated entirely by CD4 T cells. However, $CD8^+$ T-cell clones alone can also transfer T1D (Wong et al., 1996) and, when polyclonal T cells are used, both $CD4^+$ and $CD8^+$ T cells are necessary to obtain transfer of diabetes (Bendelac et al., 1987), even though insulitis can be obtained with $CD4^+$ T cells alone (Thivolet et al., 1991).

Among $CD4^+$ T cells, the major contribution is from Th1 cells. The progression of diabetes is associated with increased intra-islet expression of IFN-γ (Rabinovitch et al., 1995), and anti-IFN-γ neutralizing antibodies prevent disease progression (Debray-Sachs et al., 1991). IFN-$\gamma^{-/-}$ and IFN-γ $R^{-/-}$ NOD mice still develop diabetes (Serreze et al., 2000), but gene redundancy cannot be excluded. Additionally, converging evidence shows a central role for IL-12, the major Th1-cell differentiation factor, in driving T1D in NOD mice. The disease is accelerated by IL-12 administration (Trembleau et al., 1995). Th2 cytokine expression is not enhanced at the time of diabetes onset and such cytokines inhibit disease onset when administered in young NOD mice (Rapoport et al., 1993; Pennline et al., 1994). Note lastly that anti-insulin and anti-GAD antibodies show a Th1-dependent IgG2a isotype.

Enumeration of diabetogenic $CD8^+$ T cells in NOD mice has been made possible by use of MHC class I tetramers. Numbers of $CD8^+$ T cells with high avidity for the target antigens increase with time during progression of insulitis, peaking at the time of diabetes onset (Amrani et al., 2000). The availability of tetramers has also shown the prevalence of $CD8^+$ T cells specific for limited number of islet-cell antigens, particularly insulin and IGRP (see page 488).

β-Cell Antigens Drive the Diabetogenic T-Cell Response

Indirect evidence suggests that β cells are important for stimulating islet-specific autoantibody production in T1D. Thus patients with long-standing T1D who are likely to have lost all their islet cells show a clear decrease in levels of islet-specific antibody production (Maugendre et al., 1999). Importantly, these autoantibodies reappear after pancreas transplantation (Bosi et al., 2001). Also, as mentioned, the HLA–T1D association further supports the "driving role" for β-cell autoantigens, as does the restriction of TCR heterogeneity in young NOD mice (Shizuru et al., 1991; Baker et al., 2002), a finding which, however, could not be confirmed in adult NOD mice (Sarukhan et al., 1995). In a more direct approach using NOD mice, we have shown that diabetogenic T-cell clones, which normally expand in irradiated recipients, are exhausted and progressively disappear after being "parked" in β-cell-depleted irradiated mice; that is, mice whose β cells had been destroyed by alloxan, a selective β-cell toxin (Larger et al., 1995).

Particular attention has been directed to the initial cellular events developing locally in the pancreas, or its vicinity, in the first weeks of life in NOD mice and, notably, the wave of β-cell apoptosis observed at 2–3 weeks of age just before onset of insulitis (Trudeau et al., 2000). It has been proposed

that $CD8^+$ T cells have a disease-triggering effect in inducing the initial β-cell lesion with B cells as antigen-presenting cells. The initial inductive cellular events do not take place in the islet itself, at least for the most part, but in peripancreatic lymph nodes which show intensive T-cell proliferation. (Hoglund et al., 1999).

If β-cell antigens do trigger the diabetogenic response, the nature of the responsible β-cell autoantigen is a central question. Several antigens expressed in β cells elicit B- and T-cell reactivity either in NOD mice and/or in man: insulin, GAD (Baekkeskov et al., 1990), and IA-2 (Lan et al., 1996), a tyrosine phosphatase-like molecule identified on the basis of its selective expression by β cells. Insulin and GAD can induce disease protection provided they are administered to young (prediabetic) NOD mice (Zhang et al., 1991; Kaufman et al., 1993; Tisch et al., 1993; Daniel and Wegmann, 1996). A specific role has been proposed for proinsulin in man (Narendran et al., 2003) and preproinsulin 1 in NOD mice. Mice genetically deficient in this protein are protected from diabetes, whereas mice genetically deficient in preproinsulin 2 show accelerated disease onset (Moriyama et al., 2003). A novel and likely important β-cell candidate (T-cell) autoantigen is islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP), recently described as the target molecule of the majority of $CD8^+$ diabetogenic T cells in NOD mice (Martin et al., 2001; Lieberman et al., 2003). Yet the problem remains that no decisive priority can be given to any of these autoantigens in contributing to disease induction, and the respective autoantibodies do not appear in the same sequential order in all patients with T1D.

Two explanations can be proposed for these intriguing findings. First, autoimmunization extends from one to another specificity by antigen (epitope) spreading. Thus, a primary antigen induces the differentiation of diabetogenic T-cell clones which induce a β-cell lesion and which, ultimately, lead to the recruitment of autoreactive T cells with other β-cell-associated specificities.

Second, the initial triggering event is not related to any particular autoantigen but could be essentially nonspecific, meaning that a pancreatic viral infection could increase the expression and "availability" of the various molecules necessary for antigen recognition by T cells, i.e., class I and class II MHC molecules, costimulatory molecules, adhesion molecules, and others.

In any event, there are no data as yet to incriminate a primary β-cell autoantigen in T1D even though the HLA–T1D association in humans would suggest that such an autoantigen must exist. Box 36.1 presents a partial list of the many candidate autoantigens which have been proposed in human or murine T1D, and Table 36.1 gives the main features of the four best-characterized of these.

Thus, T1D is marked by a selective breakdown of self-tolerance to β-cell antigens. As already discussed, however, there is no clear predominance of autoreactivity against a well-defined autoantigen. If any one were to be selected at present, it would be insulin. However, all in all, T1D appears as the result of the widespread rupture of the state of tolerance that normally exists to β-cell autoantigens, without excluding that the initial breakdown of tolerance takes place against a primary autoantigen which then extends through

Box 36.1 A nonexhaustive list of T1D candidate autoantigens

1. Insulin (proinsulin)
2. Glutamic acid decarboxylase (GAD) (GAD65/GAD67)
3. IA-2/ICA512/phogrin
4. Hsp 65/p277
5. Islet-specific glucose 6 phosphatase catalytic subunit related protein (IGRP) (T cells)
6. GLIMA (38 kDa)

TABLE 36.1 Requirements for candidate β-cell autoantigens

	Insulin	GAD	IA-2	IGRP
Distribution	β cells	β cells/brain	β cells	β cells
Autoreactivity in human T1D				
Autoantibodies	+	+	+	?
T cells	+	+	+	?
Protective tolerance in NOD mice				
Peripheral	+	+	?	?
Central (transgenic mice)	+	−	?	?
Specific pathogenic T-cell clones	+	+	?	+ (CD8)
Triggering of insulitis in non-autoimmune-prone mice	+ (+poly IC)	−	?	?

antigen spreading (Vanderlugt and Miller, 2002) to other β-cell autoantigens.

Rupture of Self-Tolerance to β Cells

The concomitant presence in NOD mice of a β-cell autoantigen and of T cells expressing the relevant specific TCR is not sufficient to trigger diabetes onset. This important statement is supported (as mentioned above) by the presence of β-cell-specific autoreactive T cells in healthy human subjects (Endl et al., 1997; Bach et al., 1997; Peakman et al., 1999) and by the absence of diabetes in double transgenic RIP-LCMV mice, until they are infected by LCMV (Ohashi et al., 1991). Two factors appear to be determinant for the triggering event. First, activation of β-cell-specific T cells, so bypassing the state of ignorance described above, and second, permissiveness of regulatory mechanisms which normally prevent the development of pathogenic autoimmunity.

In healthy subjects, humans and mice, tolerance to self-antigens normally involves initially the intrathymic destruction (negative selection) of T cells with high-affinity receptors for self-peptides present in the thymus, followed by mechanisms of peripheral tolerance that regulate the autoreactive T cells which have escaped intrathymic negative selection. The question posed is the mechanisms by which tolerance to β-cell autoantigens is lost in T1D: defect in deletion, regulation, or both.

T-Cell Activation

The multiplicity of concomitant β-cell-specific immune responses discussed above indicates that the most likely mechanism leading to T-cell activation involves islet inflammation, for example by a pancreatotropic virus, yet no direct evidence has been obtained to formally incriminate any particular virus in human T1D (see pages 492 and 493) or in NOD mouse T1D. The increased expression of IFN-α observed in islets of recently diagnosed diabetic patients (Foulis et al., 1987) is suggestive of a viral mechanism. Other mechanisms of non-antigen-specific T-cell activation could be considered, such as superantigens, but no evidence supports this hypothesis except the unsubstantiated report of a selective expression of Vβ7, which could be interpreted as a result of the expansion of T cells interacting with a specific endogenous retrovirus.

Arguments for Defective Intrathymic Negative Selection

The derivation of insulin- and GAD-reactive T-cell lines from normal peripheral blood lymphocytes in diabetic patients but also in healthy subjects (Endl et al., 1997; Peakman et al., 1999) clearly indicates that thymic negative selection does not fully operate for β-cell-specific autoantigens, as also pertains to other autoantigens. It might be, however, that negative selection is less effective for such antigens in T1D than in normal subjects. This notion is supported by some observations made in the NOD mouse, showing that NOD mouse thymocytes have a lower rate of apoptosis than normal strains (Kishimoto and Sprent, 2001; Bergman et al., 2003). The defect affects both Fas-dependent and Fas-independent pathways of apoptosis. For Fas-dependent apoptosis, the defective apoptosis of NOD thymocytes correlated with the strong T-cell receptor-mediated upregulation of caspase8-homologous FLICE (Fas-associated death-domain-like interleukin I beta-converting enzyme) inhibitory protein (Kishimoto and Sprent, 2001). Additionally, use of an RIP double transgenic model as described above shows that non-MHC genes from the NOD strain promote a failure to delete high-avidity autoreactive T cells during their development in the thymus, with subsequent spontaneous breakdown of $CD4^+$ T-cell tolerance to the islet antigen and formation of intra-islet germinal centers (Lesage et al., 2002). A failure of thymic negative selection is indirectly supported by the finding that human subjects with autoimmune polyendocrine syndrome I (APECED) may present diabetes due to a mutation on the AIRE gene (Peterson et al., 2004); AIRE is demonstrated in the mouse to control the intensity of negative selection against certain antigens, and mRNA expression for some autoantigens is decreased in $AIRE^{-/-}$ mice (Anderson et al., 2002) (see Chapter 38).

Lastly, some of the β-cell candidate autoantigens (insulin or proinsulin, GAD, and IA2) are expressed in the thymus in a subset of medullary antigen-presenting cells, either as the total protein or as fragments due to alternative splicing, and the level of such expression is correlated with predisposition genes for T1D, notably the insulin promoter gene (Diez et al., 2001; Pugliese et al., 2001). Presumably, the higher the level of the thymic expression of these autoantigens, the more efficient would be the negative selection for the corresponding autoreactive T cells. Thus defective expression of β-cell autoantigens in the thymus might well contribute to the pathogenesis of T1D.

Failure of Regulatory T Cells

Diabetes onset is accelerated by various manipulations that deplete certain T-cell subsets, including thymectomy at 3 weeks (Dardenne et al., 1989), blockade of costimulatory pathways (Lenschow et al., 1996) or cyclophosphamide treatment (Yasunami and Bach, 1988). Similarly, the backcrossing to immunoincompetent mice ($RAG^{-/-}$, SCID, TCR $Ca^{-/-}$) of transgenic mice expressing a diabetogenic TCR dramatically increases rapidity and incidence of diabetes (Kurrer et al., 1997; You et al., 2004). This observation illustrates the role of T cells having undergone endogenous TCR

rearrangements in disease control probably through immunoregulation, since diabetes associated with their absence is prevented by infusion of $CD4^+CD62L^+$ regulatory T cells (You et al., 2004).

Direct evidence for control of T1D by regulatory T cells has been obtained by showing the ability of $CD4^+$ T cells from prediabetic NOD mice to protect from diabetes in a cotransfer model in which such T cells are administered to NOD/SCID or irradiated NOD recipients concurrently with diabetogenic T cells derived from the spleen of NOD diabetic mice (Boitard et al., 1989). These regulatory T cells include not only $CD25^+$ T cells but also $CD25^-CD62L^+$ T cells (Alyanakian et al., 2003) and that regulation depends on TGF-β but not on Th2 cytokines (Chatenoud, in preparation; Green et al., 2003). However, Th2 cytokines do appear important for tolerance induction and disease prevention after giving soluble β-cell autoantigens, since such tolerance is not obtained in IL-$4^{-/-}$ NOD mice (Tisch et al., 1999). The role of Th2 cytokines in the physiologic control of the spontaneous disease is unlikely, as indicated by the normal disease incidence observed in IL-$4^{-/-}$ (Wang et al., 1998) and IL-$10^{-/-}$ (Serreze et al., 2001) NOD mice. Even so, this is an argument to consider with caution, because of the occurrence of autoimmune diabetes in IFN-$\gamma^{-/-}$ and IFN-$\gamma R^{-/-}$ NOD mice (Serreze et al., 2000). Also, a role for NKT cells has been suggested in the control of T1D. The number and function of these cells are deficient in NOD mice (Gombert et al., 1996). Diabetes onset is accelerated in NKT-cell-deficient $CD1^{-/-}$ NOD mice (Wang et al., 2001) and is slowed down in wild-type NOD mice by α-galactosyl-ceramide, a selective NKT-cell ligand (Hong et al., 2001; Sharif et al., 2001). A defect in NKT-cell function has been described in human T1D (Wilson et al., 1998; Kukreja et al., 2002). At present, however, some uncertainties persist on whether the involvement of NKT cells in the pathogenesis of T1D is really significant.

β-Cell Lesion

As extensively discussed above, T1D is the consequence of damage to β cells by β-cell-specific T cells. A central question is to determine the fine mechanisms at the origin of the reduction of insulin production.

First, what are the respective roles of β cell destruction ("atrophy") versus functional (reversible) inhibition of insulin production by residual living β-cells, i.e., inflammation? The existence of inflammation can be assessed in the NOD mouse and to a lesser extent in human T1D by the rapid recovery of insulin production observed after T-cell targeted intervention. Recent-onset diabetic NOD mice recover normal glycemia within 24–48 h of administration of anti-TCR (Sempe et al., 1991) or anti-CD3 monoclonal antibodies (Chatenoud et al., 1994) in the absence of insulin therapy. The recovery of insulin production by β cells derived from recently diabetic NOD mice, after *in vitro* culture in the absence of aggressive T cells, also suggests the persistence of functional β cells at the time of diabetes onset (Strandell et al., 1990).

The questions remain, however, of the percentage of intact residual β cells at diabetes onset and what the kinetics of β-cell destruction in the months and years preceding T1D onset are. Is there a regularly progressing destruction with a superimposed inflammation or does disease progression suddenly accelerate just before diabetes onset? If so, what is the triggering mechanism at the origin of this burst of β-cell aggression? Morphometric data available in the NOD mouse (Sreenan et al., 1999), and to a lesser degree in human T1D (Hanafusa et al., 1990; Imagawa et al., 2001), are not very informative on this point, especially since the authors use insulin as a β-cell marker. Indeed, this may create a bias since living β cells subjected to inflammation may drastically reduce their level of insulin production. Particular attention should be given to noninvasive approaches to determine β-cell mass, such as those using β-cell-specific radiolabeled monoclonal antibodies (Moore et al., 2001). At the molecular level, considerable effort has been devoted to understanding the mechanisms of β-cell destruction. Several studies shed light on the events leading to β-cell apoptosis; some show the important role of nitric oxide and free radicals (Brenner et al., 1993) in addition to the *putative* mechanisms usually associated with $CD8^+$ T-cell mediated β-cell lysis, perforin or Fas mediated. The role of cytokines, notably IL-1, has been documented by *in vitro* studies using normal β cells (Mandrup-Poulsen, 2003) but it is difficult to determine what their role is *in vivo.*

Lastly, there is a growing interest in the role of chemokines and their receptors in the attraction of effector cells into the islet (Atkinson and Wilson, 2002), exemplified by the suggested role of CXCR3 and CCR4 in the initiation of insulitis in NOD mice (Kim et al., 2002; Frigerio et al., 2002).

ETIOLOGY

T1D is a multifactorial polygenic disease. It is the result of the interaction of predisposing genes with unfavorable environmental factors, probably both triggering and permissive. We shall successively discuss genetic and environmental factors in light of the knowledge gained from experimental models and human T1D.

Genetics of T1D

The Weight of Heredity

When one member of a pair of monozygotic twins develops T1D, the other twin will develop the disease in

approximately 35–50% of cases, and more often (up to 60%) if the twins are DR3/DR4 heterozygotes (Bach, 1994; Ide and Eisenbarth, 2003). This high concordance rate provides a quantification of the hereditary nature of the disease and the disease penetrance. The fact that in these conditions as many as 7% of non-twin siblings are concordant for the disease indicates in a first analysis the existence of a limited number of disease predisposition genes, the more so since half of the genetic predisposition is due to genes of the major histocompatibility complex (MHC). HLA identical siblings have a concordance rate of approximately 16%, nearly half the monozygotic twin concordance rate (Bach, 1994; Ide and Eisenbarth, 2003).

We note that about 5% of children from diabetic patients become diabetic with, interestingly (and so far unexplained), a higher risk when the diabetic parent is the father (Warram et al., 1984). Perhaps the immune system of the embryo is influenced by its interaction with the mother's islet-specific antibodies, whatever the mechanisms of the apparent protection conferred might be. This interpretation is in opposition to the hypothesis, proposed on the basis of indirect data, that there is a diabetes-promoting effect of maternal anti-islet antibodies in NOD mice (Greeley et al., 2002).

Role of MHC Genes

The association between HLA antigens and T1D was discovered in 1974 (Nerup et al., 1974) but it proved difficult to identify the actual loci of the predisposing genes due to major linkage disequilibrium within the MHC. Convergent data indicate that predisposing genes are located at the DQ locus, though there is no absolute certainty about the absence of effects conferred by other loci, especially since some data argue for a haplotype effect wherein two genes on the same haplotype may be operational in a synergistic fashion. The two major predisposing haplotypes are DQA1*0501 DQB1*0201 and DQA1*0301 DQB1*0302 but other predisposing haplotypes exist in non-white populations (See Chapter 74). Particular attention is presently being paid to a locus located between HLA-B and TNF (Lie et al., 1999; Johansson et al., 2003). This haplotype effect would provide a good explanation for the discrepancy observed between the relatively low risk contribution of each HLA predisposition gene and the major risk among sibpairs associated with the sharing of the two HLA haplotypes (independently of DR3/DR4 heterozygosity). The HLA–T1D association also includes a major protective effect of HLA DR2 or the associated DQB1*0602 gene. Table 36.2 presents the list of HLA T1D predisposition genes as well as that of disease protection genes.

Postulated mechanisms of T1D–HLA association include presentation of β-cell antigen peptides by the corresponding HLA molecules (see Chapter 5). The sequence analysis of T1D-associated DQ and DR molecules has revealed the importance of particular amino acid residues. Thus T1D is strongly associated with the absence of aspartic acid in position 57 of the DQβ chain, a critical position for peptide binding to the DQ molecule (Todd et al., 1987). The protective effect is less easily explained but may involve peptide capture by the protective HLA molecule or the induction of regulatory T cells. But caution is necessary since, as stated above, there may be a strong haplotype effect with the possible role of non-HLA MHC genes influencing immune responsiveness in a non-antigen-specific fashion, as

TABLE 36.2 Association of HLA genes and human type 1 diabetes (IDDM1) in white people (after Pociot and McDermott, 2002)

HLA-DQ alleles	HLA-DR alleles	Relative risk
Positive associations (susceptibility haplotypes)		
A1*0301-B1*0302	DRB1*04	2.5–9.5
A1*0501-B1*0201	DRB1*301	2.5–5.0
A1*0501-B1*0302	DRB1*301/DRB1*04	12.0–32.0
A1*0301-B1*0201	DRB1*301/DRB1*04	
A1*0301-B1*0402	DRB1*04/DRB1*801	4.0–15.0
A1*0301-B1*0201	DRB1*701	8.0–13.0
A1*0301-B1*0201	DRB1*901	5.5
A1*0301-B1*0401	DRB1*04	3.5–4.5
A1*0301-B1*0303	DRB1*901	2.0–4.5
Negative associations (protective haplotypes)		
A1*0102-B1*0602	DRB1*1501	0.03–0.2
A1*0103-B1*0603	DRB1*1301	0.05–0.25
A1*0301-B1*0301	DRB1*04	0.2–0.5
A1*0501-B1*0301	DRB1*1101	0.05–0.5

indicated by studies in the NOD mouse (Boulard et al., 2002).

Role of Non-MHC Genes

The difference observed between concordance rates in monozygotic twins (35–40%) and HLA identical siblings (16%) demonstrates the role of non-MHC genes in disease predisposition, as described in Chapter 21. Recent updates on the state of the art can be found in reviews by Pociot and by Eisenbarth. The study of candidate non-MHC genes has not been very fruitful since only two have been clearly identified, insulin and CTLA-4, and for both there is a low relative risk and no absolute exclusion of neighboring genes. Also incriminated are the IL-12 p40 gene (Davoodi-Semiromi et al., 2002) and the IL-4 receptor gene (Mirel et al., 2002). Genome scanning approaches have been more successful, since both in NOD mice and human T1D more than 20 chromosomal regions have been characterized (Table 36.3) (Wicker et al., 1995; Todd, 1999; Pociot and McDermott, 2002; Ide and Eisenbarth, 2003). However, not all of these regions have been confirmed in various large-scale studies performed in the US and Europe (Pociot and McDermott, 2002), and the relative risk associated with each of these regions is relatively small.

The problem is complicated by the clustering of several predisposition genes in single regions, as in the case of Idd5 in the NOD mouse. Returning to the relatively high disease concordance rate in HLA identical siblings, it seems likely that only a few of these predisposition genes are important for disease predisposition or that many of them are present in a very high proportion of the general population. Converging evidence suggests that most, if not all, T1D-predisposing genes code for polymorphic variance of normal proteins. The hope is that recent progress in genome typing, together with the knowledge of the human genome and the haplotype map, will clarify genetic predisposition to T1D. It will also be important to relate the genes in question to their putative involvement in disease pathogenesis, which has so far essentially been possible only in the NOD mouse.

Environmental Factors

Since more than 50% of monozygotic twins are discordant for T1D, the importance of environment in the etiology of T1D seems substantial. However, it is difficult to distinguish whether this effect arises from abnormal triggering of the immune response by extrinsic factors or from a permissiveness due to insufficient stimulation of immunoregulation.

Triggering Factors

Viruses

Viruses have been considered major potential candidates for the etiology of T1D for several decades. The viral hypothesis was initially based on the temporal relationship between defined virus infections and onset of overt diabetes (Jun and Yoon, 2003). This sequence was notably invoked for Coxsackie B4 virus (Asplin et al., 1982) but the argument is not very robust when one considers, as mentioned above, that T-cell-mediated islet aggression probably begins many years before clinical onset in most patients and thus before the incriminated infection. The infection may at most exacerbate an already established anti-islet response and thereby accelerate disease onset. One should add that

TABLE 36.3 Non-MHC gene loci in type 1 human diabetes (after Pociot and McDermott, 2002)

Locus	Chromosome	Markers	Significance level
IDDM2	11	INS	LOD ≥ 2.0
IDDM3	15	D15S107	LOD ≥ 2.0
IDDM4	11	D11S1296-FGF3	LOD ≥ 3.6
IDDM5	6	D6S476-D6S473	LOD ≥ 3.6
IDDM7–IDDM12	2	HOXD8-D2S72-CTLA4	LOD ≥ 3.6
IDDM8	6	D6S446-D6S281	LOD ≥ 3.6
IDDM9	3	D3S1279	LOD ≥ 1.0
IDDM10	10	D10S191-D10S220	LOD ≥ 3.6
IDDM11	14	D14S67	LOD ≥ 3.6
IDDM13	2	D2D137-D2S301	LOD ≥ 2.0
IDDM15	6	D6S283	LOD ≥ 3.6
IDDM16	14	D14S292-D14S293	
IDDM17	10	D10S1750-D10S1773	
IDDM18	5	IL12B	LOD ≥ 3.6

serological evidence, i.e., the detection of antiviral antibodies in T1D patients, has always been indecisive, as are the episodic claims of virus isolation from pancreatic tissue (Burch and Harb, 1975). The interest in enteroviruses has recently been renewed by observations that the frequency of enterovirus infections studied using both serology and testing for the presence of enterovirus RNA could be correlated with islet-specific autoantibodies in subjects at risk for developing T1D (Honeyman et al., 2000; Lonnrot et al., 2000). Also, an increased T-cell response to Coxsackie B4 antigens was observed in recent-onset diabetic patients (Juhela et al., 2000). These data are interesting but their contribution to the etiologic role of enteroviruses remains indirect, since positive results are also observed in a large percentage of the normal population.

At the experimental level, the data are also rather limited. The encephalomyocarditis virus is said to induce T1D in mice (Craighead and McLane, 1968) but T1D here is of the cytopathic type without immunologic involvement. The Coxsackie B4 virus can induce diabetes in mice with some features of autoimmune T1D (Coleman et al., 1974) but it is not clear whether disease pathogenesis involves a direct cytopathic effect or an immune β-cell attack secondary to virus-induced inflammation. This interpretation is compatible with the report by Horwitz that Coxsackie B4 infection of BDC2.5 mice which have a limited nontransgenic TCR repertoire shows accelerated diabetes (Horwitz et al., 1998).

Other interesting data have been derived from the RIP-LCMV transgenic mice that develop T1D after LCMV infection according to a hit-and-run mechanism (Von Herrath et al., 1997; Von Herrath, 2002) (see Chapter 26). The virus infection stimulates the induction of LCMVgp-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) which cause the disease, even though the virus is rapidly cleared through the action of such CTLs.

Collectively, these data are compatible with, but certainly not proof of, a viral etiology for T1D, noting also that the diabetogenic virus (or viruses) is still unidentified. It might well be that the etiologic infection takes place many years before clinical onset, calling into question the data on preclinical infections and seasonal prevalences. This lag time would explain the difficulty in identifying the diabetogenic viruses, which in addition might not be unique. Several pancreatotropic viruses could operate through nonspecific inflammatory mechanisms, as discussed earlier. There is also a possibility for antigen (epitope) mimicry but there is no good evidence for this hypothesis in man, except a homology between a Coxsackie B4 virus protein and GAD (Atkinson et al., 1994), and virtually no evidence in the mouse. We note that T1D is accelerated in BB rats infected with Kilham's virus (Guberski et al., 1991) but in this case the impact of the virus is on the immune system, rather than on islet β cells.

Diet

Cow's milk accelerates the onset of T1D in BB rats (Elliott and Martin, 1984). A gluten-free diet prevents T1D in NOD mice (Funda et al., 1999). These observations have led to a number of experimental studies and therapeutic trials in humans that are still in progress. Concerning cow's milk, a peptide from lactalbumin (called ABBOS) was shown to cross-react with a β-cell autoantigen named p69, suggesting again a possible form for antigen mimicry (Karjalainen et al., 1992).

Toxic Agents

Multiple low doses of STZ regularly induce T1D in susceptible strains of mice and rats, which suggests that toxic agents can induce T1D (see page 489). There is no evidence, however, documenting this hypothesis in human T1D.

Permissive Factors

As mentioned above, there has been a steady increase in the incidence of T1D over the last three decades in Western countries in line with the increase in incidence of other autoimmune and allergic diseases (EURODIAB, 2000; Bach, 2002b). T1D is more common in developed than in developing countries and within Europe there is a clear north–south gradient, as seen with other autoimmune diseases. These data suggest the role of a permissive environmental factor in the etiology of T1D.

The Nature of Protective Environmental Factors

Many differences exist between countries of the northern and southern hemispheres, including genetic traits, socio-economic level, climate, diet, and more generally, lifestyle. The role of genetic factors is probably not critical if we consider that migrating populations from low-incidence to high-incidence countries acquire a disease incidence similar to that of the inhabitants of the adopted country (Staines et al., 1997). Socio-economic factors are probably central, as demonstrated among countries by a correlation between the incidence of T1D and prosperity, but also among more limited neighboring regions within countries (Bach, 2002b). The most plausible explanation for this socio-economic influence is the high rate of infections, especially during childhood, in low socio-economic areas. This interpretation has been directly demonstrated in Northern Ireland for T1D (Patterson et al., 1996). A role for infectious agents was suggested for predisposition to atopic dermatitis and Crohn's disease by studies showing a correlation between sanitary conditions and disease incidence. In addition to this, the occurrence of T1D is greatest for the firstborn of large families, probably because of lower exposure to infections.

Animal Models

It has repeatedly been shown that NOD mice and BB rats have a diabetes incidence highly related to the sanitary conditions of breeding and maintenance: the cleaner these are, the higher the disease incidence (Bach, 2002b). Additionally, infection of NOD mice with bacteria, viruses or parasites totally prevents disease onset (Oldstone, 1990; Qin et al., 1993; Zaccone et al., 2003). The best-studied model is that of the prevention of T1D in NOD mice by administration of complete Freund's adjuvant (Qin et al., 1993).

Mechanisms

Infections may prevent insulitis progression in several ways. First, there is the possible role of homeostatic competition, wherein the anti-infectious immune response competes with autoreactive T cells for homeostatic maintenance signals such as IL-7 or peptide MHC recognition (King et al., 2004). Various other cellular or molecular protagonists involved in the immune response, notably in antigen processing, the binding of autoantigen peptides to HLA molecules, as well as the consumption of IL-2 or other helper cytokines could also play a role. Second, anti-infectious responses could elicit suppressor mechanisms which could extend their inhibitory effect to diabetogenic autoimmune responses through bystander suppression, whatever the underlying mechanisms might be: IL-10, TGF-β, NKT cells or other(s). Third, infectious agents could stimulate Toll-like receptors with convergent induction of suppressor mechanisms, as shown in our laboratory (unpublished) with agonists of TLR2, TLR3, and TLR4.

Conclusions

The two sets of considerations in regard to infections are not necessarily contradictory, even for those infectious agents that have been discussed among both triggering and protecting factors. The triggering of the T1D process could relate to some specific pancreatotropic viruses, whereas conversely, islet-specific immune responses could be inhibited non-antigen specifically by a spectrum of bacteria, viruses, and parasites.

IMMUNOTHERAPY OF TYPE 1 DIABETES

This aspect is treated in depth in two other chapters (Chapters 75 and 76). However, in brief, immunotherapy of T1D can be divided into five main approaches, as follows.

Non-Antigen-Specific Suppression of Islet-Specific Immune Responses

This is best achieved with conventional chemical immunosuppressive agents such as cyclosporin A and azathioprine which have both successfully been used in human T1D (Feutren et al., 1986; Silverstein et al., 1988; CERCTG, 1988). The problem is that the effect does not extend beyond the treatment period, imposing chronic treatment with its potential toxicity and risk of over-immunosuppression.

Antigen-Induced Tolerance

The second (much more attractive) approach consists of inducing antigen-specific tolerance to β-cell antigen peptides. This approach has proven very successful in the NOD mouse (Kaufman et al., 1993; Tisch et al., 1993; Muir et al., 1995; Daniel and Wegmann, 1996; Alleva et al., 2002) but its application to man is still limited, with the single exception of a trial using a heat shock protein peptide in recently diagnosed patients with T1D (Raz et al., 2001), but this is still to be confirmed. Several trials have been performed using GAD or insulin. In prediabetic or recently diagnosed diabetic patients, none so far has shown well-proven efficacy (see Chapter 75). As mentioned above, the mechanisms of such intervention probably involve stimulation of Th2 cells.

Combination of Immediate Freezing of the Islet Response and Ultimate Induction of Regulatory T Cells

This dual effect was obtained with anti-CD3 antibodies, first in NOD mice (Chatenoud et al., 1994; Belghith et al., 2003) then in man (Herold et al., 2002; Keymeulen et al., 2005). The effect is remarkable because it is immediate and does not expose patients to long-term immunosuppression, since the non-antigen-specific effect of the treatment is immediately reversible when the anti-CD3 antibody disappears from serum. Ultimately, regulatory mechanisms take over. The latter mechanism is probably islet specific, the antibody acting preferentially on preactivated T cells which mainly comprise islet-specific T cells. This is discussed in further detail in Chapter 76.

Replacement of the Protective Effect of Infections

The approach was explored with the bacillus Calmette–Guerin (BCG) (Allen et al., 1999) and Q fever vaccines (Bonn, 1999) but the duration of immunostimulation was probably too short to be effective. Hopefully, bacterial extracts or TLR agonists will be used in the future, as successfully done in the NOD mouse.

Inhibition of β-Cell Apoptosis

This has proven a difficult approach. Nicotinamide was used on this premise in major studies, but these have failed (Lampeter et al., 1998; Gale et al., 2004).

Other Measures

A multitude of other measures have been proposed and implemented in the NOD mouse. Caution is needed in the interpretation of the results obtained in the NOD model which is unusually sensitive to immunointervention except for mice at an advanced stage of the disease (Bach, 2002b).

GENERAL CONCLUSIONS

Whatever its etiology, it can be clearly stated that T1D is a T-cell-mediated autoimmune disease. Its evolution involves a slow progression of insulitis with both inflammation and destruction of β cells. The two timely questions concern triggering mechanisms, i.e., the event(s) that stimulate(s) the differentiation of β-cell-specific T cells, and the nature of the maneuvers that could arrest or reverse the otherwise inevitable progression that ultimately leads to the complete and selective destruction of β cells. Several strategies are being investigated, with those aimed at stimulating regulatory T cells using either β-cell antigen peptides or anti-CD3 monoclonal antibodies seeming particularly attractive. It is hoped that when these methods have proven their efficacy and safety, they will be applicable to prediabetic subjects, contingent on the predictive markers being sufficiently robust, as discussed in Chapter 74.

References

Adams, T.E., Alpert, S. and Hanahan, D. (1987). Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. *Nature 325*, 223–228.

Allen, H.F., Klingensmith, G.J., Jensen, P., Simoes, E., Hayward, A. and Chase, H.P. (1999). Effect of Bacillus Calmette-Guerin vaccination on new-onset type 1 diabetes. A randomized clinical study. *Diabetes Care 22*, 1703–1707.

Alleva, D.G., Gaur, A., Jin, L., Wegmann, D., Gottlieb, P.A., Pahuja, A., Johnson, E.B., Motheral, T., Putnam, A., Crowe, P.D., Ling, N., Boehme, S.A. and Conlon, P.J. (2002). Immunological characterization and therapeutic activity of an altered-peptide ligand, NBI–6024, based on the immunodominant type 1 diabetes autoantigen insulin B-chain (9–23) peptide. *Diabetes 51*, 2126–2134.

Alyanakian, M.A., You, S., Damotte, D., Gouarin, C., Esling, A., Garcia, C., Havouis, S., Chatenoud, L. and Bach, J.F. (2003). Diversity of regulatory CD4+T-cells controlling distinct organ-specific autoimmune diseases. *Proc. Natl. Acad. Sci. USA 100*, 15806–15811.

Amrani, A., Verdaguer, J., Serra, P., Tafuro, S., Tan, R. and Santamaria, P. (2000). Progression of autoimmune diabetes driven by avidity maturation of a T-cell population. *Nature 406*, 739–742.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., Von Boehmer, H., Bronson, R., Dierich, A., Benoist, C. and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science 298*, 1395–1401.

Asano, M., Toda, M., Sakaguchi, N. and Sakaguchi, S. (1996). Autoimmune disease as a consequence of developmental abnormality of a T-cell subpopulation. *J. Exp. Med. 184*, 387–396.

Asplin, C.M., Cooney, M.K., Crossley, J.R., Dornan, T.L., Raghu, P. and Palmer, J.P. (1982). Coxsackie B4 infection and islet-cell antibodies three years before overt diabetes. *J. Pediatr. 101*, 398–400.

Atkinson, M.A. and Wilson, S.B. (2002). Fatal attraction: chemokines and type 1 diabetes. *J. Clin. Invest. 110*, 1611–1613.

Atkinson, M.A., Kaufman, D.L., Campbell, L., Gibbs, K.A., Shah, S.C., Bu, D.F., Erlander, M.G., Tobin, A.J. and MacLaren, N.K. (1992). Response of peripheral-blood mononuclear cells to glutamate decarboxylase in insulin-dependent diabetes. *Lancet 339*, 458–459.

Atkinson, M.A., Bowman, M.A., Campbell, L., Darrow, B.L., Kaufman, D.L. and MacLaren, N.K. (1994). Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J. Clin. Invest. 94*, 2125–2129.

Bach, J.F. (1994). Insulin-dependent diabetes mellitus as an autoimmune disease. *Endocr. Rev. 15*, 516–542.

Bach, J.F. (2002a). Immunotherapy of type 1 diabetes: lessons for other autoimmune diseases. *Arthritis Res. 4*, S3–S15.

Bach, J.F. (2002b). The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med. 347*, 911–920.

Bach, J.F. and Mathis, D. (eds) (1997). The NOD mouse. 70th Forum in Immunology. *Res. Immunol. 148,* 285–366.

Baekkeskov, S., Aanstoot, H.J., Christgau, S., Reetz, A., Solimena, M., Cascalho, M., Folli, F., Richter-Olesen, H. and de Camilli, P. (1990). Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature 347*, 151–156.

Baker, F.J., Lee, M., Chien, Y.H. and Davis, M.M. (2002). Restricted islet-cell reactive T-cell repertoire of early pancreatic islet infiltrates in NOD mice. *Proc. Natl. Acad. Sci. USA 99*, 9374–9379.

Baxter, A.G. and Mandel, T.E. (1991). Hemolytic anemia in non-obese diabetic mice. *Eur. J. Immunol. 21*, 2051–2055.

Baxter, A.G., Horsfall, A.C., Healey, D., Ozegbe, P., Day, S., Williams, D.G. and Cooke, A. (1994). Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice. *Immunology 83*, 227–231.

Belghith, M., Bluestone, J.A., Barriot, S., Megret, J., Bach, J.F. and Chatenoud, L. (2003). TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat. Med. 9*, 1202–1208.

Bendelac, A., Carnaud, C., Boitard, C. and Bach, J.F. (1987). Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both L3T4+ and Lyt–2+ T-cells. *J. Exp. Med. 166*, 823–832.

Bendelac, A., Boitard, C., Bedossa, P., Bazin, H., Bach, J.F. and Carnaud, C. (1988). Adoptive T-cell transfer of autoimmune nonobese diabetic mouse diabetes does not require recruitment of host B lymphocytes. *J. Immunol. 141*, 2625–2628.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F. and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet. 27*, 20–21.

Bergman, M.L., Duarte, N., Campino, S., Lundholm, M., Motta, V., Lejon, K., Penha Goncalves, C. and Holmberg, D. (2003). Diabetes protection and restoration of thymocyte apoptosis in NOD Idd6 congenic strains. *Diabetes 52*, 1677–1682.

Boitard, C., Yasunami, R., Dardenne, M. and Bach, J.F. (1989). T-cell-mediated inhibition of the transfer of autoimmune diabetes in NOD mice. *J. Exp. Med. 169*, 1669–1680.

Bonn, D. (1999). Q fever vaccine on trial for type I diabetes. *Mol. Med. Today 5*,143.

Bosi, E., Braghi, S., Maffi, P., Scirpoli, M., Bertuzzi, F., Pozza, G., Secchi, A. and Bonifacio, E. (2001). Autoantibody response to islet transplantation in type 1 diabetes. *Diabetes 50*, 2464–2471.

Bottazzo, G.F., Florin-Christensen, A. and Doniach, D. (1974). Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet 2*, 1279–1283.

Bottazzo, G.F., Dean, B.M., McNally, J.M., MacKay, E.H., Swift, P.G. and Gamble, D.R. (1985). *In situ* characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. *N. Engl. J. Med. 313*, 353–360.

Boulard, O., Damotte, D., Deruytter, N., Fluteau, G., Carnaud, C. and Garchon, H.J. (2002). An interval tightly linked to but distinct from the H2 complex controls both overt diabetes (Idd16) and chronic experimental autoimmune thyroiditis (Ceat1) in nonobese diabetic mice. *Diabetes 51*, 2141–2147.

Bradley, B.J., Haskins, K., La Rosa, F.G. and Lafferty, K.J. (1992). CD8 T-cells are not required for islet destruction induced by a CD4+islet-specific T-cell clone. *Diabetes 41*, 1603–1608.

Brenner, H.H., Burkart, V., Rothe, H. and Kolb, H. (1993). Oxygen radical production is increased in macrophages from diabetes prone BB rats. *Autoimmunity 15*, 93–98.

Burch, G.E. and Harb, J.M. (1975). Coxsackie B4 virus crystal formation in mouse pancreas. *Proc. Soc. Exp. Biol. Med. 149*, 893–895.

Buschard, K. and Rygaard, J. (1978). T-lymphocytes transfer streptozotocin induced diabetes mellitus in mice. *Acta Pathol. Microbiol. Scand. 86C*, 277–282.

Campbell, I.L., Oxbrow, L., Koulmanda, M. and Harrison, L.C. (1988). IFN-gamma induces islet-cell MHC antigens and enhances autoimmune, streptozotocin-induced diabetes in the mouse. *J. Immunol. 140*, 1111–1116.

CERCTG (1988). The Canadian-European Randomized Control Trial Group. Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes 37*, 1574–1582.

Chatenoud, L., Thervet, E., Primo, J. and Bach, J.F. (1994). Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc. Natl. Acad. Sci. USA 91*, 123–127.

Coleman, T.J., Taylor, K.W. and Gamble, D.R. (1974). The development of diabetes following Coxsackie B virus infection in mice. *Diabetologia 10*, 755–759.

Craighead, J.E. and McLane, M.F. (1968). Diabetes mellitus: induction in mice by encephalomyocarditis virus. *Science 162*, 913–914.

Crisa, L., Mordes, J.P. and Rossini, A.A. (1992). Autoimmune diabetes mellitus in the BB rat. *Diabetes Metab. Rev. 8*, 9–37.

Daniel, D. and Wegmann, D.R. (1996). Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9–23). *Proc. Natl. Acad. Sci. USA 93*, 956–960.

Dardenne, M., Lepault, F., Bendelac, A. and Bach, J.F. (1989). Acceleration of the onset of diabetes in NOD mice by thymectomy at weaning. *Eur. J. Immunol. 19*, 889–895.

Davoodi-Semiromi, A., Yang, J.J. and She, J.X. (2002). IL-12p40 is associated with type 1 diabetes in Caucasian-American families. *Diabetes 51*, 2334–2336.

DCCT (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N. Engl. J. Med. 329*, 977–986.

Debray-Sachs, M., Carnaud, C., Boitard, C., Cohen, H., Gresser, I., Bedossa, P. and Bach, J.F. (1991). Prevention of diabetes in NOD mice treated with antibody to murine IFN gamma. *J. Autoimmun. 4,* 237–248.

Diez, J., Park, Y., Zeller, M., Brown, D., Garza, D., Ricordi, C., Hutton, J., Eisenbarth, G.S. and Pugliese, A. (2001). Differential splicing of the IA-2 mRNA in pancreas and lymphoid organs as a permissive genetic mechanism for autoimmunity against the IA-2 type 1 diabetes autoantigen. *Diabetes 50*, 895–900.

Durinovic-Bello, I., Hummel, M. and Ziegler, A.G. (1996). Cellular immune response to diverse islet-cell antigens in IDDM. *Diabetes 45*, 795–800.

Elliott, R.B. and Martin, J.M. (1984). Dietary protein: a trigger of insulin-dependent diabetes in the BB rat? *Diabetologia 26*, 297–299.

Endl, J., Otto, H., Jung, G., Dreisbusch, B., Donie, F., Stahl, P., Elbracht, R., Schmitz, G., Meinl, E., Hummel, M., Ziegler, A.G., Wank, R. and Schendel, D.J. (1997). Identification of naturally processed T-cell epitopes from glutamic acid decarboxylase presented in the context of HLA-DR alleles by T lymphocytes of recent onset IDDM patients. *J. Clin. Invest. 99*, 2405–2415.

EURODIAB (2000). Variation and trends in incidence of childhood diabetes in Europe. EURODIAB ACE Study Group. *Lancet 355*, 873–876.

Falorni, A. and Calcinaro, F. (2002). Autoantibody profile and epitope mapping in latent autoimmune diabetes in adults. *Ann. N. Y. Acad. Sci. 958*, 99–106.

Feutren, G., Papoz, L., Assan, R., Vialettes, B., Karsenty, G., Vexiau, P., Du Rostu, H., Rodier, M., Sirmai, J., Lallemand, A. and Bach, J.F. (1986). Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet 2*, 119–124.

Flier, J.S., Kahn, C.R., Roth, J. and Bar, R.S. (1975). Antibodies that impair insulin receptor binding in an unusual diabetic syndrome with severe insulin resistance. *Science 190*, 63–65.

Foulis, A.K., Farquharson, M.A. and Meager, A. (1987). Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. *Lancet 2*, 1423–1427.

Fowell, D. and Mason, D. (1993). Evidence that the T-cell repertoire of normal rats contains cells with the potential to cause diabetes. Characterization of the CD4+T-cell subset that inhibits this autoimmune potential. *J. Exp. Med. 177*, 627–636.

Frigerio, S., Junt, T., Lu, B., Gerard, C., Zumsteg, U., Hollander, G.A. and Piali, L. (2002). Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nat. Med. 8*, 1414–1420.

Funda, D.P., Kaas, A., Bock, T., Tlaskalova-Hogenova, H. and Buschard, K. (1999). Gluten-free diet prevents diabetes in NOD mice. *Diabetes Metab. Res. Rev. 15*, 323–327.

Gale, E.A. (2002). The rise of childhood type 1 diabetes in the 20th century. *Diabetes 51*, 3353–3361.

Gale, E.A., Bingley, P.J., Emmett, C.L. and Collier, T. (2004). European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes. *Lancet 363*, 925–931.

Gerling, I.C., Friedman, H., Greiner, D.L., Shultz, L.D. and Leiter, E.H. (1994). Multiple low-dose streptozocin-induced diabetes in NOD-scid/scid mice in the absence of functional lymphocytes. *Diabetes 43*, 433–440.

Gombert, J.M., Herbelin, A., Tancrede-Bohin, E., Dy, M., Carnaud, C. and Bach, J.F. (1996). Early quantitative and functional deficiency of NK1(+)-like thymocytes in the NOD mouse. *Eur. J. Immunol. 26*, 2989–2998.

Gonzalez, C., Menissier de Murcia, J., Janiak, P., Bidouard, J.P., Beauvais, C., Karray, S., Garchon, H.J. and Levi Strauss, M. (2002). Unexpected sensitivity of nonobese diabetic mice with a disrupted poly(ADP-Ribose) polymerase-1 gene to streptozotocin-induced and spontaneous diabetes. *Diabetes 51*, 1470–1476.

Gottlieb, P.A. and Eisenbarth, G.S. (1998). Diagnosis and treatment of pre-insulin dependent diabetes. *Annu. Rev. Med. 49*, 391–405.

Greeley, S.A., Katsumata, M., Yu, L., Eisenbarth, G.S., Moore, D.J., Goodarzi, H., Barker, C.F., Naji, A. and Noorchashm, H. (2002). Elimination of maternally transmitted autoantibodies prevents diabetes in nonobese diabetic mice. *Nat. Med. 8*, 399–402.

Green, E.A., Gorelik, L., McGregor, C.M., Tran, E.H. and Flavell, R.A. (2003). CD4+CD25+ T regulatory cells control anti-islet CD8+ T-cells through TGF-beta–TGF-beta receptor interactions in type 1 diabetes. *Proc. Natl. Acad. Sci. USA 100*, 10878–10883.

Guberski, D.L., Thomas, V.A., Shek, W.R., Like, A.A., Handler, E.S., Rossini, A.A., Wallace, J.E. and Welsh, R.M. (1991). Induction of type I diabetes by Kilham's rat virus in diabetes-resistant BB/Wor rats. *Science 254*, 1010–1013.

Halonen, M., Kangas, H., Ruppell, T., Ilmarinen, T., Ollila, J., Kolmer, M., Vihinen, M., Palvimo, J., Saarela, J., Ulmanen, I. and Eskelin, P. (2004). APECED-causing mutations in AIRE reveal the functional domains of the protein. *Hum. Mutat. 23*, 245–257.

Hanafusa, T., Miyazaki, A., Miyagawa, J., Tamura, S., Inada, M., Yamada, K., Shinji, Y., Katsura, H., Yamagata, K., Itoh, N., Asakawa, H., Nakagawa, C., Otsuka, A., Kawata, S., Kono, N. and Tarui, S. (1990). Examination of islets in the pancreas biopsy specimens from newly diagnosed type 1 (insulin-dependent) diabetic patients. *Diabetologia 33*, 105–111.

Haskins, K. and McDuffie, M. (1990). Acceleration of diabetes in young NOD mice with a CD4+ islet-specific T-cell clone. *Science 249*, 1433–1436.

Herold, K.C., Montag, A.G. and Fitch, F.W. (1987). Treatment with anti-T-lymphocyte antibodies prevents induction of insulitis in mice given multiple doses of streptozocin. *Diabetes 36*, 796–801.

Herold, K.C., Hagopian, W., Auger, J.A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., Gitelman, S.E., Harlan, D.M., Xu, D.L., Zivin, R.A. and Bluestone, J.A. (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med. 346*, 1692–1698.

Hoglund P., Mintern J., Waltzinger C., Heath W., Benoist C., Mathis D. (1999) Initiation of autoimmune diabetes by developmentally regulated presentation of islet-cell antigens in the pancreatic lymph nodes. *J. Exp. Med. 189*, 331–339.

Honeyman, M.C., Coulson, B.S., Stone, N.L., Gellert, S.A., Goldwater, P.N., Steele, C.E., Couper, J.J., Tait, B.D., Colman, P.G. and Harrison, L.C. (2000). Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes 49*, 1319–1324.

Hong, S., Wilson, M.T., Serizawa, I., Wu, L., Singh, N., Naidenko, O.V., Miura, T., Haba, T., Scherer, D.C., Wei, J., Kronenberg, M., Koezuka, Y. and Van Kaer, L. (2001). The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat. Med. 7*, 1052–1056.

Hornum, L., Jackerott, M. and Markholst, H. (1995). The rat T-cell lymphopenia resistance gene (Lyp) maps between D4Mit6 and Npy on RN04. *Mamm. Genome 6*, 371–372.

Horwitz, M.S., Bradley, L.M., Harbertson, J., Krahl, T., Lee, J. and Sarvetnick, N. (1998). Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nat. Med. 4*, 781–785.

Ide, A. and Eisenbarth, G.S. (2003). Genetic susceptibility in type 1 diabetes and its associated autoimmune disorders. *Rev. Endocr. Metab. Disord. 4*, 243–253.

Imagawa, A., Hanafusa, T., Tamura, S., Moriwaki, M., Itoh, N., Yamamoto, K., Iwahashi, H., Yamagata, K., Waguri, M., Nanmo, T., Uno, S., Nakajima, H., Namba, M., Kawata, S., Miyagawa, J.I. and Matsuzawa, Y. (2001). Pancreatic biopsy as a procedure for detecting *in situ* autoimmune phenomena in type 1 diabetes: close correlation between serological markers and histological evidence of cellular autoimmunity. *Diabetes 50*, 1269–1273.

Johansson, S., Lie, B.A., Todd, J.A., Pociot, F., Nerup, J., Cambon-Thomsen, A., Kockum, I., Akselsen, H.E., Thorsby, E. and Undlien, D.E. (2003). Evidence of at least two type 1 diabetes susceptibility genes in the HLA complex distinct from HLA-DQB1, -DQA1 and -DRB1. *Genes Immun. 4,* 46–53.

Juhela, S., Hyoty, H., Roivainen, M., Harkonen, T., Putto Laurila, A., Simell, O. and Ilonen, J. (2000). T-cell responses to enterovirus antigens in children with type 1 diabetes. *Diabetes 49*, 1308–1313.

Jun, H.S. and Yoon, J.W. (2003). A new look at viruses in type 1 diabetes. *Diabetes Metab. Res. Rev. 19,* 8–31.

Karjalainen, J., Martin, J.M., Knip, M., Ilonen, J., Robinson, B.H., Savilahti, E., Akerblom, H.K. and Dosch, H.M. (1992). A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N. Engl. J. Med. 327*, 302–307.

Karvonen, M., Sekikawa, A., La Porte, R., Tuomilehto, J. and Tuomilehto-Wolf, E. (2001). Type 1 diabetes: global epidemiology. In J.M. Ekoe, P. Zimmet and R. Williams (eds), *The Epidemiology of Diabetes Mellitus. An International Perspective* (pp. 71–102). Chichester: Wiley.

Katz, J.D., Wang, B., Haskins, K., Benoist, C. and Mathis, D. (1993). Following a diabetogenic T-cell from genesis through pathogenesis. *Cell 74*, 1089–1100.

Kaufman, D.L., Clare-Salzler, M., Tian, J., Forsthuber, T., Ting, G.S.P., Robinson, P., Atkinson, M.A., Sercarz, E.E., Tobin, A.J. and Lehmann, P.V. (1993). Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature 366*, 69–72.

Keymeulen, B., Vandemeulebroucke, E., Ziegler, A.G., Mathieu, C., Kaufman, L., Hale, G., Gorus, F., Goldman, M., Walter, M., Candon, S., Schandene, L., Crenier, L., De Block, C., Seigneurin, J-M., De Pauw, P., Pierard, D., Weets, I., Rebellow, P., Bird, P., Berrie, E., Frewin, M., Waldmann, H., Bach, J-F., Pipeleers, D., and Chatenoud, L. (2005). Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N. Engl. J. Med. 352,* 2598–2608.

Kim, S.H., Cleary, M.M., Fox, H.S., Chantry, D. and Sarvetnick, N. (2002). CCR4-bearing T-cells participate in autoimmune diabetes. *J. Clin. Invest. 110*, 1675–1686.

King, C., Ilic, A., Koelsch, K. and Sarvetnick, N. (2004). Homeostatic expansion of T-cells during immune insufficiency generates autoimmunity. *Cell 117*, 265–277.

Kishimoto, H. and Sprent, J. (2001). A defect in central tolerance in NOD mice. *Nat. Immunol. 2*, 1025–1031.

Kukreja, A., Cost, G., Marker, J., Zhang, C., Sun, Z., Lin Su, K., Ten, S., Sanz, M., Exley, M., Wilson, B., Porcelli, S. and MacLaren, N. (2002). Multiple immuno-regulatory defects in type-1 diabetes. *J. Clin. Invest. 109*, 131–140.

Kurrer, M.O., Pakala, S.V., Hanson, H.L. and Katz, J.D. (1997). Beta cell apoptosis in T-cell-mediated autoimmune diabetes. *Proc. Natl. Acad. Sci. USA 94*, 213–218.

Lampeter, E.F., Klinghammer, A., Scherbaum, W.A., Heinze, E., Haastert, B., Giani, G. and Kolb, H. (1998). The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. *Diabetes 47*, 980–984.

Lan, M.S., Wasserfall, C., MacLaren, N.K. and Notkins, A.L. (1996). IA-2, a transmembrane protein of the protein tyrosine phosphatase family, is a major autoantigen in insulin-dependent diabetes mellitus. *Proc. Natl. Acad. Sci. USA 93*, 6367–6370.

Larger, E., Becourt, C., Bach, J.F. and Boitard, C. (1995). Pancreatic islet beta cells drive T-cell-immune responses in the nonobese diabetic mouse model. *J. Exp. Med. 181*, 1635–1642.

Lenschow, D.J., Herold, K.C., Rhee, L., Patel, B., Koons, A., Qin, H.Y., Fuchs, E., Singh, B., Thompson, C.B. and Bluestone, J.A. (1996). CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. *Immunity 5*, 285–293.

Lesage, S., Hartley, S.B., Akkaraju, S., Wilson, J., Townsend, M. and Goodnow, C.C. (2002). Failure to censor forbidden clones of CD4 T-cells in autoimmune diabetes. *J. Exp. Med. 196*, 1175–1188.

Lie, B.A., Todd, J.A., Pociot, F., Nerup, J., Akselsen, H.E., Joner, G., Dahl-Jorgensen, K., Ronningen, K.S., Thorsby, E. and Undlien, D.E. (1999). The predisposition to type 1 diabetes linked to the human leukocyte antigen complex includes at least one non-class II gene. *Am. J. Hum. Genet. 64*, 793–800.

Lieberman, S.M., Evans, A.M., Han, B., Takaki, T., Vinnitskaya, Y., Caldwell, J.A., Serreze, D.V., Shabanowitz, J., Hunt, D.F., Nathenson, S.G., Santamaria, P. and Dilorenzo, T.P. (2003). Identification of the beta cell antigen targeted by a prevalent population of pathogenic CD8+ T-cells in autoimmune diabetes. *Proc. Natl. Acad. Sci. USA 100*, 8384–8388.

Like, A.A., Rossini, A.A., Guberski, D.L., Appel, M.C. and Williams, R.M. (1979). Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. *Science 206*, 1421–1423.

Liu, C.P., Jiang, K., Wu, C.H., Lee, W.H. and Lin, W.J. (2000). Detection of glutamic acid decarboxylase-activated T-cells with I-Ag7 tetramers. *Proc. Natl. Acad. Sci. USA 97*, 14596–14601.

Lonnrot, M., Korpela, K., Knip, M., Ilonen, J., Simell, O., Korhonen, S., Savola, K., Muona, P., Simell, T., Koskela, P. and Hyoty, H. (2000). Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. *Diabetes 49*, 1314–1318.

Makino, S., Kunimoto, K., Muraoka, Y., Mizushima, Y., Katagiri, K. and Tochino, Y. (1980). Breeding of a non-obese, diabetic strain of mice. *Exp. Anim. 29*, 1–13.

Mandrup-Poulsen, T. (2003). Apoptotic signal transduction pathways in diabetes. *Biochem. Pharmacol. 66*, 1433–1440.

Martin, C.C., Bischof, L.J., Bergman, B., Hornbuckle, L.A., Hilliker, C., Frigeri, C., Wahl, D., Svitek, C.A., Wong, R., Goldman, J.K., Oeser, J.K., Lepretre, F., Froguel, P., O'Brien, R.M. and Hutton, J.C. (2001). Cloning and characterization of the human and rat islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) genes. *J. Biol. Chem. 276*, 25197–25207.

Maugendre, D., Sonnet, E., Derrien, C., Le Golff, M.C., Grais, M.C., Allannic, H. and Delamaire, M. (1999). Combined analysis of long-term anti-beta-cell humoral reactivity in type 1 diabetes with and without thyroid disease. *Diabetes Metab. 25*, 28–33.

Mirel, D.B., Valdes, A.M., Lazzeroni, L.C., Reynolds, R.L., Erlich, H.A. and Noble, J.A. (2002). Association of IL4R haplotypes with type 1 diabetes. *Diabetes 51*, 3336–3341.

Moore, A., Bonner Weir, S. and Weissleder, R. (2001). Noninvasive *in vivo* measurement of beta-cell mass in mouse model of diabetes. *Diabetes 50*, 2231–2236.

Moriyama, H., Wen, L., Abiru, N., Liu, E., Yu, L., Miao, D., Gianani, R., Wong, F.S. and Eisenbarth, G.S. (2002). Induction and acceleration of insulitis/diabetes in mice with a viral mimic (polyinosinic-polycytidylic acid) and an insulin self-peptide. *Proc. Natl. Acad. Sci. USA 99*, 5539–5544.

Moriyama, H., Abiru, N., Paronen, J., Sikora, K., Liu, E., Miao, D., Devendra, D., Beilke, J., Gianani, R., Gill, R.G. and Eisenbarth, G.S. (2003). Evidence for a primary islet autoantigen (preproinsulin 1) for insulitis and diabetes in the nonobese diabetic mouse. *Proc. Natl. Acad. Sci. USA 100*, 10376–10381.

Muir, A., Peck, A., Clare-Salzler, M., Song, Y.H., Cornelius, J., Luchetta, R., Krischer, J. and MacLaren, N. (1995). Insulin immunization of nonobese diabetic mice induces a protective insulitis characterized by diminished intraislet interferon-gamma transcription. *J. Clin. Invest. 95*, 628–634.

Nakhooda, A.F., Like, A.A., Chappel, C.I., Murray, F.T. and Marliss, E.B. (1977). The spontaneously diabetic Wistar rat. Metabolic and morphologic studies. *Diabetes 26*, 100–112.

Narendran, P., Mannering, S.I. and Harrison, L.C. (2003). Proinsulin – a pathogenic autoantigen in type 1 diabetes. *Autoimmun. Rev. 2*, 204–210.

Nerup, J., Platz, P., Andersen, O.O., Christy, M., Lyngsoe, J., Poulsen, J.E., Ryder, L.P., Nielsen, L.S., Thomsen, M. and Svejgaard, A. (1974). HL-A antigens and diabetes mellitus. *Lancet 2*, 864–866.

Ohashi, P.S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C.T., Odermatt, B., Malissen, B., Zinkernagel, R.M. and Hengartner, H. (1991). Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell 65*, 305–317.

Oldstone, M.B. (1990). Viruses as therapeutic agents. I. Treatment of nonobese insulin-dependent diabetes mice with virus prevents insulin-dependent diabetes mellitus while maintaining general immune competence. *J. Exp. Med. 171*, 2077–2089.

Orlow, S., Yasunami, R., Boitard, C. and Bach, J.F. (1987). Induction précoce du diabète chez la souris NOD par la streptozotocine. *C. R. Acad. Sci. III 304*, 77–78.

Paik, S.G., Fleischer, N. and Shin, S.I. (1980). Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell-mediated autoimmune processes. *Proc. Natl. Acad. Sci. USA 77*, 6129–6133.

Patterson, C.C., Carson, D.J. and Hadden, D.R. (1996). Epidemiology of childhood IDDM in Northern Ireland 1989–1994: low incidence in areas with highest population density and most household crowding. *Diabetologia 39*, 1063–1069.

Peakman, M., Stevens, E.J., Lohmann, T., Narendran, P., Dromey, J., Alexander, A., Tomlinson, A.J., Trucco, M., Gorga, J.C. and Chicz, R.M. (1999). Naturally processed and presented epitopes of the islet-cell autoantigen IA-2 eluted from HLA-DR4. *J. Clin. Invest. 104*, 1449–1457.

Pennline, K.J., Roque-Gaffney, E. and Monahan, M. (1994). Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin. Immunol. Immunopathol. 71*, 169–175.

Peterson, P., Pitkanen, J., Sillanpaa, N. and Krohn, K. (2004). Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED): a model disease to study molecular aspects of endocrine autoimmunity. *Clin. Exp. Immunol. 135*, 348–357.

Pociot, F. and McDermott, M.F. (2002). Genetics of type 1 diabetes mellitus. *Genes Immun. 3*, 235–249.

Poussier, P., Nakhooda, A.F., Falk, J.A., Lee, C. and Marliss, E.B. (1982). Lymphopenia and abnormal lymphocyte subsets in the "BB" rat: relationship to the diabetic syndrome. *Endocrinology 110*, 1825–1827.

Pozzilli, P. and Di Mario, U. (2001). Autoimmune diabetes not requiring insulin at diagnosis (latent autoimmune diabetes of the adult): definition, characterization, and potential prevention. *Diabetes Care 24*, 1460–1467.

Pugliese, A., Brown, D., Garza, D., Murchison, D., Zeller, M., Redondo, M., Diez, J., Eisenbarth, G.S., Patel, D.D. and Ricordi, C. (2001). Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. *J. Clin. Invest. 107*, 555–564.

Qin, H.Y., Sadelain, M.W., Hitchon, C., Lauzon, J. and Singh, B. (1993). Complete Freund's adjuvant-induced T-cells prevent the development and adoptive transfer of diabetes in nonobese diabetic mice. *J. Immunol. 150*, 2072–2080.

Rabinovitch, A., Suarez-Pinzon, W.L., Sorensen, O., Bleackley, R.C. and Power, R.F. (1995). IFN-gamma gene expression in pancreatic islet-infiltrating mononuclear cells correlates with autoimmune diabetes in nonobese diabetic mice. *J. Immunol. 154*, 4874–4882.

Ramanathan, S., Bihoreau, M.T., Paterson, A.D., Marandi, L., Gauguier, D. and Poussier, P. (2002). Thymectomy and radiation-induced type 1 diabetes in nonlymphopenic BB rats. *Diabetes 51*, 2975–2981.

Rapoport, M.J., Jaramillo, A., Zipris, D., Lazarus, A.H., Serreze, D.V., Leiter, E.H., Cyopick, P., Danska, J.S. and Delovitch, T.L. (1993). Inter-

leukin 4 reverses T-cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J. Exp. Med. 178*, 87–99.

Raz, I., Elias, D., Avron, A., Tamir, M., Metzger, M. and Cohen, I.R. (2001). Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet 358*, 1749–1753.

Reijonen, H., Novak, E.J., Kochik, S., Heninger, A., Liu, A.W., Kwok, W.W. and Nepom, G.T. (2002). Detection of GAD65-specific T-cells by major histocompatibility complex class II tetramers in type 1 diabetic patients and at-risk subjects. *Diabetes 51*, 1375–1382.

Roep, B.O., Atkinson, M.A., Van Endert, P.M., Gottlieb, P.A., Wilson, S.B. and Sachs, J.A. (1999). Autoreactive T-cell responses in insulin-dependent (Type 1) diabetes mellitus. Report of the first international workshop for standardization of T-cell assays. *J. Autoimmun. 13*, 267–282.

Rose, N.R. and Bona, C. (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today 14*, 426–430.

Sarukhan, A., Bedossa, P., Garchon, H.J., Bach, J.F. and Carnaud, C. (1995). Molecular analysis of TCR junctional variability in individual infiltrated islets of non-obese diabetic mice: evidence for the constitution of largely autonomous T-cell foci within the same pancreas. *Int. Immunol. 7*, 139–146.

Scheinin, T. (1988). Characteristics of blood cells responding to insulin in children with insulin-dependent diabetes. *Immunol. Lett. 17*, 273–277.

Schloot, N.C., Meierhoff, G., Karlsson Faresjo, M., Ott, P., Putnam, A., Lehmann, P., Gottlieb, P., Roep, B.O., Peakman, M. and Tree, T. (2003). Comparison of cytokine ELISpot assay formats for the detection of islet antigen autoreactive T-cells. Report of the third immunology of diabetes society T-cell workshop. *J. Autoimmun. 21*, 365–376.

Scott, B., Liblau, R., Degermann, S., Marconi, L.A., Ogata, L., Caton, A.J., McDevitt, H.O. and Lo, D. (1994). A role for non-MHC genetic polymorphism in susceptibility to spontaneous autoimmunity. *Immunity 1*, 73–82.

Sempe, P., Bedossa, P., Richard, M.F., Villa, M.C., Bach, J.F. and Boitard, C. (1991). Anti-alpha/beta T-cell receptor monoclonal antibody provides an efficient therapy for autoimmune diabetes in nonobese diabetic (NOD) mice. *Eur. J. Immunol. 21*, 1163–1169.

Serreze, D.V., Fleming, S.A., Chapman, H.D., Richard, S.D., Leiter, E.H. and Tisch, R.M. (1998). B lymphocytes are critical antigen-presenting cells for the initiation of T-cell-mediated autoimmune diabetes in nonobese diabetic mice. *J. Immunol. 161*, 3912–3918.

Serreze, D.V., Post, C.M., Chapman, H.D., Johnson, E.A., Lu, B.F. and Rothman, P.B. (2000). Interferon-gamma receptor signaling is dispensable in the development of autoimmune type 1 diabetes in NOD mice. *Diabetes 49*, 2007–2011.

Serreze, D.V., Chapman, H.D., Post, C.M., Johnson, E.A., Suarez-Pinzon, W.L. and Rabinovitch, A. (2001). Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. *J. Immunol. 166*, 1352–1359.

Shah, S.C., Malone, J.I. and Simpson, N.E. (1989). A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N. Engl. J. Med. 320*, 550–554.

Sharif, S., Arraeza, G.A., Zucker, P., Mi, Q.S., Sondhi, J., Naidenko, O.V., Kronenberg, M., Koezuka, Y., Delovitch, T.L., Gombert, J.M., Leite de Moraes, M., Gouarin, C., Zhu, R., Hameg, A., Nakayama, T., Taniguchi, M., Lepault, F., Lehuen, A., Bach, J.F. and Herbelin, A. (2001). Activation of natural killer T-cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat. Med. 7*, 1057–1062.

Shizuru, J.A., Taylor-Edwards, C., Livingstone, A. and Fathman, C.G. (1991). Genetic dissection of T-cell receptor V beta gene requirements for spontaneous murine diabetes. *J. Exp. Med. 174*, 633–638.

Silverstein, J., MacLaren, N., Riley, W., Spillar, R., Radjenovic, D. and Johnson, S. (1988). Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N. Engl. J. Med. 319*, 599–604.

Sreenan, S., Pick, A.J., Levisetti, M., Baldwin, A.C., Pugh, M. and Polonsky, K.S. (1999). Increased beta-cell proliferation and reduced mass before diabetes onset in the nonobese diabetic mouse. *Diabetes 48*, 989–996.

Srikanta, S., Ricker, A.T., McCulloch, D.K., Soeldner, J.S., Eisenbarth, G.S. and Palmer, J.P. (1986). Autoimmunity to insulin, beta cell dysfunction, and development of insulin-dependent diabetes mellitus. *Diabetes 35*, 139–142.

Staines, A., Hanif, S., Ahmed, S., McKinney, P.A., Shera, S. and Bodansky, H.J. (1997). Incidence of insulin dependent diabetes mellitus in Karachi, Pakistan. *Arch. Dis. Child. 76*, 121–123.

Strandell, E., Eizirik, D.L. and Sandler, S. (1990). Reversal of beta-cell suppression *in vitro* in pancreatic islets isolated from nonobese diabetic mice during the phase preceding insulin-dependent diabetes mellitus. *J. Clin. Invest. 85*, 1944–1950.

Tarbell, K.V., Lee, M., Ranheim, E., Chao, C.C., Sanna, M., Kim, S.K., Dickie, P., Teyton, L., Davis, M. and McDevitt, H. (2002). CD4(+) T-cells from glutamic acid decarboxylase (GAD)65-specific T-cell receptor transgenic mice are not diabetogenic and can delay diabetes transfer. *J. Exp. Med. 196*, 481–492.

Thivolet, C., Bendelac, A., Bedossa, P., Bach, J.F. and Carnaud, C. (1991). CD8+ T-cell homing to the pancreas in the nonobese diabetic mouse is CD4+ T-cell-dependent. *J. Immunol. 146*, 85–88.

Tisch, R., Yang, X.D., Singer, S.M., Liblau, R.S., Fugger, L. and McDevitt, H.O. (1993). Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. *Nature 366*, 72–75.

Tisch, R., Wang, B. and Serreze, D.V. (1999). Induction of glutamic acid decarboxylase 65-specific Th2 cells and suppression of autoimmune diabetes at late stages of disease is epitope dependent. *J. Immunol. 163*, 1178–1187.

Todd, J.A. (1999). From genome to aetiology in a multifactorial disease, type 1 diabetes. *Bioessays 21*, 164–174.

Todd, J.A., Bell, J.I. and McDevitt, H.O. (1987). HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature 329*, 599–604.

Trembleau, S., Penna, G., Bosi, E., Mortara, A., Gately, M.K. and Adorini, L. (1995). Interleukin 12 administration induces T helper type 1 cells and accelerates autoimmune diabetes in NOD mice. *J. Exp. Med. 181*, 817–821.

Trudeau, J.D., Dutz, J.P., Arany, E., Hill, D.J., Fieldus, W.E. and Finegood, D.T. (2000). Neonatal beta-cell apoptosis: a trigger for autoimmune diabetes? *Diabetes 49*, 1–7.

Turner, R., Stratton, I., Horton, V., Manley, S., Zimmet, P., MacKay, I.R., Shattock, M., Bottazzo, G.F. and Holman, R. (1997). UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. *Lancet 350*, 1288–1293.

Umpierrez, G.E., Latif, K.A., Murphy, M.B., Lambeth, H.C., Stentz, F., Bush, A. and Kitabchi, A.E. (2003). Thyroid dysfunction in patients with type 1 diabetes—a longitudinal study. *Diabetes Care 26*, 1181–1185.

Vanderlugt, C.L. and Miller, S.D. (2002). Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat. Rev. Immunol. 2*, 85–95.

Verge, C.F., Vardi, P., Babu, S., Bao, F., Erlich, H.A., Bugawan, T., Tiosano, D., Yu, L., Eisenbarth, G.S. and Fain, P.R. (1998). Evidence for oligogenic inheritance of type 1 diabetes in a large Bedouin Arab family. *J. Clin. Invest. 102*, 1569–1575.

Von Herrath, M.G. (2002). Regulation of virally induced autoimmunity and immunopathology: contribution of LCMV transgenic models to understanding autoimmune insulin-dependent diabetes mellitus. *Curr. Top. Microbiol. Immunol. 263*, 145–175.

Von Herrath, M.G., Homann, D., Gairin, J.E. and Oldstone, M.B. (1997). Pathogenesis and treatment of virus-induced autoimmune diabetes: novel insights gained from the RIP-LCMV transgenic mouse model. *Biochem. Soc. Trans. 25*, 630–635.

Wang, B., Gonzalez, A., Hoglund, P., Katz, J.D., Benoist, C. and Mathis, D. (1998). Interleukin-4 deficiency does not exacerbate disease in NOD mice. *Diabetes 47*, 1207–1211.

Wang, B., Geng, Y.B. and Wang, C.R. (2001). CD1-restricted NK T-cells protect nonobese diabetic mice from developing diabetes. *J. Exp. Med. 194*, 313–320.

Warram, J.H., Krolewski, A.S., Gottlieb, M.S. and Kahn, C.R. (1984). Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. *N. Engl. J. Med. 311*, 149–152.

Wen, L., Chen, N.Y., Tang, J., Sherwin, R. and Wong, F.S. (2001). The regulatory role of DR4 in a spontaneous diabetes DQ8 transgenic model. *J. Clin. Invest. 107*, 871–880.

Wicker, L.S., Todd, J.A. and Peterson, L.B. (1995). Genetic control of autoimmune diabetes in the NOD mouse. *Annu. Rev. Immunol. 13*, 179–200.

Wildin, R.S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J.L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., Perroni, L., Bricarelli, F.D., Byrne, G., McEuen, M., Proll, S., Appleby, M. and Brunkow, M.E. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet. 27*, 18–20.

Wilson, S.B., Kent, S.C., Patton, K.T., Orban, T., Jackson, R.A., Exley, M., Porcelli, S., Schatz, D.A., Atkinson, M.A., Balk, S.P., Strominger, J.L. and Hafler, D.A. (1998). Extreme Th1 bias of invariant V-alpha–24J-alpha-Q T-cells in type 1 diabetes. *Nature 391*, 177–186.

Wong, F.S., Visintin, I., Wen, L., Flavell, R.A. and Janeway, C.A. (1996). CD8 T-cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in NOD mice in the absence of CD4 cells. *J. Exp. Med. 183*, 67–76.

Wong, S., Guerder, S., Visintin, I., Reich, E.P., Swenson, K.E., Flavell, R.A. and Janeway, C.A. (1995). Expression of the co-stimulator molecule B7-1 in pancreatic beta-cells accelerates diabetes in the NOD mouse. *Diabetes 44*, 326–329.

Yang, Y. and Santamaria, P. (2003). Dissecting autoimmune diabetes through genetic manipulation of non-obese diabetic mice. *Diabetologia 46*, 1447–1464.

Yasunami, R. and Bach, J.F. (1988). Anti-suppressor effect of cyclophosphamide on the development of spontaneous diabetes in NOD mice. *Eur. J. Immunol. 18*, 481–484.

You, S., Slehoffer, G., Barriot, S., Chatenoud, L. and Bach, J.F. (2004). Unique role of CD4+ CD62L+ regulatory T-cells in the control of autoimmune diabetes in T-cell receptor transgenic mice. *Proc. Natl. Acad. Sci. USA, 101* (Suppl 2),14580–14585.

Zaccone, P., Fehervari, Z., Jones, F.M., Sidobre, S., Kronenberg, M., Dunne, D.W. and Cooke, A. (2003). *Schistosoma mansoni* antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *Eur. J. Immunol. 33*, 1439–1449.

Zhang, Z.J., Davidson, L., Eisenbarth, G. and Weiner, H.L. (1991). Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc. Natl. Acad. Sci. USA 88*, 10252–10256.

CHAPTER

37

Adrenalitis

CORRADO BETTERLE

Chair of Clinical Immunology
Department of Medical and Surgical Sciences, University of Padova, Padova, Italy

RENATO ZANCHETTA

Endocrinologist, Endocrine Unit, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy

In 1564, Bartolomeo Eustachius wrote of the discovery of the "*glandulae quae renibus incumbent*" and subsequently Casserius (1561–1616) validated the discovery, depicting and naming them as "*corpuscola reni incumbentia sive renes succenturiati*" (Hiatt and Hiatt, 1997).

In 1855, Thomas Addison first proved that adrenals were vital organs when he described the symptoms and signs of patients with "anaemia . . . feebleness of the heart action . . . a peculiar change of colour in the skin occurring in connection with a diseased condition of the suprarenal capsules." He called this disorder "melasma suprarenale," postulating that it may be due to abnormal lesions in the adrenal glands. In this first description from the autopsies of 11 patients, he found six cases with tuberculosis, three with malignancies, one with adrenal hemorrhage, and one with adrenal fibrosis of an unknown origin. Probably, this last case was the first description of an autoimmune adrenalitis. In this case Addison reported: "The two adrenals together weighed 49 grains, they appeared exceedingly small and atrophied, so that the diseased condition did not result as usual from a deposit either of a strumous or malignant character, but appears to have been occasioned by an actual inflammation, that inflammation having destroyed the integrity of the organs, which finally led to their contraction and atrophy" (Addison, 1868). In 1856, the adrenal insufficiency was named "Addison's disease" by Trousseau.

ANATOMY AND PHYSIOLOGY OF THE ADRENALS

The adrenal glands develop from the mesenchyme: the outer part (cortex) from the mesoderm and the central part (medulla) from the neuroectoderm, which comprises part of the chromaffin system (Kannan, 1988).

The cortex is divided into three layers. The zona glomerulosa (5–10% of the cortex) comprises discontinuous subcapsular aggregates of small cells, containing less cytoplasm than the other cortical cells; the middle zone or fasciculata (70% of the cortex), is formed by radial cords of large cells arranged in columns with abundant lipid-filled cytoplasm; the inner cortical zone or zona reticularis is composed of cells arranged in cords with compact, finely granular, eosinophilic cytoplasm (Figure 37.1A).

The adrenal cortex synthesizes three main groups of hormones (the glucocorticoids, the mineralocorticoids, and the adrenal androgens) (Auchus and Miller, 2001). The homeostasis of glucocorticoids is regulated by a feedback mechanism through the hypothalamus by means of corticotropin-releasing hormone (CRH), the pituitary gland by ACTH, and the adrenal cortex by cortisol (Koch, 2004). The main steps in the synthesis of the adrenal cortex hormones and the enzymes involved are described in Figure 37.2.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

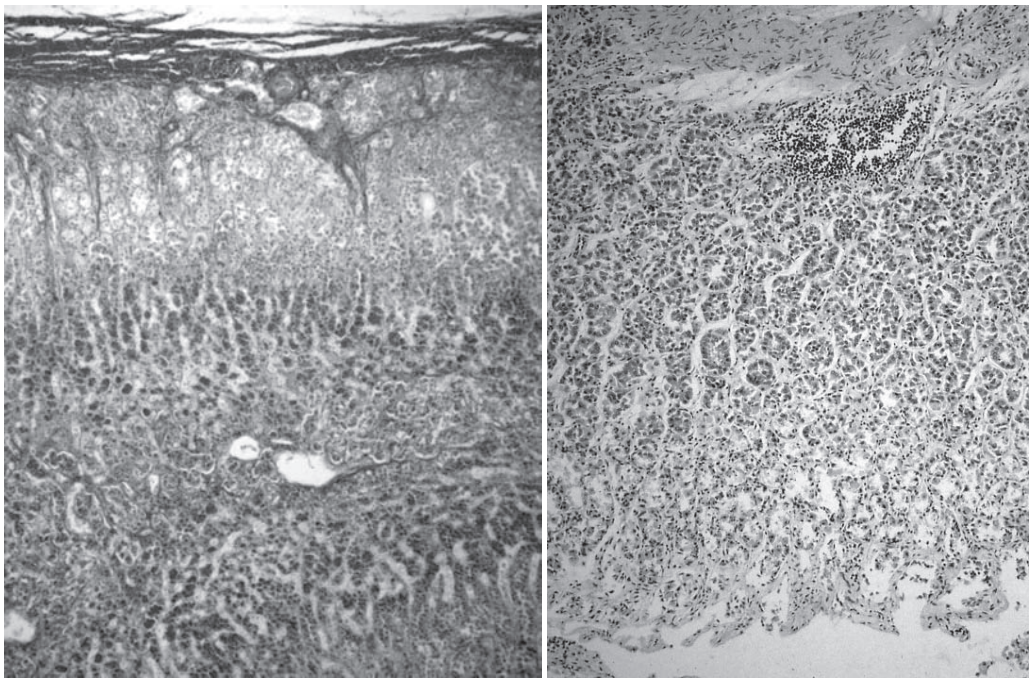


FIGURE 37.1 *A,* Histopathology of the normal adrenal cortex showing the typical three layers. *B,* Adrenal cortex from a patient with AAD showing atrophy of the cortex and a diffuse lymphocytic infiltration and, at top, a lymphoid follicle.

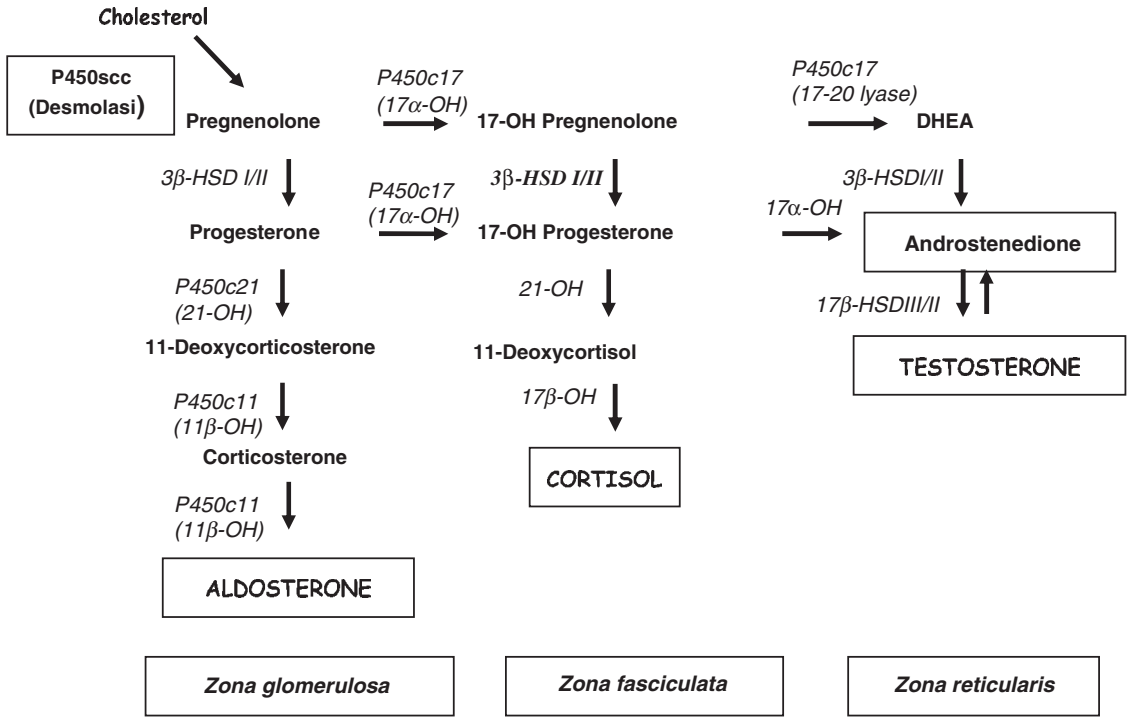


FIGURE 37.2 Main steps of the pathway of adrenal cortex hormone synthesis and the respective enzymes.

By immunocytochemical techniques, the normal adrenal cortex shows the presence of many proteins (Thiebaut et al., 1987; Henzen-Logmans et al.,1988; Sasano et al.,1989; 1994; Muscatelli et al.,1994; Fogt et al.,1998; Pelkey et al.,1998). Immunopositivity has been demonstrated for the class II major histocompatibility (MHC) complexes (found in 10–20% of the cortical cells) including the human antigen D-related leukocyte (HLA DR) (McNicol, 1986; Jackson et al., 1988) and interleukin-6 (Gonzalez-Hernandez et al.,1994) which is involved in the communication process between the immune and endocrine system. Using specific antibodies against the steroidogenic enzymes and cytochrome P450 (Sasano et al., 1992), it was demonstrated that cytochrome P450 is involved in the adrenal steroid biosynthesis process, while the AdBP/SF-1 transcription factor regulates the expression of the CYP genes (Orth and Kavacs, 1998).

EPIDEMIOLOGY OF ADDISON'S DISEASE AND AUTOIMMUNE ADRENALITIS

Addison's disease (AD) is a very rare disorder and formerly tuberculosis was the most frequent cause. Of the 11 cases of AD in 1855, six (55%) had tuberculosis and only one (9%) had autoimmune AD (AAD). Guttman (1930) examined 566 autopsied patients with AD and found 70% had tuberculous adrenalitis. Dunlop (1963) reviewed 86 cases of AD and reported 79% with tuberculous adrenalitis. In London, Mason et al. (1968) reported that 31% of cases had the tuberculous form of AD. In Denmark, Nerup (1974) found that tuberculosis was present in 17% of patients with AD. In more recent years, AAD has become the most frequent cause of AD. In Europe, from 1974 to 2002, 1557 patients with AD were evaluated and the frequency of AAD ranged from 44.5% to 94%; in contrast, AD due to tuberculosis was found with a frequency ranging from 0% to 33%, and the remaining forms of AD ranged from 1% to 22% (Betterle et al., 2002).

Despite a constant decrease of tuberculous forms of AD in recent years, the prevalence of AD has increased and this may be due to the absolute increase of AAD. Indeed, in London in 1968, Mason et al. found a prevalence of AD of 39 cases per million. In 1974 in Denmark, Nerup calculated a prevalence of 60 cases per million, while in Coventry in 1997, Willis and Vince found a prevalence of 93 cases per million. In Nottingham in 1994, Kong and Jeffocoate reported a prevalence of AD of 110 cases per million, while in Italy Laureti et al. (1999) calculated 117 cases per million. Recently the prevalence of AD was reported to be 140 cases per million in a population of western Norway with a calculated incidence, in the past decade, of 6.2 new cases per million per year (Løvås and Husebye, 2002).

The frequency of AD varies also in relation to different geographic areas: in New Zealand, Eason et al. (1982) calculated it to be 4.5 cases per million; in Japan it is five cases per million (Takayanagi et al., 2000); in the USA 50 cases per million (Jacobson et al., 1997); and in North Europe, more than 100 cases per million, as mentioned above (Kong and Jeffocoate, 1994; Laureti et al., 1999; Løvås and Husebye, 2002).

AUTOIMMUNE ADDISON'S DISEASE (AAD)

AAD depends on a combination of genetic, environmental, and endogenous factors able to both induce a break of immune tolerance and initiate an autoimmune attack on the adrenal cortex, as for other autoimmune diseases (Kamradt and Mitchinson, 2001).

Histopathology

Focal Lymphocytic Adrenalitis

In 1933 (Duff and Bernstein, 1933) and in 1969 (Kiaer and Rytter Norgaard, 1969) workers described a focal adrenalitis in patients without signs or symptoms of AD. Petri and Nerup (1971), studying two groups of patients (413 and 161 miscellaneous cases), reported the presence of very small numbers of lymphocytes in 15% and 18.6% of the adrenal glands, respectively. Subsequently, in up to half of the autopsied patients without AD, a focal accumulation of lymphocytes and plasma cells was demonstrated in the adrenal cortex, associated with chronic inflammatory diseases in the retroperitoneum (Fidler, 1977; Orth and Kavacs, 1998). The percentage of these infiltrates was similar to that reported in focal thyroiditis. In 1989, Hayashi et al. evaluated 174 cases at autopsy and demonstrated that mononuclear cell infiltration in the adrenal cortex increased with age, being present in about in 7.4% of those aged over 49 years and 63% aged over 60 years. Immunohistochemical studies revealed that the infiltrating mononuclear cells were mainly composed of $CD3^+$ T cells. The major proportion of $CD3^+$ T cells express the CD4 phenotype, whereas $CD8^+$ T cells were fewer. A proportion of the $CD4^+$ T cells were activated (Hayashi et al., 1989). These findings indicate that the focal lymphocytic infiltration of the adrenal glands is not rare and may represent a latent adrenalitis, which, however, rarely reaches clinical expression since symptomatic autoimmune adrenalitis (AA) is extremely rare in the population.

Diffuse Lymphocytic Adrenalitis

The pathologic findings initially described by Addison (1855) as "idiopathic" are constantly present in the adrenal

glands of patients affected by AA. On macroscopic examination, both the adrenals are small (weight 1–2 g) and sometimes it is difficult to identify them in the retroperitoneal tissue. The capsule is fibrous, so that the adrenal glands are not detectable macroscopically and adrenal tissue is not detectable in multiple sections (Drury et al., 1979).

On microscopic examination, there is complete destruction of the three-layer architecture. The adrenal cortical cells are single, enlarged or pleomorphic, with increased eosinophilia, depleted in lipids or present as part of a cluster. Residual cortical nodules are seen as the disease progresses. The tissue is diffusely infiltrated by small lymphocytes, plasma cells, and macrophages (Figure 37.1B) (McNicol and Laidler, 1996). The histopathologic finding of infiltrating lymphocytes sometimes is associated with follicle formation and fibrosis. The medulla remains normal. This pattern is present in patients with AA, either isolated or associated in the context of autoimmune polyendocrine syndromes (McIntyre Gass, 1962; Irvine and Barnes 1975; Betterle et al., 2002).

Animal Models

Induced Immunity

Colover and Glynn (1958) reported isoimmunization in guinea pigs by injection of adrenal antigens and complete Freund's adjuvant, with distinctive and "specific" lesions of the adrenals. Subsequently, many authors (for example, Steiner et al., 1960; Barnett et al., 1963) reported examples of experimental autoimmune adrenalitis (EAA) in different species (rabbits, guinea pigs, rats, monkeys), including an antibody reaction in the guinea pigs and a lymphocytic infiltration in the rabbit adrenal tissue (Witebsky and Milgrom, 1962; Barnett et al., 1963). The adrenal lesions in rabbits were characterized by foci of lymphocytes and histiocytes, with a small number of plasma cells and eosinophils, and degenerative changes in adrenal cells. In 1968 (Andrada et al., 1968) and in 1970 (Werdelin and Witebsky, 1970), EAA in a Lewis rat model was induced: adrenalitis occurred at day 7 after immunization. The histology was studied by autoradiographic tracing of H_3 thymidine and H_3 adenosine-labeled cells and demonstrated that adrenalitis was initiated with the appearance of a few specifically reactive lymphocytes, followed by an infiltration of mononuclear cells, mainly lymphocytes and plasma cells, throughout the adrenal cortex. Eosinophilia, cytoplasmic vacuolization, and a loss of nuclear definition were evident in cortical cells.

Using electron microscopy, Hoenig et al. (1970) studied the inflammatory lesions in paraffin-embedded tissues; 5 days after immunization, lymphocytes were present in sinusoids and adrenal parenchyma where damage was in the vicinity of lymphocytes, with enlargement of intercellular spaces and ischemic areas with inflammatory cells and fibrin.

It was demonstrated by Fujii et al. (1992) that in mice repeated immunizations caused a delayed type of hypersensitivity to adrenal antigens, and that transfer of adrenalitis from an affected to a healthy animal was not possible by serum but was possible using spleen cells, confirming previous reports (Levine and Wenk, 1968; Werdelin et al., 1971) in which the disease was passively transferred by immunocytes derived from lymph nodes.

Spontaneous Immunity

Spontaneous AA occurs in dogs (Harlton, 1976; Kaufman, 1984; Little et al., 1989; Kintzer and Peterson, 1994; Sadek and Schaer, 1996; Dunn and Herrtage, 1998) and cats (Kaufman, 1984; Peterson et al., 1989; Tasker et al., 1999; Stonehewer and Tasker, 2001) with hypoadrenocorticism due to immune-mediated destruction of the adrenal glands. While biochemical laboratory data were reported, none of the authors described positivity for adrenal cortex autoantibodies, but only the presence of lymphocytic infiltration in the adrenal glands, at autopsy.

Beales et al. (2002) found the adrenal glands of a nonobese diabetic (NOD) mouse to contain a mononuclear cell infiltration in the adrenal cortex, but without signs of hypoadrenalism. Thus the NOD mouse was proposed as a spontaneous model suitable for investigating mechanisms involved in diffuse lymphocytic infiltration of the adrenal glands.

Different Clinical Presentations of AAD

AAD can develop as a sole disease or associated with other autoimmune diseases. A review of the literature on 1557 patients with AA revealed that Hashimoto' thyroiditis was present in 3.7–32%, Graves' disease in 2–22.7%, atrophic gastritis in 25%, chronic candidiasis in 0.8–21%, diabetes mellitus in 1.2–20.4%, hypoparathyroidism in 1.2–20%, hypergonadotropic hypogonadism in 4.5–17.6%, vitiligo in 0.8–16%, alopecia in 0.8–12%, celiac disease in 1.2–8%, pernicious anemia in 0.8–6%, multiple sclerosis in 3.7%, inflammatory bowel diseases in 2.4%, Sjögren syndrome in 2.4%, chronic hepatitis in 1.6–3%, and lymphocytic hypophysitis in 0.8% (Betterle et al. 2002).

Neufeld and Blizzard (1980) proposed the classification of autoimmune syndromes into four main groups, involving different endocrine glands, and named them "autoimmune polyglandular syndromes" (APS) (see Box 37.1). According to this classification, AAD can be associated with type 1 and 2 APS.

Betterle et al. (2002), in a review of APS, proposed the existence of three different types of APS involving AAD (type 1, type 2, and type 4). So, AAD can present in four

TABLE 37.1 Four different clinical presentations of autoimmune adrenalitis

Characteristics	Type 1 APS	Type 2 APS	Type 4 APS	Isolated AD
Mean age at onset	13 years	36 years	36 years	30 years
F : M ratio	F ≥ M	F > M	F > M	M > F
Association with:				
1. Hypoparathyroidism and/or candidiasis	+	Absent	Absent	Absent
2. TAD and/or type 1 diabetes	Rare	+	Absent	Absent
3. Other disease (hypergonadotropic hypogonadism, vitiligo, alopecia, atrophic gastritis, pernicious anemia, celiac disease, myasthenia gravis)	Frequent (up to 70%)	Rare (up to 12%)	+	Absent
4. Ectodermal dystrophy	+	Absent	Absent	Absent
Genetic				
1. Mutations in the AIRE gene	+	Absent	Absent	Absent
2. HLA	No correlations	DR3 and/or DR4	?	DR3
Immunology				
ACA and/or 21-OHAbs at the onset of AD	>90%	>90%	>90%	80%
Histopathology				
Lymphocytic adrenalitis	+	+	+	+
Adrenal atrophy	+	+	+	+

Box 37.1 Clinical classification of APS according to Neufeld and Blizzard, 1980 (modified)

APS-1 Chronic candidiasis, chronic hypoparathyroidism, Addison's disease *(at least two diseases must be present)*

APS-2 Addison's disease *(always present)* + thyroid autoimmune diseases and/or type 1 diabetes mellitus

APS-3 Thyroid autoimmune diseases + other autoimmune diseases *(excluding Addison's disease and/or hypoparathyroidism)*

APS-4 Combinations of other autoimmune diseases not included in previous classifications

main clinical forms: isolated or associated with type 1, 2 or 4 APS.

The clinical and immunologic features of type 1, 2, 4 APS and of isolated AAD are summarized in Table 37.1. Below we describe isolated AAD; for APS see Chapter 38.

Isolated AAD

Isolated AAD is characterized by the presence of AAD without other clinically evident autoimmune disease. However, before considering such cases as true isolated AA, it is important to test for autoantibodies because in about one-third, a positive result will point to a potential APS (Betterle et al., 2002). Isolated AAD is more prevalent in males with a F : M ratio of 0.8 and a median age at onset of 30 years (Betterle et al., 2002). In isolated AD, HLA DR3-DQ2 and DR4-DQ8 were reported to be increased and DR1-DQ5 significantly reduced (Myhre et al., 2002). We studied 22 patients with isolated AD and found a significant correlation only with HLA DRB1*03-DQB1*02 (Albergoni et al., 2003).

In our experience, during the period 1968–2004, 434 cases of AD were seen and 353 (81%) were affected by AAD, 51 (12%) by tuberculosis, 12 by adrenoleukodystrophy (ALD) (2.8), 12 by other diseases (2.8%) (cancer 1.6%, infectious 0.5%, vascular 0.2%, genetic 0.2%) and six (1.4%) were unclassifiable, confirming that autoimmunity is by far the most frequent cause of AD in Italy.

Immunologic Studies

Cellular Immunity

The early studies on AD, using the assay for migration inhibition factor, reported cell-mediated immunity in affected patients, with claims for organ-specific hypersensitivity (Nerup and Bendixen, 1969). Subsequent studies, by means of an intracutaneous test with adrenal extracts, showed a cutaneous delayed-type hypersensitivity reaction but no collateral evidence based on blast transformation experiments (Nerup et al., 1970). Other studies reported a decrease in suppressor T-cell function (Verghese et al., 1980) and an increase in circulating Ia-positive T lymphocytes (Rabinowe et al., 1984), which indicated the involvement of cellular immunity. However, more thorough studies of cellular immunity in AA were not carried out until more recently (Freeman and Weetman, 1992) when a proliferative

T-cell response to an adrenal-specific protein fraction of 18–24 kDa molecular weight was described. However, T-cell reactivity to specific adrenal autoantigens has not yet been reported. As hypothesized by Volpé (1994), cell-mediated immunity seems to be the main pathogenic cause of organ-specific autoimmune diseases, involving both T-cell subsets (Liblau et al., 1995).

Humoral Immunity

Anderson et al. (1957), using a complement fixation test on a homogenate of adrenal cortex tissue, demonstrated that 2/8 (25%) of patients with idiopathic AD had antibodies (adrenal cortical antibodies – ACA). From 1957 to 1970, using this technique, ACA were cumulatively reported in 57/159 (36%) patients with idiopathic AD and in 2/23 (9%) of those with tuberculous form, reviewed by Betterle (2004). Subsequently, Blizzard and Kyle (1963), using indirect immunofluorescence (IIF) on animal adrenal gland sections, demonstrated that ACA are organ-specific antibodies which react with all three layers of the adrenal cortex, producing a homogenous cytoplasmic staining pattern (Figure 37.3). Sometimes, there is reactivity against one or two of the three layers of the cortex (Irvine and Barnes, 1975; Sotsiu et al., 1980). The autoantibodies also react with the surface of living cortical cells in culture (Khoury et al., 1981), indicating the existence of microsomal antigens on the surface of adrenal cells. Between 1963 and 2002, IIF was used on human or animal adrenal tissues in 1637 patients with autoimmune and 267 with tuberculous AD, and ACA were detected in 61% and 6.7% respectively, as reviewed by Betterle (2004).

The prevalence of ACA has varied considerably between different laboratories because of differences in the substrates used (animal or human), time of incubation, geographic or racial origins of individuals, gender, age of onset, duration of the disease or whether other associated diseases were present (Rees Smith and Furmaniak, 1995; Betterle et al., 2002; Nigam et al., 2003). Other studies (Kendall-Taylor et al., 1988; Wulfraat et al., 1989) have reported the presence of autoantibodies blocking the ACTH receptor in 90% of patients affected by AAD, but these data could not be confirmed (Wardle et al., 1993). Finally, autoantibodies to hydrocortisone were detected in patients with an Addison-like syndrome and AIDS (Salim et al., 1988).

In 1968, steroid-producing cell antibodies (StCA) were first described (Anderson et al., 1968) in males affected by AAD without gonadal failure (Figure 37.4). Subsequently, StCA were detected in 60–80% of the patients affected by type 1 APS, in 25–40% of those with type 2 APS and 18%

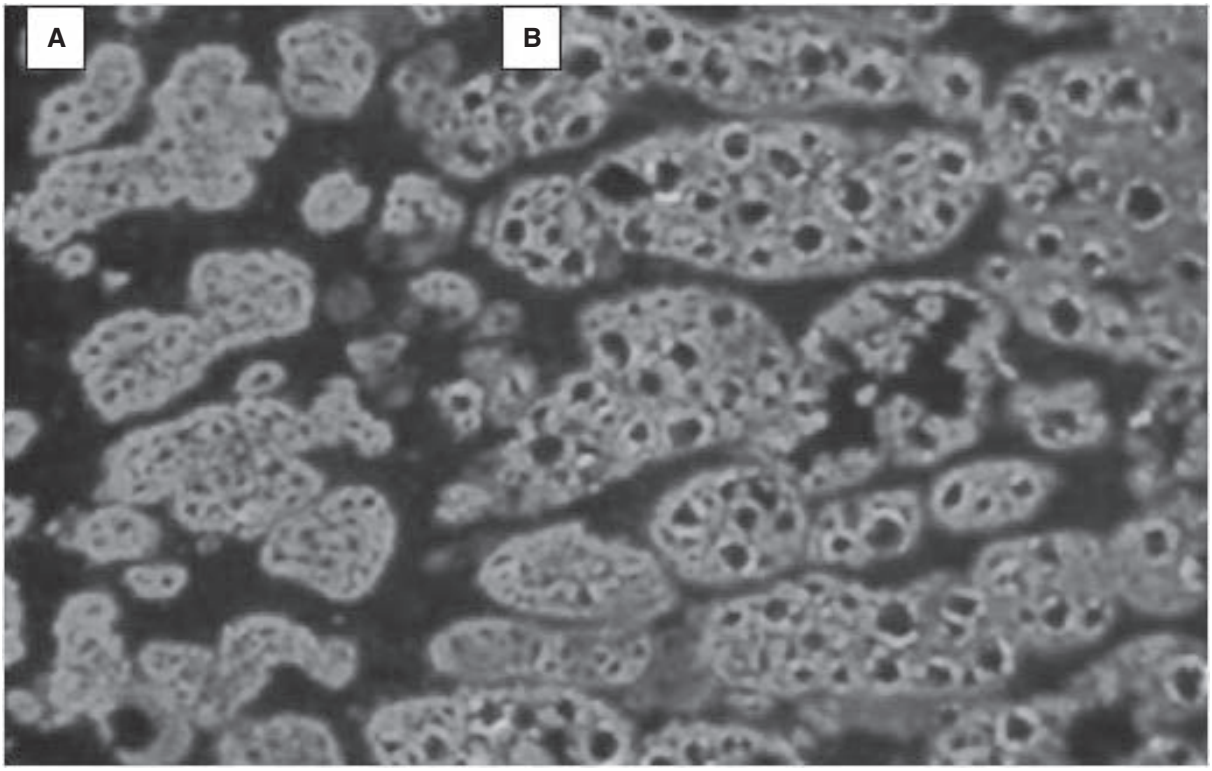


FIGURE 37.3 Immunofluorescence pattern on normal human adrenal cortex given by serum of a patient with AAD. This serum reacted with the cytoplasm of cells in all three layers of the cortex, but shown here are only glomerulosa (*A*) and fasciculata (*B*). See color plate section.

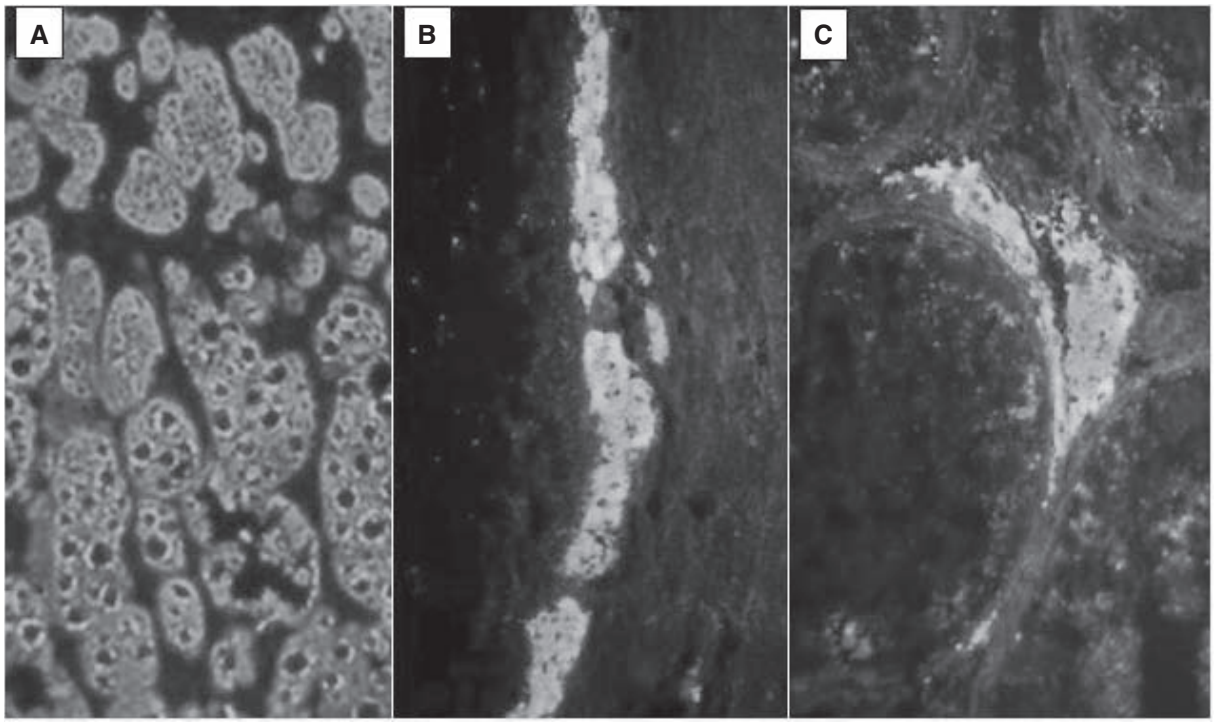


FIGURE 37.4 Immunofluorescence pattern given by serum of a patient with AAD and premature ovarian failure. This serum reacted against adrenal cortex (*A*), follicular theca of the ovary (*B*) and Leydig cells of the testis (*C*), and reflects autoantibodies to steroid-producing cells (StCA). See color plate section.

of those with isolated AA (Betterle et al., 2002; Betterle, 2004; Betterle et al., 2005). The presence of these antibodies correlates with premature ovarian failure characterized by lymphocytic oophoritis (Betterle et al., 1993; Hoek et al., 1997; Betterle and Volpato, 1998). These antibodies are not described in patients without AA or negative for ACA.

With regard to the identification of adrenal autoantigens, in 1988 Furmaniak et al. described a specific 55 kDa protein in human adrenal microsomes which was reactive with the ACA. In 1992, two independent laboratories (Baumann-Antczak et al., 1992; Bednarek et al., 1992; Winqvist et al., 1992) demonstrated by means of purification of native 21-hydroxylase (21-OH) that this adrenal enzyme is a major antigen of adrenal cortical cells. Subsequently, this was confirmed in experiments with specific absorption with purified human 21-OH, using sera from six patients with different forms of AAD (Morgan et al., 2000).

21-OH is an adrenal-specific enzyme of the cytochrome P450 family and plays a key role in the synthesis of the cortical hormones (Furmaniak et al., 1999; Furmaniak and Rees Smith, 2002). There are two 21-OH genes (CYP21): 21-OH is encoded by the CYP21B gene, whereas the CYP21A gene is inactive (Wilson et al., 1995). 21-OH catalyzes the conversion of progesterone and 17-hydroxyprogesterone into 11-deoxycorticosterone and 11-deoxycortisol (see Figure 37.2). It is a 55 kDa microsomal protein containing a heme group, located in the active site of the C-terminal end of the molecule that is important for autoantibody binding (Wedlock et al., 1993; Asawa et al., 1994) and in oxidation-reduction reactions (Picado-Leonard and Miller, 1987; Lin et al., 1994).

Analysis of autoantibody-binding sites on 21-OH indicated that the epitopes on 21-OH were conformational (Wedlock et al., 1993) and confirmed the participation of both the central and C-terminal parts of the molecule. These studies identified the presence of three different, short 5-, 6-, and 15-amino acid sequences in the C-terminal part of the 21-OH involved in the binding of antibodies to 21-OH (Chen et al., 1998). These observations are important in relation to effects of 21-OH antibodies on 21-OH enzyme activity. In fact, in studies *in vitro*, using sera positive for 21-OHAbs from patients with AA, a dose-dependent blocking activity was identified (Furmaniak et al., 1994), although this is not usually evident *in vivo* (Boscaro et al., 1996).

Following the discovery that 21-OH is the major adrenal cortex autoantigen, a specific and sensitive technique was described, by labeling the protein with ^{35}S-methionine in an *in vitro* transcription translation (TnT) system, and using an radioimmunoprecipitation assay (RIA) for the detection of antibodies (Colls et al., 1995; Falorni et al., 1995, Chen et al., 1996). Thereafter, because of certain limitations of this technique, a more convenient assay to measure 21-OHAbs

was developed based on the use of ^{125}I-labeled recombinant human 21-OH and the precipitation of the immuncomplexes using solid-phase protein A (RIA) (Tanaka et al., 1997). Using these techniques, from 1995 to 2002, a group of 572 patients with AAD and 76 with tuberculosis were studied; 78% and 1.9% respectively were 21-OHAbs positive, reviewed by Betterle (2004).

In order to compare techniques for ACA and 21-OHAbs determinations, we studied 165 patients with AD, with different durations of disease, and found that 81% of those with AAD were positive by both techniques, whereas none with non-AAD was positive (Betterle et al., 1999). The prevalence varied in relation to both the clinical presentation (type 1 or 2 APS or isolated AAD) and the duration of the disease, being higher (100%) in patients with recent onset than in those with long-standing disease (79%) and in those with type 2 APS than in those with isolated AD (Betterle et al., 2002). In these studies, results using IIF for ACA and IPA and RIA for 21-OHAbs were in good agreement.

Some discrepancies were reported by Falorni et al. (1997) on relationships between ACA measured using IIF and 21-OHAbs measured by RIA, probably related to laboratory-dependent technical aspects of the ACA measured by IIF. It would be very useful to set up an international proficiency program for adrenal autoantibody measurement, so that data across the world could be compared.

With regard to adrenal autoantigens, in addition to 21-OH, Krohn et al. (1992) reported that screening of a human fetal adrenal cDNA expression library with sera from patients with APS type 1 identified a protein with high homology to 17α-hydroxylase (17α-OH) reacting also with a fragment of recombinant 17α-OH expressed in bacteria. 17α-OH, coded by a single gene on the human chromosome 10, showed 30% homology with 21-OH antigen.

Winqvist et al. (1992), using immunoblotting and immunoprecipitation studies, also observed a reactivity of sera from APS type 1 patients with a cytocrome P450 side chain cleavage enzyme (P450scc), a heme-binding protein coded by a single gene on human chromosome 15, that showed 20% homology with 21-OH sequence (Chung et al., 1986).

The binding sites of these two antigens and their enzyme activity have not been studied, even though Peterson and Krohn (1994) reported the presence of four distinct, reactive regions in the 17α-OH molecule and an inhibiting effect *in vivo* of the P450sccAbs in the type 1 APS serum (Winqvist et al., 1993). The 17α-OHAbs and P450sccAbs are detectable by RIA, using recombinant human antigens, and are correlated with StCA detected by IIF (Chen et al., 1996).

In addition to the main antigens mentioned above, other autoantigens have also been discovered. Winqvist et al. (1996) identified an autoantibody of 51 kDa, recognizing the aromatic L-amino acid decarboxylase (Anti-AADC) involved in the generation of serotonin and dopamine. AADC was reported in the sera of patients with type 1 APS in association with chronic hepatitis, vitiligo, and type 1 diabetes mellitus (Husebye et al., 1997).

Other authors (Ekwall et al., 1998; 1999; Dal Pra et al., 2004) reported the presence of autoantibodies against a 230 kDa enzyme, tryptophan hydroxylase (TPH) involved in the synthesis of serotonin, or against histidine decarboxylase (Skoldberg et al., 2003) in patients with type 1 APS, who have a gastrointestinal dysfunction.

Again, patients with type 1 APS and autoimmune hepatitis recognize the cytochromes P450 CYPIA2 and CYP2A6 as autoantigens (Clemente et al., 1997; 1998).

Recently, an autoantibody to SOX9 and SOX10 was reported in patients with type 1 APS and vitiligo (Hedstrand et al., 2001), and an antibody to tyrosine hydroxylase in those with alopecia areata (Hedstrand et al., 2000).

Some authors reported reactivities against other antigens: to 3β-hydroxysteroid dehydrogenase (Arif et al., 1996), to candidal antigens, heat shock protein 90, pyruvate kinase and alcohol dehydrogenase, in patients with AAD and chronic mucocutaneous candidiasis (Peterson et al., 1996). Two different studies (Song et al., 1994; Peterson et al., 1997) were unable to confirm the reactivity to other enzymes (11α-hydroxylase, aromatase, adrenodoxin) in patients with AAD.

Natural History of AAD

Apart from the patients with AA, ACA can be detected in 0.2% of normal controls, in 4% of first-degree relatives of AA patients, 4% of hospitalized patients, and 1.3% of patients with organ-specific autoimmune disease, with a much higher prevalence (2.5–20%) in premature ovarian failure or idiopathic hypoparathyroidism (Nerup, 1974; De Bellis et al., 1993; Betterle et al., 1997a; 1997b).

AAD is a chronic disease with a long silent period marked by the presence of ACA. The natural history of the disease could entail five main phases: one potential, three subclinical, and one clinical (Betterle et al., 1988).

To recognize these different phases, it is necessary to basally measure levels of cortisol, ACTH, plasma renin activity (PRA) and aldosterone and cortisol 30–60 min after an intravenous injection of 250 μg of cosyntropin (α1-24-corticotropin) (ACTH test) (Stewart et al., 1988; Grinspoon and Biller, 1994). For the study of patients with ACA/21-OHAbs, it was proposed to use an ACTH test with a low dose (1 μg) of ACTH (Laureti et al., 2000), since the test with high doses might have inadequate sensitivity. However, the test with low doses of ACTH did not reveal differences in the cortisol response either in normal controls or in patients with ACA/21-OHAbs compared with results obtained using the test with high doses.

Stage 0 is characterized by the presence of adrenal-specific autoantibodies and a normal adrenal cortical mass, in the absence of detectable dysfunction of the adrenal glands by ACTH test, representing the "potential" phase of chronic adrenalitis. Stage 1 is characterized by an increase in PRA, together with normal/decreased levels of aldosterone; stage 2 by normal ACTH values with normal basal cortisol but a low cortisol peak; and stage 3 by an increase in ACTH and normal level of basal cortisol, representing subclinical adrenal function. Stage 4, the clinical adrenalitis stage, is when the ACTH is significantly increased and the basal cortisol is very low. Stage 1 (increase of PRA and normal/low levels of aldosterone) reveals that what is first affected by the autoimmune attack is the zona glomerulosa of the adrenal glands, perhaps because of its greater sensitivity to diffuse lymphocyte infiltration, but another explanation is that the other zones are protected by either locally produced high concentrations of corticosteroid hormones or the ability to self-regenerate (Betterle et al., 2002).

From 1983 to 2000, 236 ACA-positive patients were followed in 12 studies and 28% developed AAD, but the evolution varied greatly from 0% to 90% (Betterle, 2004; Betterle et al., 2005). The highest risk was found in children with high antibody titers and DR1*0404 (Betterle et al., 1997a; 1997b; Laureti et al., 1998; Yu et al., 1999).

Recently, in order to estimate risk of AD, 100 ACA-positive patients (21 children and 79 adults) with autoimmune diseases but without clinical AAD were studied over a period of 19 years; 31 of the seropositive patients developed clinical AAD. The overall cumulative risk of AAD in ACA/21-OHAb-positive patients was 47.5%, with an annual incidence of 4.9%. We assessed various indices, including clinical (gender and age), immunologic (type of preexisting autoimmune disease, titers of autoantibodies), genetic (HLA status) and functional (level of adrenal cortical function) by multivariate analysis. This demonstrated that the occurrence of AAD was independently associated with:

- the presence of chronic hypoparathyroidism and/or mucocutaneous candidiasis;
- thyroid autoimmune disease and/or type 1 diabetes mellitus;
- high antibody titers;
- abnormal adrenal function at entry.

The probability of developing AAD can now be estimated by a mathematical model. This risk evaluation yielded two practical outcomes:

1. individulization of the patients with different risk grades in order to monitor them at different rates;
2. selection of patients to begin an immuno-intervention trial treatment in order to prevent the development of the disease (Betterle et al., unpublished observations).

Diagnosis of AAD

Clinical Manifestations

AAD has a long preclinical period and clinical features do not appear until 90% or more of the adrenal cortex is destroyed. The main clinical signs at onset are general malaise, fatigue, weakness (99%), anorexia, nausea and vomiting (90%), weight loss (97%), cutaneous and mucosal hyperpigmentation (Figure 37.5) caused by the enhanced stimulation of the skin MC1-receptor by ACTH and other pro-opiomelanocortin-related peptides (98%), and severe hypotension (87%). Other signs (abdominal pain, salt craving, diarrhea, constipation, syncope) have a variable frequency (34–39%) (Williams and Dluhy, 1998). In women a loss of axillary and pubic hair, dry skin, reduced libido, and an impairment of well-being also occur.

General Biochemical Indices

In AAD at diagnosis, the serum levels of sodium, chloride and bicarbonate are reduced, while those of potassium are elevated. The hyponatremia (in 100% of patients) is due to loss of sodium in urine and increase in both plasma vasopressin and angiotensin II which impair free water clearance; hyperkalemia (in 50–70%) is due to aldosterone deficiency, impaired glomerular filtration, and acidosis. From 10% to 20% of patients have a mild or moderate hypercalcemia, for unknown reasons (Williams and Dluhy, 1998). Anemia is present in 40–50%, and eosinophilia and lymphocytosis in 10–15% of the cases.

Hormonal Tests

The association between a morning (8 am) immunometric determination of both plasma ACTH and basal cortisol levels differentiates cases of primary adrenal failure from both a healthy status and other types of adrenal disease (Oelkers et al., 1992).

An increase in levels of ACTH (higher than 22.0 pmol/L) and decrease (below 165 nmol/L) in basal cortisol indicate primary adrenal failure. In the initial phases of AD, plasma renin activity increases above the normal range, and serum aldosterone is either subnormal or low-to-normal. In relation to differential diagnosis, in the case of secondary adrenal failure, levels of both ACTH and cortisol are low and, in general, aldosterone and plasma renin activity are normal. Dehydroepiandrosterone, which is the major precursor of sex steroid synthesis, is involved in the adrenal failure, causing a pronounced androgen deficiency in women, with the loss of both axillary and pubic hair, dry skin, reduced libido and, frequently, an impairment in well-being (Arlt and Allolio, 2003).

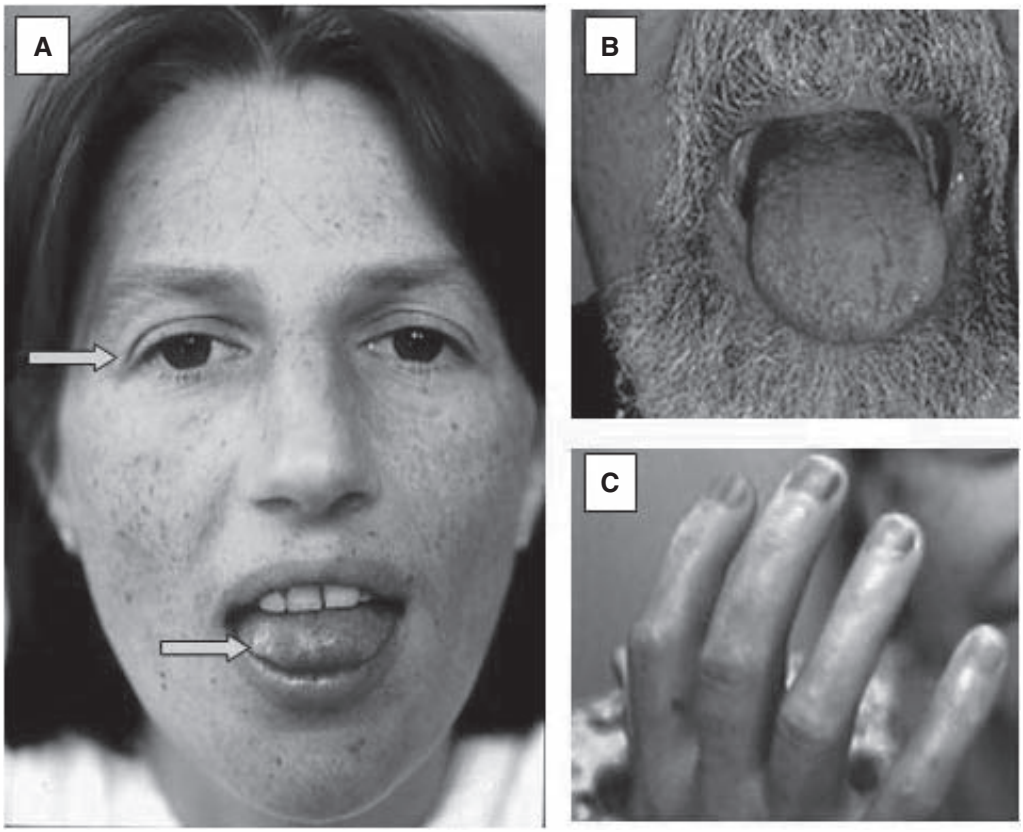


FIGURE 37.5 Clinical manifestations of AAD at onset. *A,* Hyperpigmentation of the skin, melanosis of the tongue, and enophthalmos as a sign of dehydration. *B,* Male showing a melanosis of the tongue. *C,* Hyperpigmentation of the nails. See color plate section.

In cases in which there are no clear clinical manifestations of AD, and/or in the presence of adrenal cortex antibodies, it may be necessary to perform an ACTH test (see above, Natural History). TSH levels are increased in 30% of patients, because of the lack of inhibiting effect of cortisol on TSH production or the presence of coexisting autoimmune hypothyroidism (Orth and Kavacs, 1998).

Imaging

Computed tomography (CT) and nuclear magnetic resonance (NMR) show the adrenals with optimal resolution and clarity, and greatly facilitate the diagnosis and characterization of adrenal insufficiency. In patients with AAD, either isolated or as a component of APS syndromes, the adrenal glands appear bilaterally miniscule without calcifications (Doppman, 2001) (Figure 37.6).

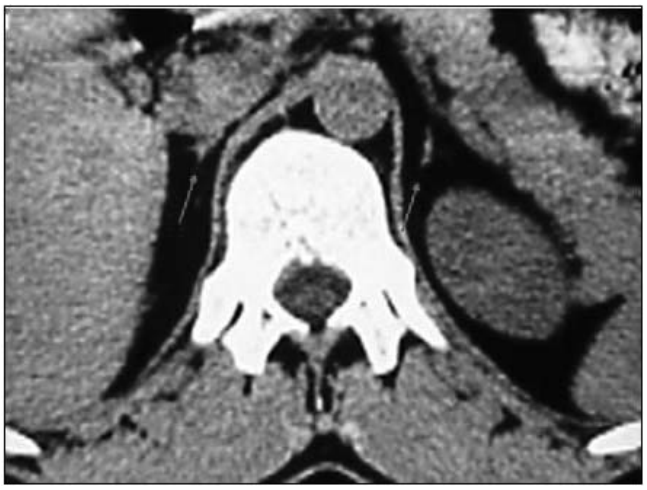


FIGURE 37.6 Adrenal imaging. CT of the adrenal glands of a patient with autoimmune Addison's disease at diagnosis. The adrenals are small (see arrows).

Therapy

The life-saving therapy for patients with chronic adrenal insufficiency is two or three daily doses of a specific hormone replacement (Groves et al., 1988; Williams and

Dluhy, 1998; Arlt and Allolio, 2003). 20–30 mg of hydrocortisone or 25–50 mg of cortisone acetate are required. The first dose is administered in the morning and the second in the afternoon, about 6–8 hours after the first, and the third at 6 pm or at least 4 hours before sleep. Substitutive glucocorticoid therapy is not taken with food. The dosage must be increased for obese individuals and those taking other drugs. Patients should be given relatively small doses to avert weight gain and osteoporosis. ACTH cannot be used for the purposes of treatment surveillance, whereas a 24-hour urinary free-cortisol test is a good marker for correct therapy (Burch, 1982; Howlett, 1997). Mineralocorticoid replacement is usually necessary and can be administered as a single daily dose of 0.05–0.1 mg of fludrocortisone or another aldosterone substitute. Patients should also receive an ample amount of sodium (3–4 g/daily), assessed by measurement of blood pressure, serum electrolytes, and plasma renin activity levels (Williams and Dluhy, 1998).

Complications are rare with this type of therapy. However, possible hypokalemia, hypertension, sodium retention or heart disease should be monitored. Patients with AAD should carry a medical identification card, stating the current therapy and recommendations for emergency situations such as febrile illnesses, injury, vomiting, surgical interventions, dental extractions or pregnancies, when the intake of glucocorticoids must be doubled or tripled (Oelkers, 1996).

Acknowledgments

We would like to thank all those who have cooperated with us over the years regarding the study of the clinical, genetic, immunologic, morphologic, and endocrine aspects of AD. The authors are also grateful to Mrs Helen Maguire for her assistance in reviewing this manuscript.

This work was partially funded by a Grant "Sugli aspetti immunologici della menopausa precoce" from MURST (Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

References

Addison, T. (1868). On the constitutional and local effects of disease of the suprarenal capsules. In a collection of the published writings of the late Thomas Addison MD, physician to Guy's Hospital. London: New Sydenham Society.

Albergoni, P., Gazzola, M.V., Slanzi, E., Carcassi, C., Dal Pra, C., Moscon, A., and Betterle, C. (2003). HLA-DR and DQ associations with autoimmune Addison's disease in Italian patients. *Genes Immun. 4* (Suppl.1), S35.

Anderson, J.R., Goudie, R.B., Gray, K.G., and Timbury, G.C. (1957). Autoantibodies in Addison's disease. *Lancet 1*, 1123–1124.

Anderson, J.U., Goudie, R.B., Gray, K., and Stuart Smith, D.A. (1968). Immunological features of idiopathic Addison's disease: an antibody to cells producing steroid hormones. *Clin. Exp. Immunol. 3*, 107–117.

Andrada, J.A., Skelton, F.R., Andrada, E.C., Milgrom, F., and Witebsky, E. (1968). Experimental autoimmune adrenalitis in rats. *Lab. Invest. 19*, 460–465.

Arif, S., Vallian, S., Farzaneh, F., Zanone, M.M., James, S.L., Pietropaolo, M., Hettiarachchi, S., Vergani, D., Conway, G.S., and Peakman, M. (1996), Identification of 3-beta-hydroxysteroid dehydrogenase as a novel target of steroid cell autoantibodies: association of autoantibodies with endocrine autoimmune disease. *J. Clin. Endocrinol. Metab. 81*, 4439–4445.

Arlt, W. and Allolio, B. (2003). Adrenal insufficiency. *Lancet 361*, 1881–1893.

Asawa, T., Wedlock, N., Baumann-Antczak, A., Rees Smith, B., and Furmaniak, J. (1994). Naturally occurring mutations in human steroid 21-hydroxylase influence adrenal autoantibody binding. *J. Clin. Endocrinol. Metab. 79*, 372–376.

Auchus, R.J. and Miller, W.L. (2001). The principles, pathways and enzymes of human steroidogenesis. In L.J. DeGroot and J.L. Jameson (eds), *Endocrinology*, vol. 2, 4th ed. Philadelphia: W.B. Saunders.

Barnett, E.V., Dumonde, D.C., and Glynn, L.E. (1963). Induction of autoimmunity to adrenal gland. *Immunology, 6*, 382.

Baumann-Antczak, A., Wedlock, N., Bednarek, J., Kiso, Y., Krishnan, H., Fowler, S., Rees Smith B., and Furmaniak, J. (1992). Autoimmune Addison's disease and 21-hydroxylase. *Lancet 340,* 429–430.

Beales, P.E., Castri, F., Valiani, A., Rosignoli, G., Buckley, L., and Pozzilli, P. (2002). Adrenalitis in the non-obese diabetic mouse. *Autoimmunity 35(5),* 329–333.

Bednarek, J., Furmaniak, J., Wedlock, N., Kiso, Y., Baumann-Antczak, A., Fowler, S., Krishnan, H., Craft, J.A., and Rees Smith, B. (1992). Steroid 21-hydroxylase is a major autoantigen involved in adult onset autoimmune Addison's disease. *FEBS Lett. 309*, 51–55.

Betterle, C. (2004). Addison's disease and autoimmune polyglandular syndromes. In V. Geenen, and G. Chrosus (eds), *Immunoendocrinology in Health and Disease*. New York: Marcel Dekker.

Betterle, C. and Volpato, M. (1998). Adrenal and ovarian autoimmunity. *Eur. J. Endocrinol. 138*, 16–25.

Betterle, C., Scalici, C., Presotto, F., Pedini, B., Moro, L., Rigon, F., and Mantero, F**.** (1988). The natural history of adrenal function in autoimmune patients with adrenal autoantibodies. *J. Endocrinol. 117*, 467–475.

Betterle, C., Rossi, A., Dalla Pria, S., Artifoni, L., Pedini, B., Gavasso, S., and Caretto, A. (1993). Premature ovarian failure: autoimmunity and natural history of the disease. *Clin. Endocrinol. 39*, 35–43.

Betterle, C., Volpato, M., Rees Smith, B., Furmaniak, J., Chen, S., Greggio, N.A., Sanzari, M., Tedesco, F., Pedini, B., Boscaro, M., and Presotto, F. (1997a). I. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. *J. Clin. Endocrinol. Metab. 82*, 932–938.

Betterle, C., Volpato, M., Rees Smith, B., Furmaniak, J., Chen, S., Zanchetta, R., Greggio, N.A., Pedini, B., Boscaro, M., and Presotto, F. (1997b). II. Adrenal cortex and steroid 21-hydroxylase autoantibodies in children with organ-specific autoimmune diseases: markers of high progression to clinical Addison's disease. *J. Clin. Endocrinol. Metab. 82*, 939–942.

Betterle, C., Volpato, M., Pedini, B., Chen, S., Rees Smith, B., and Furmaniak, J. (1999). Adrenal-cortex autoantibodies and steroid producing cells autoantibodies in patients with Addison's disease: comparison of immunofluorescence and immunoprecipitation assays. *J. Clin. Endocrinol. Metab. 84*(2), 618–622.

Betterle, C., Dal Pra, C., Mantero, F., and Zanchetta, R. (2002). Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr. Rev. 23*, 327–364.

Betterle, C. (2004). Addison's disease and autoimmune polyglandular syndromes. In V. Geenen and G. Chrousos, *Immunoendocrinology in health and disease*. NY: M. Dekker.

Betterle, C., Coco, G., and Zanchetta, R. (2005). Adrenal cortex autoantibodies in subjects with normal adrenal function. *Best. Pract. Res. Clin. Endocrinol. Metab. 19*, 85–99.

Blizzard, R.M. and Kyle, M. (1963). Studies on adrenal antigens and autoantibodies in Addison's disease. *J. Clin. Invest. 42*, 1653–1660.

Boscaro, M., Betterle, C., Volpato, M., Fallo, F., Furmaniak, J., Rees Smith, B., and Sonino, N. (1996). Hormonal responses during various phases of autoimmune adrenal failure: no evidence for 21-hydroxylase enzyme activity inhibition *in vivo*. *J. Clin. Endocrinol. Metab. 81*, 2801–2804.

Burch, W.M. (1982). Urine free-cortisol determination: a useful tool in the management of chronic hypoadrenal states. *JAMA 247*, 2002–2004.

Casserius, J. *Tabulae Anatomicae LXXIIX*, West Germany: Druckerei Holzer, Copy 1283.

Chen, Q.J., Lan, M.S., She, J.X., and Maclaren, N.K. (1998). The gene responsible for autoimmune polyglandular syndrome type 1 maps to chromosome 21q22.3 in US patients. *J. Autoimmun. 11,* 117–183.

Chen, S., Sawicka, J., Betterle, C., Powell, M., Prentice, L., Volpato, M., Rees Smith, B., and Furmaniak, J. (1996). Autoantibodies to steroidogenic enzymes in autoimmune polyglandular syndrome, Addison's disease, and premature ovarian failure. *J. Clin. Endocrinol. Metab. 83,* 2977–2986.

Chung, B.C., Matteson, K.J., Voutilainen, R., Mohandas, T.K., and Miller, W.L. (1986). Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. *Proc. Natl Acad. Sci. USA 83*, 8962–8966.

Clemente, M.G., Obermyer-Straub, P., Meloni, A., Strassburg, C.P., Arangino, V., Tukey, R.H., De Virgilis, S., and Manns, M.P. (1997). Cytochrome P450 IA2 is a hepatic autoantigen in autoimmune polyglandular syndrome type 1. *J. Clin. Endocrinol. Metab. 82*, 1353–1361.

Clemente, M.G., Meloni, A., Obermyer-Straub, P., Frau, F., Manns, M.P., and De Virgilis, S. (1998). Two cytochrome P450 are major hepatocellular autoantigens in autoimmune polyglandular syndrome type 1. *Gastroenterology 114*, 324–328.

Colls, J., Betterle, C., Volpato, M., Rees Smith, B., and Furmaniak, J. (1995). A new immunoprecipitation assay for autoantibodies to steroid 21-hydroxylase in Addison's disease. *Clin. Chem. 41,* 375–380.

Colover, J. and Glynn, L.E. (1958). Experimental iso-immune adrenalitis. *Immunology 2,* 172–178.

Dal Pra, C., Chen, S., Betterle, C., Zanchetta, R., McGrath, V., Furmaniak, J., and Rees Smith, B. (2004). Autoantibodies to human tryptophan hydroxylase and aromatic L-amino acid decarboxylase. *Eur. J. Endocrinol. 150*, 313–321.

De Bellis, A., Bizzarro, A., Rossi, R., Paglionico, V.A., Criscuolo, T., Lombardi, G., and Bellastella, A. (1993). Remission of subclinical adrenocortical failure in subjects with adrenal autoantibodies. *J. Clin. Endocrinol. Metab. 76*, 1002–1007.

Doppman, J.L. (2001). Adrenal imaging. In L.J. DeGroot and J.L. Jameson (eds), *Endocrinology*, vol. 2, 4th ed. Philadelphia: W.B. Saunders.

Duff, G.L. and Bernstein, C. (1933). Five cases of Addison's disease with so-called atrophy of the adrenal cortex. *Bull. Johns Hopkins Hosp. 52*, 67.

Dunlop, D. (1963). Eighty-six cases of Addison's disease. *Br. Med. J. 3,* 887–891.

Dunn, K.J. and Herrtage, M.E. (1998) Hypocortisolaemia in a Labrador retriever. *J. Small Anim. Pract. 39*, 90–93.

Drury, M.J., Keelan, D.M., Timoney, F.J., and Irvine, W.J. (1979) Case report. Juvenile familial endocrinopathy. *Clin. Exp. Immunol. 7*, 125–132.

Eason, R.J., Croxon, M.S., Perry, M.C., and Somerfield, S.D. (1982). Addison's disease, adrenal autoantibodies and computerized adrenal tomography. *NZ Med. J. 95*, 569–573.

Ekwall, O., Hedstrand, H., Grimelius, L., Haavik, J., Perheentupa, J., Gustafsson, J., Husebye, E., Kämpe, O., and Rorsman, F. (1998). Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet 352*, 279–283.

Ekwall, O., Sjoberg, K., Mirakian, R., Rorsman, F., and Kampe, O. (1999). Tryptophan hydroxylase autoantibodies and intestinal disease in autoimmune polyendocrine syndrome type I. *Lancet 354*, 568.

Eustachius, B. (1564) Opuscula anatomica de renum structura, efficio et adminstratione. Venice: V.V. Luchino.

Falorni, A., Nikoshokov, A., Laureti, S., Grenbäck, E., Hulting, A.L., and Casucci, G. (1995). High diagnostic accuracy for idiopathic Addison's disease with a sensitive radiobinding assay for autoantibodies against recombinant human 21-hydroxylase. *J. Clin. Endocrinol. Metab. 80*, 2752–2755.

Falorni, A., Laureti, S., Nikoshkov, A., et al. for the Belgian Diabetes Registry. (1997). 21-hydroxylase autoantibodies in adult patients with endocrine autoimmune diseases are highly specific for Addison's disease. *Clin. Exp. Immunol. 107*, 341–346.

Fidler, W.J. (1977). Ovarian thecal metaplasma in adrenal glands. *Am. J. Clin. Pathol. 67,* 318–322.

Fogt, F., Vortmeyer, A.O., Poremba, C., Minda, M., Harris, C.A., and Tomaszewski, J.E. (1998) Bcl2 expression in normal adrenal glands and in adrenal neoplasms. *Mod. Pathol. 11*, 716–720.

Freeman, M. and Weetman, A.P. (1992). T and B cell reactivity to adrenal antigens in autoimmune Addison's disease. *Clin. Exp. Immunol. 88*, 275–279.

Fujii, Y., Kato, N., Kito, J., Asai, J., and Yokochi, T. (1992). Experimental autoimmune adrenalitis: a murine model for Addison's disease. *Autoimmunity 12*, 47–52

Furmaniak, J. and Rees Smith, B. (2002). Addison's disease. In R.G. Gill (ed.), *Immunologically Mediated Endocrine Diseases*. Philadelphia: Lippincott Williams and Williams.

Furmaniak, J., Talbot, D., Reinwein, D., Benker, G., Creag, F.M., and Smith, B. (1988). Immunoprecipitation of human adrenal microsomal antigen. *FEBS Lett. 232*, 25–28.

Furmaniak, J., Kominani, S., Asawa, T., Wedlock, N., Colls, J., and Rees Smith, B. (1994). Autoimmune Addison's disease. Evidence for a role of steroid 21-hydroxylase autoantibodies in adrenal insufficiency. *J. Clin. Endocrinol. Metab. 79*, 1517–1521.

Furmaniak, J., Sanders, J., and Rees Smith, B. (1999), Autoantigens in the autoimmune endocrinopathies. In R. Volpé (ed.), *Contemporary Endocrinology: Autoimmune Endocrinopathies*. Totowa, NJ: Humana Press.

Gonzalez-Hernandez, J.A., Bornstein, S.R., Ehrhart-Bornestein, M., Spath-Scwalbe, E., Jirikowski, G., and Scherbaum, W.A. (1994). Interleukin-6 messenger ribonucleic acid expression in human adrenal gland *in vivo*: new clue to a pancreatic or autocrine regulation of adrenal function. *J. Clin. Endocrinol. Metab. 79*, 1492–1497.

Grinspoon, S.K. and Biller, B.M. (1994). Clinical review 62: laboratory assessment of adrenal insufficiency. *J. Clin. Endocrinol. Metab. 79*, 923–931.

Groves, R.W., Toms, G.C., Houghton, B.J., and Monson, J.P. (1988). Corticosteroid replacement therapy: twice or thrice daily? *J. R. Soc. Med. 81*, 514–516.

Guttman, P.H. (1930). Addison's disease: statistical analysis of 566 cases and study of the pathology. *Arch. Pathol. 10,* 742–895.

Harlton, B.W. (1976). Addison's disease in a dog. *Vet. Med. Small Anim. Clin. 71*, 285–288.

Hayashi, Y., Hiyoshi, T., Takemura, T., Kurashima, C., and Hirokawa, K. (1989). Focal lymphocytic infiltration in the adrenal cortex of the elderly: immunohistochemical analysis of infiltrating lymphocytes. *Clin. Exp. Immunol. 77*, 101–105.

Hedstrand, H., Ekwall, O., Haavik, J., Landgren, E., Betterle, C., Perheentupa, J., Gustafsson, J., Husebye, E., Rorsman, F., and Kämpe, O. (2000). Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome Type 1. *Biochem. Biophys. Res. Commun. 267*, 456–461.

Hedstrand, H., Ekwall, O., Olsson, M.J., Landgren, E., Kemp, H.E., Weetman, A., Perheentupa, J., Husebye, E., Gustafsson, J., Betterle, C., Kampe, O., and Rorsman, F. (2001). The transcription factors SOX9 and SOX10 are vitiligo autoantigens in autoimmune polyendocrine syndrome type 1. *J. Biol. Chem. 276*, 35390–35395.

Henzen-Logmans, S.C., Stel, H.V., Van Muijen, G.N., Mullink, H., and Meijer, C.J. (1988). Expression of intermediate filament proteins in adrenal cortex and related tumors. *Histopathology 12*, 359–372.

Hiatt, J.R. and Hiatt, N. (1997). The conquest of Addison's disease. *Am. J. Surg. 174*, 280–283.

Hoek, A., Schoemaker, J., and Drexhage, H.A. (1997). Premature ovarian failure and ovarian autoimmunity. *Endocr. Rev. 18*, 107–134.

Hoenig, E.M., Hirano, A., Levine, A., and Ghatak, N.R. (1970). The early development and fine structure of allergic adrenalitis. *Lab. Invest. 22*, 198–205.

Howlett, T.A. (1997). An assessment of optimal hydrocortisone replacement therapy. *Clin. Endocrinol. (Oxf.) 46*, 263–268.

Husebye, E.S., Gebre-Medhin, G., Thuomi, T., Perheentupa, J., Landin-Olsson, M., Gustafsson, J., Rorsman, F., and Kampe, O. (1997). Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab. 82*, 147–150.

Irvine, W.J. and Barnes, E.W. (1975). Addison's disease, ovarian failure and hypoparathyroidism. *Clin. Endocrinol. Metab. 4*, 379–434.

Jackson, R., McNicol, A.M., Farquarson, M., and Foulis, A.K. (1988). Class II MHC expression in normal adrenal cortex and cortical cell in autoimmune Addison's disease. *J. Pathol. 155,* 113–120.

Jacobson, D.L., Gange, S.J., Rose, N.R., and Graham, N.M.H. (1997). Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol. 84,* 223–243.

Kamradt, T. and Mitchinson, N.A. (2001). Tolerance and autoimmunity. *N. Engl. J. Med. 344*, 655–664.

Kannan, C.R. (1988). Addison's disease. In C.R. Kannan (ed.), *The Adrenal Gland*. London: Plenum.

Kaufman, J. (1984) Diseases of the adrenal cortex of dogs and cats. *Mod. Vet. Pract. 65*, 513–516.

Kendall-Taylor, P., Lambert, A., Mitchell, R., and Robertson, W.R. (1988). Antibody that blocks stimulation of cortisol secretion by adrenocorticotrophic hormone in Addison's disease. *Br. Med. J. 296*, 1489–1491.

Khoury, E.L., Hammond, L., Bottazzo, G.F., and Doniach, D. (1981). Surface-reactive antibodies to human adrenal cells in Addison's disease. *Clin. Exp. Immunol. 45*, 48–55.

Kiaer, W. and Rytter Norgaard, J.O. (1969). Granulomatous hypophysitis and thyroiditis with lymphocytyc adrenalitis. *Acta Pathol. Microbiol. Scand. 76,* 229.

Kintzer, P.P. and Peterson, M.E. (1994). Diagnosis and management of primary spontaneous hypoadrenocorticism (Addison's disease) in dogs. *Sem. Vet. Med. Surg. 9(3),* 148–152.

Koch, C.A. (2004). Adrenal cortex physiology, In L. Martini (ed.), *Encyclopedia of Endocrine Disease.* San Diego, CA: Elsevier.

Kong, M.F. and Jeffocoate, W. (1994) Eighty-six cases of Addison's disease. *Clin. Endocrinol. 41*, 757–761.

Krohn, K., Uibo, R., Aavik, E., Peterson, P., and Savilhati, K. (1992). Identification by molecular cloning of an autoantigen associated with Addison's disease as steroid 17-α-hydroxylase. *Lancet 339*, 770–773.

Laureti, S., De Bellis, A.M., Muccitelli, V.I., Calcinaro, F., Bizzaro, A., Rossi, R., Bellastella, A., Santeusanio, F., and Falorni, A. (1998). Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with preclinical Addison's disease. *J. Clin. Endocrinol. Metab. 83*, 3507–3511.

Laureti, S., Vecchi, L., Santeusanio, F., and Falorni, A. (1999). Is the prevalence of Addison's disease understimated? *J. Clin. Endocrinol. Metab. 84*, 1762.

Laureti, S., Arvat, E., Candeloro, P., Di Vito, L., Ghigo, E., Santeusanio, F., and Falorni, A. (2000). Low dose (1 g) ACTH test in the evaluation of adrenal dysfunction in pre-clinical Addison's disease. *Clin. Endocrinol. 53*, 107–115.

Levine, S. and Wenk, E.J. (1968). The production and passive transfer of allergic adrenalitis. *Am. J. Pathol. 52*, 41–53.

Liblau, R.S., Singer, S.M., and McDevitt, H.O. (1995). Th1 and Th2 CD+T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today 16*, 34–38.

Lin, D., Zhang, L., Chiao, E., and Miller, W.L. (1994) Modeling and mutagenesis of the active site of human P450c17. *Mol. Endocrinol. 8*, 392–402.

Little, C., Marshall, C., and Downs, J. (1989). Addison's disease in the dog. *Vet. Rec. 124(17),* 469–470.

Løvås, K. and Husebye, E.S. (2002). High prevalence and increasing incidence of Addison's disease in western Norway. *Clin. Endocrinol. 56*, 787–791.

Mason, A.S., Meade, T.W., Lee, J.A., and Morris, J.N. (1968). Epidemiological and clinical picture of Addison's disease. *Lancet 2*, 744–747.

McIntyre Gass, J.D. (1962). The syndrome of keratoconjunctivitis, superficial moniliasis, idiopathic hypoparathyroidism and Addison's disease. *Am. J. Ophthalmol. 54*,660–674.

McNicol, A.M. (1986). Class II MHC expression in the adrenal cortex. *Lancet 2*, 1282.

McNicol, A.M. and Laidler, P. (1996). The adrenal gland and extra-adrenal paraganglia. In P.D. Lewis (ed.), *Endocrine System*. Edinburgh: Churchill Livingstone.

Morgan, J., Betterle, C., Zanchetta, R., Dal Pra, C., Chen, S., Rees Smith, B., and Furmaniak, J. (2000). Direct evidence that steroid 21-hydroxylase (21-OH) is the major antigen recognized by adrenal cortex autoantibodies (ACA). *J. Endocrinol. 167* (Suppl), OC19.

Muscatelli, F., Strom, T.M., Walker, A.P., Zanaria. E., Recan, D., Meindl, A., Bardoni, B., Guioni, S., Zehetner, G., and Rabl, W. (1994). Mutations in the DAX-1 gene give rise to both x-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature 372*, 672–676.

Myhre, A.G., Undelien, D.A., Lovas, K., Uhlving,. S, Nedrebo, B.G., Fougner, K.J., Trovik, T., Sorheim, J.I., and Husebye, E.S. (2002). Autoimmune adrenocortical failure in Norway autoantibodies and human leukocyte antigen class II association related to clinical features. *J. Clin. Endocrinol. Metab. 87,* 618–623.

Nerup, J. (1974). Addison's disease – clinical studies. A report of 108 cases. *Acta Endocrinol. 76,* 121–141.

Nerup, J. and Bendixen, G. (1969). Anti-adrenal cellular hypersensitivity in Addison's disease. 2. Correlation with clinical and serological findings. *Clin. Exp. Immunol. 5*, 341–353.

Nerup, J., Andersen, V., and Bendixen, G. (1970). Antiadrenal cellular hypersensitivity in Addison's disease. IV. *In vivo* and *in vitro* investigations of the mitochondrial fraction. *Clin. Exp. Immunol. 6*, 733.

Neufeld, M. and Blizzard, R.M. (1980). Polyglandular autoimmune diseases. In A. Pinchera, D. Doniach, G.F. Fenzi and L. Baschieri (eds), Symposium on Autoimmune Aspects of Endocrine Disorders. New York: Academic Press.

Nigam, R., Bhatia, E., Miao, D., Brozzetti, A., Eisenbarth, G.S., and Falorni, A. (2003). Prevalence of adrenal antibodies in Addison's disease among north Indian caucasians. *Clin. Endocrinol. 59*, 593–598.

Oelkers, W. (1996). Adrenal insufficiency. *N. Engl. J. Med. 335*, 1206–1212.

Oelkers, W., Diederich, S., and Bahr, V. (1992). Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity and aldosterone. *J. Clin. Endocrinol. Metab. 75*, 259–264.

Orth, D.N. and Kavacs, W.J. (1998). The adrenal cortex. In J.D. Wilson, D.W. Foster, H.M. Kronenberg and P.R. Larse (eds), *Williams Textbook of Endocrinology*, 9th ed. Philadelphia: W.B. Saunders.

Pelkey, T.J., Frierson, H.F. Jr, Mills, S.E., and Stoler, M.H. (1998). The alpha subunit of inhibin in adrenal cortical neoplasia. *Mod. Pathol. 11*, 516–524.

Peterson, M.E., Greco, D.S., and Orth, D.N. (1989). Primary hypoadrenocorticism in ten cats. *J. Vet. Intern. Med. 3*, 55–58.

Peterson, P. and Krohn, K.J.E. (1994). Mapping of B cell epitopes on steroid 17-α-hydroxylase, an autoantigen in autoimmune polyglandular syndrome type 1. *Clin. Exp. Immunol. 98*, 104–109.

Peterson, P., Perheentupa, J., and Krohn, K.J.E. (1996). Detection of candidal antigens in autoimmune polyglandular syndrome type I. *Clin. Diagn. Lab. Immunol. 3,* 290–294.

Peterson, P., Uibo, R., Peranen, J., and Krohn, K.J.E. (1997). Immunoprecipitation of steroidogenic enzyme autoantigens with autoimmune polyglandular syndrome type 1 (APS I) sera; further evidence for independent humoral immunity to P450 c17 and P450c21. *Clin. Exp. Immunol. 107,* 335–340.

Petri, M. and Nerup, J. (1971). Addison's adrenalitis. *Acta Path. Microbiol. Scand. 79*, 381–388.

Picado-Leonard, J. and Miller, W.L. (1987). Cloning and sequence of the human gene for P450c17 (steroid 17α-hydroxylase/17,20 lyase): similarity with the gene for P450c21. *DNA Cell. Biol. 6*, 439–448.

Rabinowe, S.L., Jackson, R.A., Dluhy, R.G., and Williams, G.H. (1984). Ia-positive T lymphocytes in recently diagnosed idiopathic Addison's disease. *Am. J. Med. 77*, 597–601.

Rees Smith, B. and Furmaniak, J. (1995), Editorial: adrenal and gonadal autoimmune diseases. *J. Clin. Endocrinol. Metab. 80*, 1502–1505.

Sadek, D. and Schaer, M. (1996). Atypical Addison's disease in the dog: a retrospective survey of 14 cases. *J. Am. Anim. Hosp. Assoc. 32*, 159–163.

Salim, Y.S., Faber, V., Wiik, A., Andersewn, P.L., Hoier-Madsen, M., and Mouritsen, S. (1988). Anti-corticosteroid antibodies in AIDS patients. *Acta Pathol. Microbiol. Immunol. Scand. 96*, 889–894.

Sasano, H., Nose, M., and Sasano, N. (1989). Lectin histochemistry in adrenocortical hyperplasia and neoplasm with emphasis on carcinoma. *Arch. Pathol. Lab. Med. 113*, 68–72.

Sasano, H., Miyazaki, S., Sawai, T., Sasano, N., Nagura, H., Funahashi, H., Aiba, M., and Demura, H. (1992). Primary pigmented nodular adrenocortical disease (PPNAD): immunohistochemical and *in situ* hybridization analysis of steroidogenic enzymes in eight cases. *Mod. Pathol. 5*, 23–29.

Sasano, H., Suzui, T., Shizawa, S., Kato, K., and Natura, H. (1994). Transforming growth factor alpha, epidermal growth factor and epidermal growth factor receptor in normal and diseased human adrenal cortex by immunohistochemistry and *in situ* hybridization. *Mod. Pathol. 7,* 741–746.

Skoldberg, F., Portela-Gomes, G.M., Grimelius, L., Nilsson, G., Perheentupa, J., Betterle, C., Husebye, E.S., Gustafsson, J., Ronnblom, A., Rorsman, F., and Kampe, O. (2003). Histidine decarboxylase is a novel autoantigen of enterochromaffin-like cells in autoimmune polyendocrine syndrome type 1. *J. Clin. Endocrinol. Metab. 88*, 1445–1452.

Song, Y., Connor, E.L., Muir, A., She, J.X., Zorovich, B., Derovanesian, D., and Maclaren, N. (1994). Autoantibody epitope mapping of the 21-hydroxylase antigen in autoimmune Addison's disease. *J. Clin. Endocrinol. Metab. 78*, 1108–1112.

Sotsiu, F., Bottazzo, G.F., and Doniach, D. (1980). Immunofluorescence studies on autoantibodies to steroid-producing cells, and to germline cells in endocrine disease and infertility. *Clin. Exp. Immunol. 39*, 97–111.

Steiner, J.W., Langer, B., Schatz, D.L., and Volpé, R. (1960). Experimental immunologic adrenal injury: a response to injections of autologous and homologous adrenal antigens in adjuvant. *J. Exp. Med. 112*, 187.

Stewart, P.M., Corrie, J., Seckl, J.R., Edwards, C.R., and Padfield, P.L. (1988). A rational approach for assessing the hypothalamo-pituitary-adrenal axis. *Lancet 1*, 1208–1210.

Stonehewer, J. and Tasker, S. (2001). Hypoadrenocroticism in a cat. *J. Small Anim. Pract. 42,* 186–190.

Takayanagi, R., Miura, K., Nakagawa, H., and Nawata, H. (2000). Epidemiological study of adrenal gland disorders in Japan. *Biomed. Pharmacother. 54*, 164–168.

Tanaka, H., Perez, M.S., Powell, M., Sandres, J.F., Sawicka, J., Chen, S., Prentice, L., Asawa, T., Betterle, C., Volpato, M., Rees Smith, B., and Furmaniak, J. (1997). Steroid 21-hydroxylase autoantibodies: measurements with a new immunoprecipations assay. *J. Clin. Endocrinol. Metab. 82,* 1440–1446.

Tasker, S., MacKay, A.D., and Sparkes, A.H. (1999). Case report. A case of feline primary hypoadrenocorticism. *J. Felin. Med. Surg. 1*, 257–260.

Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I., and Willingham, M.C. (1987). Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl Acad. Sci. USA 84*, 7735–7738.

Trousseau, A. (1856). Bronze Addison's disease. *Arch. Gen. Med. 8*, 478–485.

Verghese, M.V., Ward, F.E., and Eisenbarth, G.S. (1980). Decreased suppressor cell activity in patients with polyglandular failure. *Clin. Res. 28*, 270A.

Volpé, R. (1994). Autoimmune endocrinopathies: aspects of pathogenesis and the role of immune assays in investigation and management. *Clin. Chem. 40*, 2132–2145.

Wardle, C.A., Weetman, A.P., Mitchell, R., Peers, N., and Robertson, W.R. (1993). Adrenocorticotropic hormone receptor-blocking immunoglobulins in serum from patients with Addison's disease: a re-examination. *J. Clin. Endocrinol. Metab. 77*, 750–753.

Wedlock, N., Asawa, T., Baumann-Antczak, A., Rees Smith, B., and Furmaniak, J. (1993). Autoimmune Addison's disease. Analysis of autoantibody sites on human steroid 21-hydroxylase. *FEBS Lett. 332*, 123–126.

Werdelin, O. and Witebsky, E. (1970). Experimental allergic rat adrenalitis, a study on its elicitation and lymphokinetics. *Lab. Invest. 23*, 136–143.

Werdelin, O., Wick, G., and McCluskey, R.T. (1971). The fate of newly formed lymphocytes migration from an antigen-stimulated lymph node in rats with allergic adrenalitis. *Lab. Invest. 25,* 279–286.

Williams, G.H. and Dluhy, R.G. (1998). Disease of the adrenal cortex: In A.S. Fauci, E. Braunwald, K.J. Isselbacher, Wilson, J.D., Martin, J.B., Kasper, D.L., Hanser, S.L., and Longo, D.L. (eds), *Harrison's Principles of Internal Medicine*, 14th ed. New York: McGraw-Hill.

Willis, A.C. and Vince, F.P. (1997). The prevalence of Addison's disease in Coventry, UK. *Postgrad. Med. J. 73*, 286–288.

Wilson, R.C., Mercado, A.B., Cheng, K.C., and New, M.I. (1995). Steroid 21-hydroxylase deficiency: genotype may not predict phenotype. *J. Clin. Endocrinol. Metab. 80*, 2322–3239.

Winqvist, O., Karlsson, F.A., and Kampe, O. (1992). 21-hydroxylase, a major autoantigen in idiopathic Addison's disease. *Lancet 339*, 1559–1562.

Winqvist, O., Gustafsson, J., Rorsman, F., Karlsson, F.A., and Kampe, O. (1993). Two different cytochrome P450 enzymes are the adrenal antigens in autoimmune polyendocrine syndrome type I and Addison's disease. *J. Clin. Invest. 92* ,2377–2385.

Winqivist, O., Soderbergh, A., and Kampe, O. (1996). The autommune basis of adrenal cortical destruction in Addison's disease (review). *Mol. Med. Today 2*, 282–289.

Witebsky, E. and Milgrom, F. (1962). Immunological studies on adrenal glands: II. Immunization with adrenals of the same species. *Immunology 5*, 67–78.

Wulfraat, N.M., Drexhage, H.A., Bottazzo, G.F., Wiersinga, W.M., Jeucken, P., and Van der Gaag, R. (1989). Immunoglobulins of patients with idiopathic Addison's disease block the *in vitro* action of adrenocotropin. *J. Clin. Endocrinol. Metab. 69*, 231–238.

Yu, L., Brewer, K.W., Gates, S., Wu, A., Wang, T., Babu, S.R., Gottlieb, P.A., Freed, B.M., Noble, J., Erlich, H.A., Rewers, J., and Eisenbarth, G.S. (1999). DRB1*04 and DQ alleles: expression of 21-hydroxylase autoantibodies and risk of progression to Addison's disease. *J. Clin. Endocrinol. Metab. 84*, 328–335.

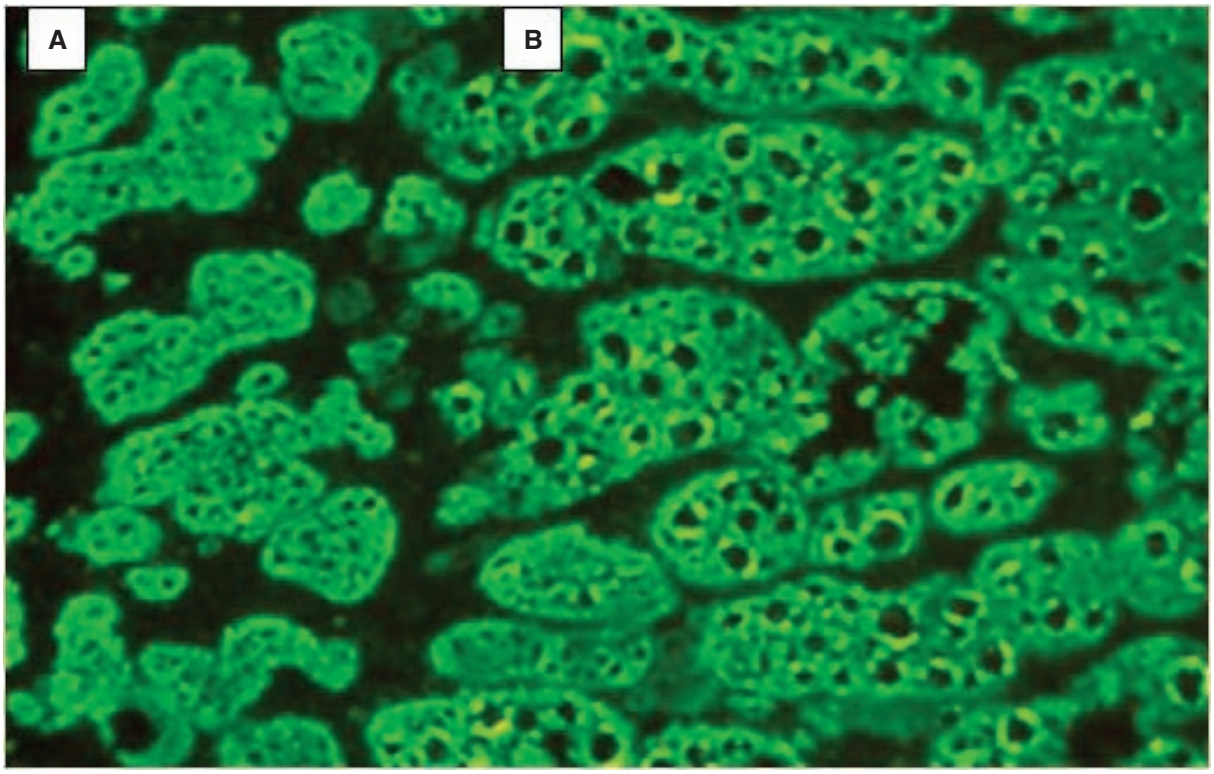


FIGURE 37.3 Immunofluorescence pattern on normal human adrenal cortex given by serum of a patient with AAD. This serum reacted with the cytoplasm of cells in all three layers of the cortex, but shown here are only glomerulosa (*A*) and fasciculata (*B*).

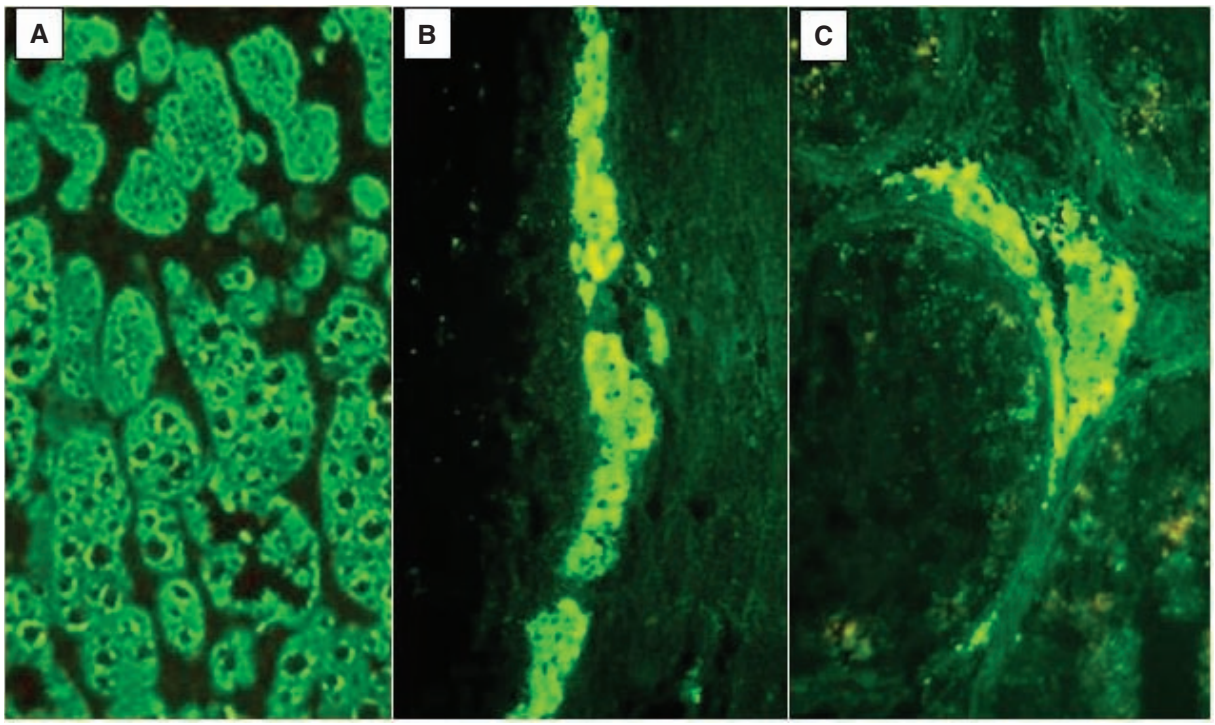


FIGURE 37.4 Immunofluorescence pattern given by serum of a patient with AAD and premature ovarian failure. This serum reacted against adrenal cortex (*A*), follicular theca of the ovary (*B*) and Leydig cells of the testis (*C*), and reflects autoantibodies to steroid-producing cells (StCA).

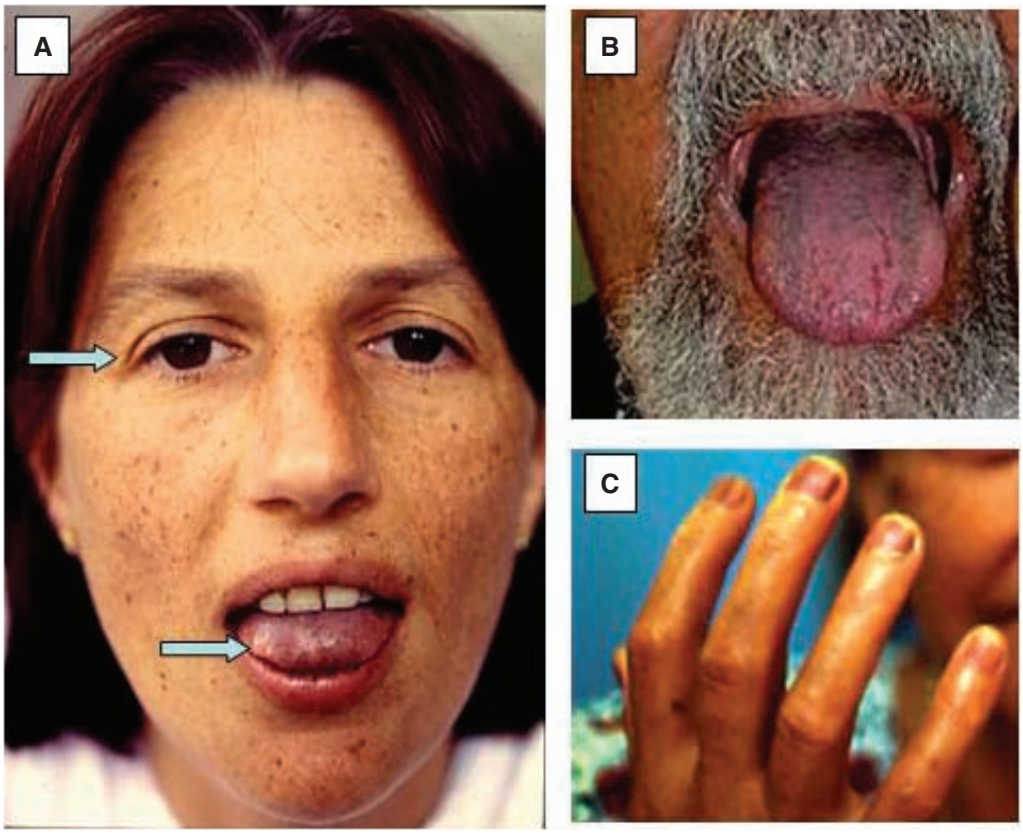


FIGURE 37.5 Clinical manifestations of AAD at onset. *A,* Hyperpigmentation of the skin, melanosis of the tongue, and enophthalmos as a sign of dehydration. *B,* Male showing a melanosis of the tongue. *C,* Hyperpigmentation of the nails.

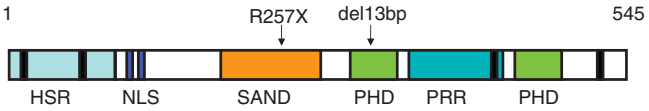


FIGURE 38.3 Schematic outline of the different domains of the AIRE protein. HSR: homogeously staining region, also present in Sp100 and Sp 140, a region that mediates homodimerization; NLS: nuclear localization signal; SAND: a putative DNA-binding region; PHD: plant homeodomain zinc fingers; PRR: proline-rich region. The four black boxes correspond to nuclear receptor binding LXXL motifs. The most common mutation in the Finnish population (R257X) and another common mutation, a 13 bp deletion, are denoted with arrows. The numbers correspond to the first and last amino acids of the protein.

CHAPTER

38

Polyendocrine Syndromes

PÄRT PETERSON*
*Department of Molecular Pathology, Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia

RAIVO UIBO†
†Department of Immunology, University of Tartu, Estonia

OLLE KÄMPE‡
‡Departmentt of Medical Sciences, Uppsala University and University Hospital, Uppsala, Sweden

In 1855, Thomas Addison published observations describing adrenocortical failure (Addison, 1855). This disorder has since been known as Addison's disease (Wilks, 1862). Schmidt (1926) later demonstrated that the detection of lymphocytic infiltration in both the adrenal and thyroid glands is more frequent than expected. The simultaneous occurrence of Addison's disease and thyroiditis became known as Schmidt syndrome (OMIM 269200). Several subsequent publications indicated that idiopathic Addison's disease and thyroid disease are often found together in one patient. In 1959 Beaven and coworkers reported that Addison's disease frequently occurred with diabetes mellitus in the same patient. Some years later, Carpenter et al. (1964) published an observation that insulin-dependent diabetes mellitus is more common in Schmidt syndrome than in the normal population, further establishing the association of the three endocrine diseases. Addison's disease was less frequently associated with diabetes than with thyroid disease, for which the majority of the cases were Hashimoto's thyroiditis.

The first clinical reports on patients with Addison's disease presenting with chronic candidal infection and hypoparathyroidism appeared in the 1950s (Etzel and Robson, 1958) and in the 1960s several reports pointed to the clinical and genetic heterogeneity among patients with Addison's disease (Spinner et al., 1968; Blizzard and Gibbs, 1968), suggesting that, depending on the associated disorders, Addison's disease can be part of two separate clinical syndromes. In 1981, on the basis of previous clinical and laboratory data, the occurrence of Addison's disease, when associated with other diseases, was divided by Neufeld and coworkers (1981) into two autoimmune polyglandular (polyendocrine) syndromes (APS), type 1 and type 2.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

APS-1 (OMIM 240300) has a broad spectrum of clinical manifestations. The disease is also referred to as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) or polyglandular autoimmune syndrome (PAS) type 1 (Table 38.1). The most frequent clinical entities include chronic mucocutaneous candidiasis, hypoparathyroidism, and Addison's disease. At least two of the three major components need to be present in order to make a diagnosis. Usually, APS-1 starts in childhood with chronic candidiasis as a first manifestation followed by hypoparathyroidism and Addison's disease. Any one of the three major diseases may be absent, and APS-1 may clinically appear in adulthood. In addition, many other components of the disorder have been described in APS-1 patients.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 38.1 Prevalences in APS-1 and APS-2 of particular clinical features and accompanying diseases (adapted from Perheentupa, 2002; Betterle et al., 2002; Schatz and Winter, 2002)

Disease components	APS-1	APS-2
Addison's disease	60–100	70–100
Hypoparathyroidism	77–100	Absent
Chronic candidiasis	73–100	Absent
Ectodermal dysplasia	10–77	Absent
Autoimmune thyroid disease	8–18	69–83
Type 1 diabetes	4–23	28–52
Hypogonadism	31–60	9
Alopecia	27–72	2–5
Vitiligo	4–26	4–11
Keratopathy	12–35	Absent
Autoimmune hepatitis	10–19	4
Pernicious anemia	12–15	2–25
Chronic gastritis	6	11

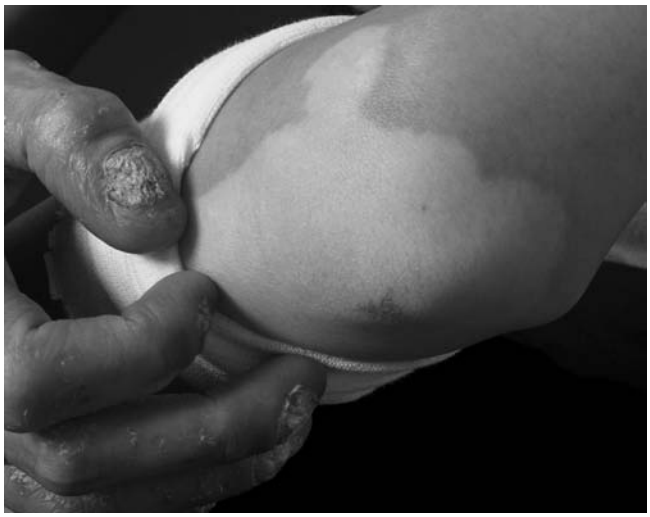


FIGURE 38.2 Vitiligo and nail candidiasis in a patient with APS-1.

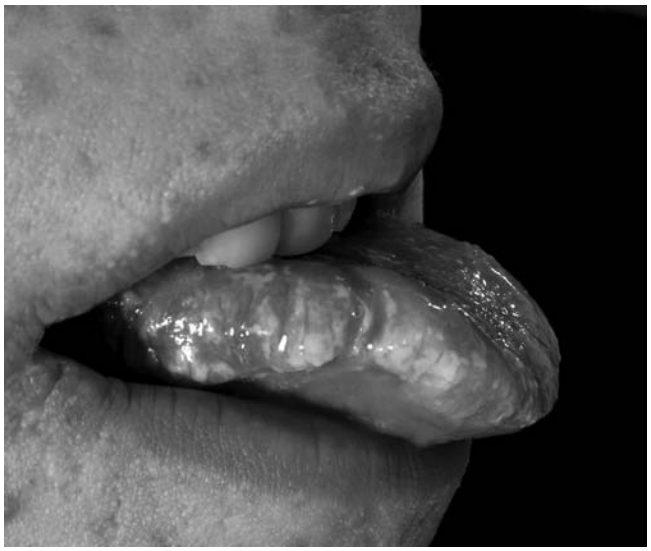


FIGURE 38.1 Mucocutaneous candidiasis in a patient with APS-1.

Candida infection appears in more chronic cases as white thrush on the tongue (Figure 38.1). Candidal esophagitis is often present and the infection may spread to nails (Figure 38.2) and in some cases even to skin of the hands and face. Several cases of oral carcinoma have been reported, suggesting that oral candidiasis might be carcinogenic (reviewed in Perheentupa, 2002). Hypoparathyroidism is the most frequent, and sometimes the only, endocrine disease seen in APS-1 patients. For example, in the majority of Iranian Jews and in approximately 20% of Finnish patients, hypoparathyroidism remained the only endocrinopathy. In some cases hypoparathyroidism is followed by adrenocortical insufficiency, with an age at onset of about 4–12 years, but in other cases it may appear at 20 years of age (Perheentupa, 2002). Premature ovarian failure in females is far more common (female-to-male ratio, 3 : 1) than primary testicular failure in males. The blood–testis barrier could be a probable explanation for this gender difference. Type 1 diabetes, hypophyseal failure, autoimmune thyroid disease, keratoconjunctivitis, pernicious anemia, autoimmune hepatitis, and gastrointestinal dysfunctions consisting of malabsorption or diarrhea are less common components of the syndrome. While the chronic candidiasis is most likely due to the defect in cell-mediated immunity, the association of ectodermal dystrophy with autoimmunity is still an open question. Ectodermal skin diseases such as alopecia with patchy loss of hair, alopecia totalis with total hair loss and vitiligo with pigment-free skin areas, enamel hypoplasia and nail dystrophy can be present. In addition, urticaria-like erythema (Perheentupa, 2002) and hyposplenism or splenic atrophy have also been reported in APS-1 patients (Friedman et al., 1991).

APS-2 is a genetically complex disease with a multifactorial etiology. The clinical onset usually occurs in adulthood, although it can start at any time during the lifespan. The classic definition of APS-2 (Neufeld et al., 1981) comprises a combination of Addison's disease with autoimmune thyroid disease or type 1 diabetes, or both (see Table 38.1), although it is obvious that any combinations of autoimmune disorders within the complex represent the same disorder with a probably inherited propensity to develop organ-specific autoimmunity (Schatz and Winter, 2002). Type 1 diabetes develops often before the Addison's disease. Other diseases such as vitiligo, alopecia, hypergonadotropic hypogonadism, and chronic gastritis with or without pernicious anemia may occur but less frequently than in APS-1 and usually not in childhood. The prevalence of APS-2 is estimated to be 1.4–2.0 in 100 000 inhabitants. APS-2 affects

in particular middle-aged Caucasian women with a male-to-female ratio of 1 : 2–3.

APS-3 was included in the original classification of autoimmune polyendocrine syndromes (Neufeld et al., 1981) to define the clinical entity of autoimmune thyroid disease and one or more other autoimmune diseases, excluding Addison's disease. The genetic and etiological background of APS-3 is similar to that of APS-2. Sometimes, APS-4 is also mentioned but we suggest that for clarity, this entire complex of disorders (APS-2, APS-3, and APS-4) should be named APS-2 as distinct from APS-1 (APECED).

AUTOIMMUNE FEATURES

Seminal evidence on the autoimmune pathogenesis of Addison's disease was provided by Anderson et al. (1957) who reported the presence of adrenocortical antibodies (ACA) in patients with Addison's disease using complement fixation. The finding of autoantibodies to adrenal cortex was later confirmed by the immunofluorescence method (Blizzard et al., 1962). With indirect immunofluorescence, ACA were found in 50–90% of patients with Addison's disease (Blizzard and Kyle, 1963; Riley et al., 1980; Sotsiou et al., 1980) but were not detected in the tuberculous form of Addison's disease (Irvine and Barnes, 1975). The autoantibodies are usually IgG (Goudie et al., 1968), predominantly of the IgG1 subclass (Boe et al., 2004), and often react with all layers of adrenal cortex (Irvine and Barnes, 1975). Immunofluorescence studies revealed that in addition to ACA, autoantibodies to the steroid-producing cells (StCA) were consistently present, reacting with the theca interna and corpus luteum of ovary, interstitial cells of testis, and placental trophoblasts (Irvine and Barnes 1975, Maclaren and Blizzard 1985).

The characteristic feature of APS-1 is the presence of high-titer autoantibodies to several tissue-specific autoantigens. The nature of the proteins recognized by the autoantibodies began to emerge in the beginning of 1990s when the adrenal cortex-specific autoantigens were identified. Steroidogenic P450 cytochrome enzymes, steroid 17α-hydroxylase (P450c17 or CYP17), steroid 21 hydroxylase (P450c21 or CYP21) and side chain cleavage enzyme (P450scc or CYP11A1) were identified as the molecular targets of the adrenal specific autoantibodies (Krohn et al., 1992; Winqvist et al., 1992; 1993; 1995; Bednarek et al., 1992; Baumann-Antczak et al., 1992; Uibo et al., 1994). All three enzymes belong to the P450 cytochrome superfamily and are central in the steroidogenic pathway occurring in the adrenal cortex. P450c21 is highly expressed in the adrenal cortex whereas P450c17 and P450scc are also expressed in the gonads.

Autoantibodies to several other self-antigens have been reported in APS-1, which often belong to related protein families. For example, autoantibodies against glutamic acid decarboxylases (GAD65 and GAD67), major autoantigens in type 1 diabetes, are common in APS-1 (Björk et al., 1994; Velloso et al., 1994). Group II pyridoxal phosphate-dependent amino acid decarboxylases are structurally related to GAD enzymes, including aromatic L-amino acid decarboxylase (AADC), histidine decarboxylase (HDC), and cysteine sulfinic acid decarboxylase (CSAD), which all react as autoantigens in APS-1 (Husebye, et al., 1997; Sköldberg et al., 2003; 2004). Another set of autoantigens comprises tryptophan and tyrosine hydroxylases (TPH and TH, respectively), which belong to a group of pteridine-dependent hydroxylases (Ekwall et al., 1998; 2000; Hedstrand et al., 2000). APS-1 patients also develop antibodies to hepatic P450 cytochromes, P4501A2 and 2A6; however, only P4501A2 is associated with autoimmune hepatitis in APS-1 (Clemente et al., 1998; Gebre-Medin et al., 1997). In addition, immunoreactivity to the tyrosine phosphatase-like protein IA-2 (IA-2), intrinsic factor, thyroid peroxidase and thyroglobulin (Perniola et al., 2000) and to a transcription factor SOX10 have been reported (Hedstrand et al., 2001). The patients with hypoparathyroidism were found to have antibodies to calcium sensing receptor protein (Li et al., 1996; Goswami et al., 2004) but the result was not confirmed by other studies (Gylling et al., 2003; Söderbergh et al., 2004). The prevalence of autoantibodies among 90 Finnish, Swedish, and Norwegian APS-1 patients is shown in Table 38.2.

In APS-2, most of the patients have autoantibodies to P450c21, the major autoantigen of Addison's disease (Winqvist et al., 1992; Bednarek et al., 1992; Baumann-Antzcak et al., 1992). The P450c21 autoantibodies have been demonstrated by several methods including immunoblotting with recombinant protein expressed in

TABLE 38.2 Prevalence of autoantibodies in APS-1 patients (adapted from Perniola et al., 2000; Ekwall et al., 2000; Söderbergh et al., 2004; Sköldberg et al., 2003, 2004)

Autoantigen	Prevalence %
Steroid 21 hydroxylase (P450c21)	66
Side chain cleavage enzyme (P450scc)	52
Steroid 17α-hydroxylase (P450c17)	44
Aromatic L-amino acid decarboxylase (AADC)	51
Glutamic acid decarboxylase (GAD65)	37
Histidine decarboxylase (HDC)	37
Cysteine sulfinic acid decarboxylase (CSAD)	3.6
Tryptophan hydroxylase (TPH)	45
Tyrosine hydroxylase (TH)	44
Thyroglobulin (TG)	36
Thyroid peroxidase (TPO)	36
Transcription factor SOX10	22
Cytochrome P4501A2 (P4501A2)	8
Tyrosine phosphatase-like protein IA-2 (IA-2)	7

bacteria, yeast or mammalian cells or by immunoprecipitation with *in vitro* translated protein (for review, see Betterle et al., 2002 and Chapter 37). The P450c21 protein is also the molecular target of the ACA that have been widely used as a diagnostic marker for Addison's disease. In approximately 15–20% of female APS-2 patients, autoantibodies to P450c17 and P450scc are detectable, which are the targets of the StCA (Hoek et al., 1997; Betterle et al., 2002). APS-2 patients with type 1 diabetes frequently have autoantibodies to GAD65 and/or IA-2 protein. Autoantibodies to thyroid peroxidase proteins are common in patients with autoimmune thyroiditis.

GENETIC FEATURES

APS-1 is inherited in an autosomal recessive manner and occurs as a defect in the autoimmune regulator (AIRE) gene (Figure 38.3). The prevalence worldwide is very low and is population dependent. The frequency is higher among certain populations such as Finns (1/25,000), Sardinians (1/14,400) and Iranian Jews (1/9000), and lower among Norwegians, (1/80,000). A high prevalence (1/4400) occurs in the small town of Bassano del Grappa in Northern Italy (Betterle et al., 2002).

AIRE, the gene mutated in APS-1, was identified by positional cloning on chromosome 21q22.3 (Nagamine et al., 1997; Finnish-German Consortium 1997). To date, around 50 mutations have been found in the AIRE gene (reviewed in Heino et al., 2001; Kumar et al., 2002). The mutations are spread throughout the coding region but two mutational hotspots have emerged. The most common mutation is amino acid arginine change to stop codon (R257X) in exon 6, which is found in 83% of the Finnish APS-1 chromosomes (Björses et al., 2000). The R257X mutation is also common in Northern Italian and Eastern European APS-1 patients (Scott et al., 1998; Björses et al., 2000; Cihakova et al., 2001). The other frequently occurring mutation, 1094-1106del13bp, is the most common mutation in Anglo-American APS-1 patients, accounting for 70% of British and 53% of North American APS-1 alleles, as well as being reported in several APS-1 cases from other populations (Wang et al., 1998; Pearce et al., 1998; Heino et al., 1999). A large proportion of the AIRE mutations are nonsense mutations or deletions/insertions, usually resulting in a truncated protein lacking the PHD fingers, one of the characteristic domains of the protein. Most of the missense mutations occur in the N-terminal of AIRE, a region responsible for the homodimerization of the protein (reviewed in Pitkänen and Peterson, 2003).

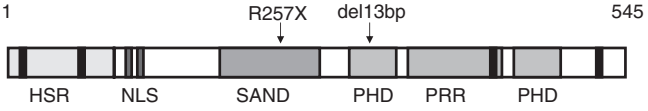


FIGURE 38.3 Schematic outline of the different domains of the AIRE protein. HSR: homogeously staining region, also present in Sp100 and Sp 140, a region that mediates homodimerization; NLS: nuclear localization signal; SAND: a putative DNA-binding region; PHD: plant homeodomain zinc fingers; PRR: proline-rich region. The four black boxes correspond to nuclear receptor binding LXXL motifs. The most common mutation in the Finnish population (R257X) and another common mutation, a 13 bp deletion, are denoted with arrows. The numbers correspond to the first and last amino acids of the protein. See color plate section.

Despite considerable variation in the APS-1 phenotype, no clear correlation with genotype has been found. The growing number of AIRE mutations will enable us to draw better conclusions in the future. However, it is evident now that other factors influence the outcome of the disease components. As an example, there is significant clinical variation among Finnish APS-1 patients carrying a homozygous R257X mutation and several reports describe intrafamilial differences among siblings having an identical AIRE genotype (Halonen et al., 2002).

APS-1 has no clear association with the HLA region, although some components of the disease seem to correlate with certain HLA alleles or haplotypes. For example, in one study, Addison's disease was found to be associated with HLA DRB1*03, alopecia with DRB1*04-DQB1*0302 and type 1 diabetes correlated negatively with DRB1*15-DQB1*0602 (Halonen et al., 2002). However, another study did not find an association with HLA alleles (Gylling et al., 2003).

APS-2, in contrast to APS-1, is a genetically complex disease. As for most autoimmune diseases, APS-2 is strongly associated with the HLA gene locus in chromosome 6p21. Genetic studies have demonstrated a consistent association of APS-2 with HLA DRB1*0301 (DR3), DQA1*0501 and DQB1*02 (DQ2) alleles, which are in strong linkage equilibrium with each other (Maclaren and Riley, 1986; Partanen et al., 1994). The haplotype is associated with isolated disease components of APS-2, type 1 diabetes, Graves' disease, autoimmune hypothyroidism, and Addison's disease. Most of the studies have not elucidated which of these three alleles is the primary etiological genetic factor. Another haplotype, consisting of DRB1*04 (DR4) DQA1*0301 DQB1*0302 (DQ8), is also associated with type 1 diabetes and Addison's disease (Partanen et al., 1994; Myhre et al., 2002) and the existence of both risk haplotypes in one individual confers high susceptibility for type 1 diabetes or Addison's disease alone or in combination. In addition, Myhre et al. (2002) reported that the haplotype DRB1*01 (DR1) DQA1*0101 DQB1*0501 (DQ5) is protective against Addison's disease. Alleles of other genes located in HLA region, such as TNF, P450c21 and MIC-A gene, have been associated with APS-2 (Partanen et al., 1994; Peterson et al., 1995; Gambelunghe et al., 1999) but

these associations are most likely to be secondary to the strong linkage disequilibrium in the HLA region and thus are not primary associations.

The cytotoxic T lymphocyte-associated protein-4 (CTLA-4) gene on chromosome 2q33 is associated with autoimmune endocrinopathies from different populations (reviewed in Vaidya and Pearce, 2004). In particular, the allelic variants of an A to G single nucleotide polymorphism at position 49 and microsatellite marker in the 3′ untranslated region of the CTLA-4 gene are associated with type 1 diabetes and thyroiditis in Italian, German, and British patients (Levin and Tomer, 2003). The CTLA-4 association with Addison's disease and APS-2 with 3′ untranslated region microsatellite was significantly increased among English but not among Norwegian, Finnish, and Estonian patients (Kemp et al., 1998). In another study on English patients with autoimmune Addison's disease and APS-2, a strong association of the G allele at position 49 was found with Addison's disease as a component of APS-2 (Vaidya et al., 2000). Recently, autoimmune disease susceptibility was mapped to a noncoding 6.1 kb 3′ region of CTLA-4, the common allelic variation of which was correlated with lower messenger RNA levels of the soluble alternative splice form of CTLA-4 (Ueda et al., 2003). Additionally, a number of other susceptibility genes have been characterized in APS-2; however, their role is not so universal and they will not be discussed here. These studies are reviewed by Redondo and Eisenbarth (2002), Bottini et al. (2004), MacMurray et al. (2004) and others.

An influence of AIRE gene polymorphisms in APS-2 or other major autoimmune diseases is possible but analyses of the AIRE mutations R257X and 967-979del13bp indicated no contribution to the susceptibility to type 1 diabetes, Graves' disease or autoimmune hepatitis (Vaidya et al., 2000; Nithiyananthan et al., 2000; Meyer et al., 2001; Djilali-Saiah et al., 2004).

ENVIRONMENTAL FEATURES

The environmental factors involved in autoimmune polyendocrine syndromes are less evident than genetic factors. A recent increase in the incidence of type 1 diabetes mellitus and probably other autoimmune endocrine diseases and polyendocrine syndromes in most of the developed countries points to the novel environmental influences as the genetic background has largely remained unchanged. The role of environment has been best studied in patients with type 1 diabetes (see Chapter 36), in which analysis of twins shows that the genetic background cannot be the only reason for the disease (Hyttinen et al., 2003). National prosperity and good hygiene levels seem to correlate in uncertain ways with type 1 diabetes (Patterson et al., 2001). The role of viral infections in polyendocrinopathies remains unclear.

ANIMAL MODELS

Spontaneous Animal Models

The most studied spontaneous animal models for polyendocrine autoimmunity are the Bio-Breeding (BB) rat, Komeda diabetes-prone (KDP) rat, nonobese diabetic (NOD) mouse, and obese-strain (OS) White Leghorn chicken.

The BB rat develops type 1 diabetes which is clinically similar to the diabetes seen in humans. In addition to destruction of the insulin-producing pancreatic β cells, BB rats frequently have lymphocytic infiltration in the thyroid gland, usually without hypothyroidism, but the thyroiditis can be potentiated by feeding rats with a high iodine diet (Allen et al., 1986). No evidence of lymphocytic infiltration to the adrenal gland has been observed but autoantibodies to gastric parietal cells and smooth muscle have been reported (Crisa et al., 1992). BB rats have severe T-cell lymphopenia which is a recessive trait and important in the development of diabetes. The mononuclear infiltration into islets starts within the first 3 weeks of life and the diabetes develops at 8–12 weeks. Genetic backcross experiments showed that both insulitis and thyroiditis develop through common immune defects involving T-cell lymphopenia, but the susceptibility of MHC class II alleles for disease development is distinct for insulitis and thyroiditis (Awata et al., 1995).

At least three susceptibility genes are important in development of diabetes in the BB rat: IDDM2 in the MHC gene region on chromosome 20, Ian5 (immune-associated nucleotide 5 on chromosome 4 (also called Ian4, IDDM1 or lyp), which is responsible for the lymphopenia (MacMurray et al., 2002; Hornum et al., 2002; Moralejo et al., 2003), and a third, so far unmapped gene on chromosome 2 (Jacob et al., 1992). Ian5 is a GTO-binding protein highly expressed in lymph node and spleen, and regulates apoptosis in the T-cell lineage (MacMurray et al., 2002).

Similarly to the BB rat, the KDP rat develops autoimmune diabetes but without significant T-cell lymphopenia. The disease onset is rapid with development of 100% diabetes by the age of 30 weeks (Yokoi et al., 1997). Both sexes are equally affected. In addition to pancreatic islets, KDP rats have severe lymphocyte infiltration into the thyroid glands and mild to moderate infiltration into the submandibular glands, kidney, adrenals, and pituitary (Yokoi et al., 1997). Most of the genetic predisposition to autoimmunity in the KDP rat is due to a specific nonsense mutation (R455X) in the Cblb (Casitas B-lineage lymphoma b) gene identified by positional cloning (Yokoi et al., 2002). Cblb is a ubiquitine ligase that negatively controls the CD28 costimulatory molecule in T-cell activation. T cells from homozygous Cblb-deficient mice do not require the CD28 costimulatory signal for activation and develop spontaneous

autoimmunity with T- and B-cell infiltrations in multiple organs (Bachmaier et al., 2000). It should be noted that neither Ian5 nor Cblb genes have been found so far as susceptibility genes for human type 1 diabetes (Payne et al., 2004; Kosoy et al., 2004) and hence it is unlikely that these genes will contribute to either type of APS.

The NOD mouse is mostly studied as a model for type 1 diabetes. In addition to pancreatic β-cell destruction, NOD mice have autoimmunity to submandibular and lacrimal glands, although to a lesser extent. The incidence of thyroiditis is low but high iodine in the diet has a triggering effect on development of thyroiditis (Many et al., 1996). Lymphocytic infiltration in the adrenal and parathyroid glands has been described in NOD mice (Krug et al., 1991; Beales et al., 2002). Insulitis starts at an early age (4–5 weeks old), reaching 70–90% by 9 weeks. Diabetes is mild and more prevalent in females (80–90%) than males (10–50%). The incidence of submandibular and lacrimal gland infiltration seems not to be secondary to insulitis and is more prevalent in females.

The genetic background of the NOD mouse has been extensively studied and approximately 20 different genetic loci have been reported (reviewed in Johansson et al., 2003, and see Chapter 36). IDDM1 located in the MHC gene region ($H2^{g7}$ on chromosome 17) is the major locus for diabetes. Additionally, the IDDM3 region and Il2 gene on chromosome 3, CTLA-4 and CD28 on chromosome 1, and CD30 and Tnfr2 on chromosome 4 have been suggested to be important. Genetic segregation experiments indicate a genetic heterogeneity in terms of different autoimmune disorders in the NOD mouse; it seems that each disorder is mainly controlled by unique sets of disease-promoting alleles but that some disorders are more similar than others (Johansson et al., 2003). In addition, many transgenic, gene-targeted and congenic variations of NOD mice have been developed with variable effects on development of diabetes (reviewed in Yang and Santamaria, 2003, and see Chapters 26 and 36). Despite the similarities between human type 1 diabetes and diabetes in the NOD mouse, it should be noted that NOD mice do not develop autoantibodies against the major human autoantigen GAD65 (Velloso et al., 1994; Mackay et al., 1996).

The OS chicken spontaneously develops lymphocytic thyroiditis and autoantibodies to adrenal gland. Mononuclear infiltration starts in the second week of life and results in complete destruction of the thyroid gland by 1–2 months of age. OS chickens, however, do not have adrenal insufficiency or type 1 diabetes.

Experimentally Induced Animal Models

One of the well-studied mouse models is thymectomy at day 3 in the BALB/C strain, which leads to multiorgan autoimmunity characterized by gastritis, thyroiditis, oophoritis, and other infiltrations. The infiltrations are T-cell dependent and the mice develop autoantibodies to affected tissues. Thymectomy-induced autoimmunity is strictly dependent on time of thymectomy, i.e., between the second and fifth days after birth. The autoimmunity occurs due to a lack of $CD4^+CD25^+$ regulatory T cells (see Chapter 26), which migrate out of the thymus in the early neonatal period, and injection of purified regulatory T cells back into the thymectomized mice prevents autoimmunity (reviewed in Sakaguchi et al., 2001).

Several transgenic and knockout animal models develop endocrine autoimmunity (reviewed in Lam-Tse et al., 2002). The transgenic models often use expression of autoantigen, MHC or costimulatory molecules in a tissue-specific manner, or overrepresentation of a certain T-cell receptor repertoire. The models are useful but often represent exaggerations of native autoimmune process. From gene knockout models, two recently generated mouse models have emerged: AIRE- and FoxP3-deficient mice, representing respectively APS-1 and IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance) diseases in humans. AIRE-deficient mice do not express self-antigens in the thymus; hence there is impaired elimination of self-reactive T cells and as a result, the mice develop lymphocytic infiltrations and autoantibodies to multiple organs (see below). FoxP3 is a forkhead transcription factor family member that is essential for development in thymus of the $CD4^+CD25^+$ regulatory T cells which participate in the maintenance of immunologic tolerance (Fontenot et al., 2003; Khattri et al., 2003; Ochs et al., 2005; see Chapter 9). $CD4^+CD25^+$ regulatory T cells have been shown to suppress several experimental models of organ-specific autoimmune diseases. Lack of FoxP3 expression in mice leads to the overproliferation of $CD4^+$ lymphocytes with multiorgan infiltration and to elevation of numerous cytokines (Gavin and Rudensky, 2003).

PATHOGENIC MECHANISMS

The endocrine glands in APS-1 and APS-2 are affected by a similar histologic autoimmune destruction. The tissue is gradually destroyed, resulting in atrophy, or parenchymal cells are replaced by fat cells (Perheentupa, 2002). Adrenalitis in APS patients appears to be diffuse, affecting all three layers of the cortex. As an indication of T-cell-mediated autoimmunity, it mainly consists of lymphocytes but macrophages and plasma cells have also been detected (Carpenter et al., 1964; Muir et al., 1993). Indeed, there is significant support for a direct pathogenic role of T cells in autoimmune endocrine diseases, exemplified by type 1 diabetes as the most studied disorder among endocrinopathies (reviewed in Roep, 2002). Treatment with immunosuppressive drugs, cyclosporine A, directed against T cells, will

produce temporary remission of the disease in APS-2 patients (Csaszar and Patakfalvi, 1992). However, it has been difficult to characterize autoreactive T cells in APS patients, partly due to the lack of specific and sensitive methods, and to T cells that, in contrast to autoantibodies, are sequestered in the specific tissue lesions (Roep, 2002). Interestingly, a recent report by Kriegel and coworkers (2004) suggested that $CD4^+CD25^+$ regulatory T cells from peripheral blood of APS-2 patients are functionally defective in their suppressive capacity despite no evident differences in their quantity or in their T-cell surface markers between the patients and controls. The results concur with depletion of regulatory T cells in mice causing a syndrome resembling APS-2 (Sakaguchi et al., 2001). Although further confirmatory experiments are needed, these data highlight the possibility that an impaired function of regulatory T cells may have a pathogenic role in APS-2.

Autoimmunity in APS-1 is due to defective central tolerance to the autoantigens. Using a microarray approach and a gene knockout mouse as a model, Mathis and coworkers showed that AIRE gene deficiency in mice results in defective expression of the tissue-specific autoantigens in thymus, where negative selection of autoreactive T cells occurs (Anderson et al., 2002). The AIRE gene is predominantly expressed in thymic medullary epithelial cells (Figure 38.4) and acts as a transcriptional activator (reviewed in Pitkänen and Peterson, 2003). The lack of the AIRE expression in thymus thus leads to a defect in clonal deletion of autoreactive T cells and ultimately to autoimmunity (Liston et al., 2003; Mathis and Benoist, 2004). AIRE may regulate the processing and/or presentation of self-proteins so that the maturing T cells can recognize these self-antigens (Kuroda et al., 2005). AIRE also seems to have a role in peripheral tolerance as the peripheral T cells of AIRE-deficient mice have increased proliferative responses to foreign antigen (Ramsey et al., 2002). However, direct data are lacking on clonal deletion in human APS-1 patients. A report by Sediva et al. (2002) detected low IFN-γ levels in patients with APS-1 and marked elevation of IgM and an increase of numbers of $CD3^+CD4^+$ lymphocytes. Interestingly, fathers of APS-1 patients had elevated levels of IgA and activated T lymphocytes, which may indicate that heterozygous AIRE mutations may contribute at least to subclinical autoimmunity.

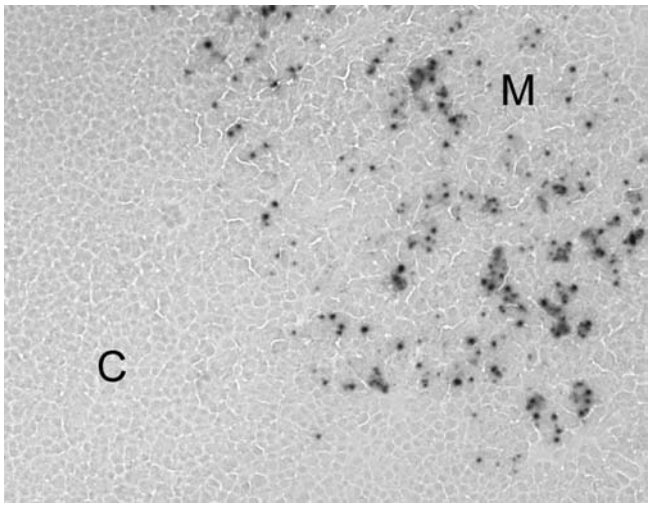


FIGURE 38.4 AIRE expression in thymic medullary epithelial cells visualized by beta-galactosidase staining of the heterozygous AIRE-deficient mouse thymus. The beta-galactosidase reporter gene has been inserted into the AIRE gene locus in these mice. C: cortex; M: medulla. ×200. Courtesy of Kaidi Möll and Dr Hamish Scott.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

Autoantibodies are often diagnostic or even predictive markers for the clinical disease. In APS-1, for example, autoantibodies to the steroidogenic enzymes (P450c21, P450c17, and P450scc) are significant markers for Addison's disease. Antibodies against at least one of the three antigens are found in 84% of patients with Addison's disease (Söderbergh et al., 2004). As another example, hypogonadism is strongly associated with the presence of autoantibodies to P450scc protein. Autoantibodies to GAD65 that have high clinical association with isolated type 1 diabetes or diabetes in APS-2 are also associated with type 1 diabetes in APS-1. Autoantibodies to the IA-2 antigen, however, seem to have the strongest association with type 1 diabetes in APS-1 (Söderbergh et al., 2004). Autoantibody analyses in patients with APS-1 indicate serologic markers for hepatitis (aromatic L-amino acid decarboxylase, cytochrome P450 1A2 or tryptophan hydroxylase), gastrointestinal dysfunction (tryptophan hydroxylase), and vitiligo (SOX10; Hedstrand et al., 2001). Table 38.3 summarizes the associations between disease components and the presence of the more common autoantibodies in APS-1.

The vast majority (up to 90%) of patients with APS-2 have ACA or autoantibodies to P450c21. The titers of autoantibodies decrease to some extent as the disease progresses and therefore, the diagnostic value of the autoantibodies is highest at the onset of Addison's disease. P450c21 autoantibodies are generally a better marker for autoimmune Addison's disease than ACA, but it has been reported that 100% of APS-2 patients at the clinical onset of Addison's disease are positive for ACA (Betterle et al., 2002). The presence of ACA or anti-P450c21 antibodies in patients with autoimmune thyroid disease or type 1 diabetes but without Addison's disease (Barker et al., 2005) would indicate subclinical hypoadrenalism and be a predictive marker for later development of overt Addison's disease, although the

TABLE 38.3 Associations between APS-1 disorders and antibody reactivity (adapted from Söderbergh et al., 2004)

Disease	Autoantigen	Sensitivity %	Specificity %	Risk ratio
Addison's disease	Steroid 21 hydroxylase (P450c21)	75	63	7.9
	Sidechain cleavage enzyme (P450scc)	68	74	7.0
	Steroid 17α-hydroxylase (P450c17)	65	68	9
Hypogonadism	Sidechain cleavage enzyme (P450scc)	75	76	12.5
Type 1 diabetes	Tyrosine phosphatase-like protein IA-2 (IA-2)	33	87	14.9
Vitiligo	Aromatic L-amino acid decarboxylase (AADC)	63	69	3.8
	Glutamic acid decarboxylase (GAD65)	56	63	3.3
Hepatitis	Tryptophan hydroxylase (TPH)	86	79	27.0
	Cytochrome P4501A2 (P4501A2)	71	76	17.5
	Aromatic L-amino acid decarboxylase (AADC)	79	75	16.4
Intestinal dysfunction	Glutamic acid decarboxylase (GAD65)	75	74	10.8
	Tryptophan hydroxylase (TPH)	80	64	7.5

progression to overt disease may take years. StCA or autoantibodies to P450c17 and/or P450scc, when occurring in APS-2 patients, are good markers for gonadal insufficiency. For example, 85–100% of APS-2 patients with premature ovarian failure had detectable StCA (Betterle et al., 2002). StCA are also found in 18–40% of APS-2 patients without gonadal insufficiency (Hoek et al., 1997) and follow-up of StCA positive patients with Addison's disease has shown a high risk of developing gonadal failure in females but not in males (Betterle et al., 2002).

TREATMENT AND OUTCOME

In patients with APS-1, anticandidal drugs such as amphotericin B, ketoconazole or fluconazole are used in treatment of the candidiasis. Itraconazole has been reported to be effective for nail candidiasis but 4–6 months' treatment is needed to eliminate the infection (Perheentupa, 2002). Clinical follow-up of oral candidal infection is needed at least once or twice a year and because of the risk of cancer, attention should be paid to suppression of the oral infection. Replacement therapy with hormones has been efficiently used for Addison's disease and hypoparathyroidism. Patients with Addison's disease are treated with hydrocortisone at the smallest dose that relieves symptoms, usually 10 + 5 + 5 mg a day. The patients should also receive fludrocortisone as a substitute for aldosterone.

The therapy of hypoparathyroidism is aimed at maintaining a normal calcium plasma level and serum calcium and phosphate levels should be monitored regularly. Therapies are calciferol sterols (vitamin D hydroxylated forms, usually calcitriol) and calcium salt preparations, preferably calcium carbonate, but these do not efficiently substitute for parathyroid hormone and are difficult to regulate. As the calcium levels may vary significantly over a short time, there are significant risks of both hypo- and hypercalcemia. The patients should receive advice and written information about the symptoms, complications, and risk elements of the disease (Perheentupa, 2002). Recently available recombinant PTH may significantly improve the treatment of hypoparathyroidism in APS-1 patients.

CONCLUDING REMARKS—FUTURE PROSPECTS

Recent years have seen remarkable progress in our knowledge of the mechanisms of autoimmune polyendocrinopathies, including a clear pathogenetic distinction between APS-1 and APS-2. The endocrine organs, which often express tissue-specific proteins, are destroyed with high precision by the autoimmune reaction. The identification of the autoantibody targets in APS-1 has revealed that the autoantigens often belong to related protein families, raising the question of exactly how the immune system selects specific targets for autoimmune reactivity. The findings in AIRE-deficient mice suggest that AIRE has an important role in T-cell negative selection in being responsible for the expression of multiple tissue-specific autoantigens in thymus. From these data, many new questions also arise, including the molecular mechanism by which AIRE activates these genes and regulation of the expression of AIRE itself. According to expression microarray experiments in the mouse, AIRE activates approximately 1000 different tissue-specific genes in the thymus, and whether all of these proteins represent potential APS-1 autoantigens remains to be clarified.

Regulatory T cells have now been convincingly demonstrated to control self-reactive T cells and prevent autoimmunity. However, their role in complex autoimmune diseases has been unclear so far. Recent data showing that

regulatory T cells from APS-2 patients are defective in their suppressive capacity provide the first progress in this direction. It remains to be studied whether AIRE-regulated tissue-specific antigens could, in addition to deletion of reactive T cells, also promote development of regulatory T cells in the thymus. Further studies are needed to confirm these possibilities.

Systematic analyses of autoantigens and autoantibodies have provided a basis for clinical diagnostic approaches and will be useful in the prediction of autoimmune disease entities in subjects at high risk of developing the diseases. Much current research effort is going into the identification of genes involved in APS-2. Clearly, MHC genes have the dominant role and the CTLA-4 gene is a good example of another gene involved. It can be expected that, with the advent of whole genome scans, more genetic associations will be identified in future. Environmental factors in APS-2 certainly remain to be clarified and deserve more attention. Although a monogenic disease, APS-1 also seems to be influenced by environmental and/or other genetic factors to account for the variety of clinical symptoms and disease expressions. The possibility that heterozygous deficiency of AIRE, in combination with other genetic defects, might contribute to the occurrence of autoimmunity is worthy of further investigation.

Acknowledgments

The authors are grateful to Professor Ian Mackay and Professor Kai Krohn for their valuable comments on the manuscript.

References

Addison, T. (1855). On the constitutional and local effects of disease of the suprarenal capsules. In a collection of the published writings of the late Thomas Addison MD, physician to Guy's Hospital. London: New Sydenham Society.

Allen, E.M., Appel, M.C. and Braverman, L.E. (1986). The effect of iodide ingestion on the development of spontaneous lymphocytic thyroiditis in the diabetes-prone BB/W rat. *Endocrinology 118*, 1977–1981.

Anderson, J.R., Goudie, R.B., Gray, K.G. and Timbury, G.C. (1957). Autoantibodies in Addison's disease. *Lancet i*i, 123–124.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C. and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science 298*, 1395–1401.

Awata, T., Guberski, D.L. and Like, A.A. (1995). Genetics of the BB rat: association of autoimmune disorders (diabetes, insulitis, and thyroiditis) with lymphopenia and major histocompatibility complex class II. *Endocrinology 136*, 5731–5735.

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*, 211–216.

Barker, J.M., Ide, A., Hostetler, C., Yu, L., Miao, D., Fain. P.R., Eisenbarth, G.S. and Gottlieb, P.A. (2005). Endocrine and immunogenetic testing in individuals with type 1 diabetes and 21-hydroxylase autoantibodies: Addison's disease in a high-risk population. *J. Clin. Endocrinol. Metab. 90,* 128–134.

Baumann-Antczak, A., Wedlock, N., Bednarek, J., Kiso, Y., Krishnan, H., Fowler, H., Rees-Smith, B. and Furmaniak, J. (1992). Autoimmune Addison's disease and 21-hydroxylase. *Lancet 340*, 429–430.

Beales, P.E., Castri, F., Valiant, A., Rosignoli, G., Buckley, L. and Pozzilli, P. (2002). Adrenalitis in the non-obese diabetic mouse. *Autoimmunity 35*, 329–333.

Beaven, D.W., Nelson, D.H., Renold, A.E. and Thorn, G.W. (1959). Diabetes mellitus and Addison's disease: a report on eight patients and a review of 55 cases in the literature. *N. Engl. J. Med. 261*, 443–454.

Bednarek, J., Furmaniak, J., Wedlock, N., Kiso, Y., Baumann-Antczak, A., Fowler, S., Krishnan, H., Craft, J.A. and Rees-Smith, B. (1992). Steroid 21-hydroxylase is a major autoantigen involved in adult onset autoimmune Addison's disease. *FEBS Lett. 309*, 51–55.

Betterle, C., Dal Pra, C., Mantero, F. and Zanchetta, R. (2002). Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr. Rev. 23*, 327–364.

Björk, E., Velloso, L.A., Kämpe, O. and Karlsson, F.A. (1994). GAD autoantibodies in IDDM, stiff-man syndrome, and autoimmune polyendocrine syndrome type I recognize different epitopes. *Diabetes 43*, 161–165.

Björses, P., Halonen, M., Palvimo, J.J., Kolmer, M., Aaltonen, J., Ellonen, P., Perheentupa, J., Ulmanen, I. and Peltonen, L. (2000). Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am. J. Hum. Genet. 66*, 378–392.

Blizzard, R.M. and Gibbs, J.H. (1968). Candidiasis: studies pertaining to its association with endocrinopathies and pernicious anemia. *Pediatrics 42,* 231–237.

Blizzard, R.M. and Kyle, M. (1963). Studies of the adrenal antigens and antibodies in Addison's disease. *J. Clin. Invest. 42*, 1653–1660.

Blizzard, R.M., Kyle, M.A., Chandler, R.W, and Hung, W. (1962). Adrenal antibodies in Addison's disease. *Lancet ii*, 901–902.

Boe, A.S., Bredholt, G., Knappskog, P.M., Hjelmervik, T.O., Mellgren, G., Winqvist, O., Kämpe, O. and Husebye, E.S. (2004). Autoantibodies against 21-hydroxylase and side-chain cleavage enzyme in autoimmune Addison's disease are mainly immunoglobulin G1. *Eur. J. Endocrinol. 150,* 49–56.

Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., Carpenter, C.C.J., Solomon, N., Silverberg, S.G., Bledsoe, T., Northcutt, R,C., Klinenberg, J.R., Bennet, I.L. and Harvey, M. (1964). Schmidt's syndrome (thyroid and adrenal insufficiency): a review of the literature and a report of fifteen new cases including ten instances of co-existent diabetes-mellitus. *Medicine 43*, 153–180.

Carpenter, C.C.J., Solomon, N., Silverberg, S.G., Bledsoe, T., Northcutt, R.C., Klinenberg, J.R., Bennet, I.L. and Harvey, M. (1964). Schmidt's syndrome (thyroid and adrenal insufficiency): a review of the literature and a report of fifteen new cases including ten instances of co-existent diabetes-mellitus. *Medicine 43*, 153–180.

Cihakova, D., Trebusak, K., Heino, M., Fadeyev, V., Tiulpakov, A., Battelino, T., Tar, A., Halasz, Z., Blumel, P., Tawfik, S., Krohn, K., Lebl, J. and Peterson, P. (2001). Novel AIRE mutations and P450 cytochrome autoantibodies in Central and Eastern European patients with APECED. *Hum. Mutat. 18*, 225–232.

Clemente, M.G., Meloni, A., Obermayer-Straub, P., Frau, F., Manns, M.P. and De Virgiliis, S. (1998). Two cytochromes P450 are major hepatocellular autoantigens in autoimmune polyglandular syndrome type 1. *Gastroenterology 114*, 324–328.

Craig, J.M., Schiff, L.H. and Boone, J.E. (1955). Chronic moniliasis associated with Addison's disease. *Am. J. Dis. Child. 89*, 669–684.

Crisa, L., Mordes, J.P. and Rossini, A.A. (1992). Autoimmune diabetes mellitus in the BB rat. *Diabetes Metab. Rev. 8*, 4–37.

Csaszar, T. and Patakfalvi. A. (1992). Treatment of polyglandular autoimmune syndrome with cyclosporin-A. *Acta Med. Hung. 49*, 187–193.

Djilali-Saiah, I., Renous, R., Caillat-Zucman, S., Debray, D. and Alvarez, F. (2004). Linkage disequilibrium between HLA class II region and autoimmune hepatitis in pediatric patients. *J. Hepatol. 40*, 904–909.

Eisenbarth, G.S. and Gottlieb, P.A. (2004). Autoimmune polyendocrine syndromes. *N. Engl. J. Med. 350*, 2068–2079.

Ekwall, O., Hedstrand, H., Grimelius, L., Haavik, J., Perheentupa, J., Gustafsson, J., Husebye, E., Kämpe, O. and Rorsman, F. (1998). Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet 352*, 279–283.

Ekwall, O., Hedstrand, H., Haavik, J., Perheentupa, J., Betterle, C., Gustafsson, J., Husebye, E., Rorsman, F. and Kämpe, O. (2000). Pteridin-dependent hydroxylases as autoantigens in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab. 85*, 2944–2950.

Etzel, B.S. and Robson, H.N. (1958). The syndrome of hypoparathyroidism, Addison's disease and moniliasis. *Australas. Ann. Med. 7*, 27–33.

Finnish-German APECED Consortium (1997). An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet. 17*, 399–403.

Fontenot, J.D., Gavin, M..A and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol. 4*, 330–336.

Friedman, T.C., Thomas, P.M. and Fleisher, T.A. (1991). Frequent occurrence of asplenism and cholelithiasis in patients with autoimmune polyglandular disease type I. *Am. J. Med. 91*, 625–630.

Gambelunghe, G., Falorni, A., Ghaderi, M., Laureti, S., Tortoioli, C., Santeusanio, F., Brunetti, P. and Sanjeevi, C.B. (1999). Microsatellite polymorphism of the MHC class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. *J. Clin. Endocrinol. Metab. 84*, 3701–3707.

Gavin, M. and Rudensky, A. (2003). Control of immune homeostasis by naturally arising regulatory CD4+ T cells. *Curr. Opin. Immunol. 15*, 690–696.

Gebre-Medhin, G., Husebye, E.S, Gustafsson, J., Winqvist, O., Gøksoyr, A., Rorsman, F. and Kämpe, O. (1997). Cytochrome P450IA2 and aromatic L-amino acid decarboxylase are hepatic autoantigens in autoimmune polyendocrine syndrome type I. *FEBS Lett. 412*, 439–445.

Goswami, R., Brown, E.M., Kochupillai, N., Gupta, N., Kifor, O. and Chattopadhyay, N. (2004). Prevalence of calcium sensing receptor autoantibodies in patiens with sporadic idiopathic hypoparathyroidism. *Eur. J. Endocrinol. 150*, 9–18.

Goudie, R.B., McDonald, E., Anderson, J.R. and Gray, K. (1968). Immunological features of idiopathic Addison's disease: characterization of adrenocortical antigens. *Clin. Exp. Immunol. 3,* 119–131.

Gylling, M., Kääriainen, E., Väisänen, R., Kerosuo, L., Solin, M.L., Halme, L., Saari, S., Halonen, M., Kämpe, O., Perheentupa, J. and Miettinen, A. (2003). The hypoparathyroidism of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protective effect of male sex. *J. Clin. Endocrinol. Metab. 88*, 4602–4608.

Halonen, M., Eskelin, P., Myhre, A.G., Perheentupa, J., Husebye, E.S, Kämpe, O., Rorsman, F., Peltonen, L., Ulmanen, I. and Partanen, J. (2002). AIRE mutations and human leukocyte antigen genotypes as determinants of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy phenotype. *J. Clin. Endocrinol. Metab. 87*, 2568–2574.

Hedstrand, H., Ekwall, O., Haavik, J., Landgren, E., Betterle, C., Perheentupa, J., Gustafsson, J., Husebye, E., Rorsman, F. and Kämpe, O. (2000). Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem, Biophys, Res, Commun. 267*, 456–461.

Hedstrand, H., Ekwall, O., Olsson, M.J., Landgren, E., Kemp, E.H., Weetman, A.P., Perheentupa, J., Husebye, E., Gustafsson, J., Betterle, C., Kämpe, O. and Rorsman, F. (2001). The transcription factors SOX9 and SOX10 are vitiligo autoantigens in autoimmune polyendocrine syndrome type I. *J. Biol. Chem. 276*, 35390–35395.

Heino, M., Scott, H.S., Chen, Q., Peterson, P., Mäenpää, U., Papasavvas, M.P., Mittaz, L., Barras, C., Rossier, C., Chrousos. G.P., Stratakis, C.A., Nagamine, K., Kudoh, J., Shimizu, N., Maclaren. N., Antonarakis, S.E. and Krohn, K. (1999). Mutation analyses of North American APS-1 patients. *Hum. Mutat. 13*, 69–74.

Heino, M., Peterson, P., Kudoh, J., Shimizu, N., Antonarakis, S.E., Scott, H.S. and Krohn, K. (2001). APECED mutations in the autoimmune regulator (AIRE) gene. *Hum. Mutat. 18*, 205–211.

Hoek, A., Schoemaker, J. and Drexhage. H.A. (1997). Premature ovarian failure and ovarian autoimmunity. *Endocr. Rev. 18*, 107–134.

Hornum, L., Romer, J. and Markholst, H. (2002). The diabetes-prone BB rat carries a frameshift mutation in Ian4, a positional candidate of IDDM1. *Diabetes 51*, 1972–1979.

Husebye, E.S, Gebre-Medhin, G., Tuomi, T., Perheentupa, J., Landin-Olsson, M., Gustafsson, J., Rorsman, F. and Kämpe, O. (1997). Autoantibodies against aromatic L-amino acid decarboxylase in autoimmune polyendocrine syndrome type I. *J. Clin. Endocrinol. Metab. 82*, 147–150.

Hyttinen, V., Kaprio, J., Kinnunen, L., Koskenvuo, M. and Tuomilehto, J. (2003). Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes 52,* 1052–1055.

Irvine, W.J. and Barnes, E.W. (1975). Addison's disease, ovarian failure and hypoparathyroidism. *Clin. Endocrinol. Metab. 4,* 379–384.

Jacob, H.J., Pettersson, A., Wilson, D., Mao, Y., Lernmark, A. and Lander, E.S. (1992). Genetic dissection of autoimmune type I diabetes in the BB rat. *Nat. Genet. 2*, 56–60.

Johansson, A.C., Lindqvist, A.K., Johannesson, M. and Holmdahl, R. (2003). Genetic heterogeneity of autoimmune disorders in the nonobese diabetic mouse. *Scand. J. Immunol. 57,* 203–213.

Kemp EH, Ajjan RA, Husebye, E.S, Peterson, P., Uibo R, Imrie H, Pearce SH, Watson PF and Weetman, A.P.. (1998). A cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphism is associated with autoimmune Addison's disease in English patients. Clin Endocrinol. (Oxf). 49, 609–613.

Khattri, R., Cox, T., Yasayko, S.A. and Ramsdell, F. (2003). An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol. 4*, 337–342.

Kosoy, R., Yokoi, N., Seino, S. and Concannon, P. (2004). Polymorphic variation in the CBLB gene in human type 1 diabetes. *Genes Immun. 5*, 232–235.

Kriegel, M.A., Lohmann, T., Gabler, C., Blank, N., Kalden, J.R. and Lorenz, H.M. (2004). Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med. 199,* 1285–1291.

Krohn, K., Uibo, R., Aavik, E., Peterson, P. and Savilahti, K. (1992). Identification by molecular cloning of an autoantigen associated with Addison's disease as steroid 17 alpha-hydroxylase. *Lancet 339,* 770–773.

Krug, J., Williams, A.J., Beales, P.E., Doniach, I., Gale, E.A. and Pozzilli, P. (1991). Parathyroiditis in the non-obese diabetic mouse—a new finding. *J. Endocrinol. 131*, 193–196.

Kumar, P.G., Laloraya, M. and She, J.X. (2002). Population genetics and functions of the autoimmune regulator (AIRE). *Endocrinol. Metab. Clin. North Am. 31*, 321–338.

Kuroda, N., Mitani, T., Takeda, N., Ishimaru, N., Arakaki, R., Hayashi, Y., Bando, Y., Izumi, K., Takahashi, T., Nomura, T., Sakaguchi, S., Ueno,

T., Takahama, Y., Uchida, D., Sun, S., Kajiura, F., Mouri, Y., Han, H., Matsushima, A., Yamada, G. and Matsumoto, M. (2005). Development of autoimmunity against transcriptionally unrepressed target antigen in the thymus of Aire-deficient mice. *J. Immunol. 174*, 1862–1870.

Lam-Tse, W.K., Lernmark, A. and Drexhage, H.A. (2002). Animal models of endocrine/organ-specific autoimmune diseases: do they really help us to understand human autoimmunity? *Springer Semin. Immunopathol. 24*, 297–321.

Levin, L. and Tomer, Y. (2003). The etiology of autoimmune diabetes and thyroiditis: evidence for common genetic susceptibility. *Autoimmun. Rev. 2*, 377–386.

Li, Y., Song, Y.H., Rais, N., Connor, E., Schatz, D., Muir, A. and Maclaren, N. (1996). Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *J. Clin. Invest. 97*, 910–914.

Liston, A., Lesage, S., Wilson, J., Peltonen, L. and Goodnow, C.C. (2003). Aire regulates negative selection of organ-specific T cells. *Nat. Immunol. 4*, 350–354.

Mackay, I.R., Bone, A., Tuomi, T., Elliott, R., Mandel, T., Karopoulos, C. and Rowley, M.J. (1996). Lack of autoimmune serological reactions in rodent models of insulin dependent diabetes mellitus. *J. Autoimmun. 9*, 705–711.

Maclaren, N.K. and Riley, W.J. (1986). Inherited susceptibility to autoimmune Addison's disease is linked to human leukocyte antigens DR3 and/or DR4, except when associated with autoimmune polyglandular disease. *J. Clin. Endocrinol. Metab. 62*, 455–459.

MacMurray, A.J., Moralejo, D.H., Kwitek, A.E., Rutledge, E.A., Van Yserloo, B., Gohlke, P., Speros, S.J., Snyder, B., Schaefer, J., Bieg, S., Jiang, J., Ettinger, R.A., Fuller, J., Daniels, T.L., Pettersson, A., Orlebeke, K., Birren, B., Jacob, H.J., Lander, E.S. and Lernmark, A. (2002). Lymphopenia in the BB rat model of type 1 diabetes is due to a mutation in a novel immune-associated nucleotide (Ian)-related gene. *Genome Res. 12*, 1029–1039.

MacMurray, J., Meloni, G.F., Lucarelli, P., Pellecchia, M., Eisenbarth, G.S., Comings, D. and Mustelin, T. (2004). A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat. Genet. 36*, 337–338.

Many, M.C., Maniratunga, S. and Denef, J.F. (1996). The non-obese diabetic (NOD) mouse: an animal model for autoimmune thyroiditis. *Exp. Clin. Endocrinol. Diabetes 104*, S3 17–20.

Mathis, D. and Benoist, C. (2004). Back to central tolerance. *Immunity 20*, 509–516.

Meeran, K., Shaw, N.J., Smith, C.S., Toft, A.D., Williams, G. and Kendall-Taylor, P. (1998). A common and recurrent 13-bp deletion in the autoimmune regulator gene in British kindreds with autoimmune polyendocrinopathy type 1. *Am. J. Hum. Genet. 63*, 1675–1684.

Meyer, G., Donner, H., Herwig, J., Bohles, H., Usadel, K.H. and Badenhoop, K. (2001). Screening for an AIRE-1 mutation in patients with Addison's disease, type 1 diabetes, Graves' disease and Hashimoto's thyroiditis as well as in APECED syndrome. *Clin. Endocrinol. (Oxf.) 54,* 335–338.

Moralejo, D.H., Park, H.A., Speros, S.J., MacMurray, A.J., Kwitek, A.E., Jacob, H.J., Lander, E.S. and Lernmark, A. (2003). Genetic dissection of lymphopenia from autoimmunity by introgression of mutated Ian5 gene onto the F344 rat. *J. Autoimmun. 21*, 315–324.

Muir, A., Schatz, D.A. and Maclaren, N.K. (1993). Autoimmune Addison's disease. *Springer Semin. Immunopathol. 14*, 275–284.

Myhre, A.G., Undlien, D.E., Lovas, K., Uhlving, S., Nedrebo, B.G., Fougner, K.J., Trovik, T., Sorheim, J.I. and Husebye, E.S. (2002). Autoimmune adrenocortical failure in Norway autoantibodies and human leukocyte antigen class II associations related to clinical features. *J. Clin. Endocrinol. Metab. 87,* 618–623.

Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K.J., Lalioti, M.D., Mullis, P.E., Antonarakis, S.E., Kawasaki, K., Asakawa, S., Ito, F. and Shimizu, N. (1997). Positional cloning of the APECED gene. *Nat. Genet. 17*, 393–398.

Neufeld, M., Maclaren, N.K. and Blizzard, R.M. (1981). Two types of autoimmune Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. *Medicine 60*, 355–362.

Nithiyananthan, R., Heward, J.M., Allahabadia, A., Barnett, A.H., Franklyn, J.A. and Gough, S.C.L. (2000). A heterozygous deletion of the autoimmune regulator (AIRE1) gene, autoimmune thyroid disease, and type 1 diabetes: no evidence for association. *J. Clin. Endocrinol. Metab 85*, 1320–1322.

Ochs, H.D., Ziegler, S.F. and Torgerson, T.R. (2005). FOXP3 acts as a rheostat of the immune response. *Immunol. Rev. 203*, 156–164.

Partanen, J., Peterson, P., Westman, P., Aranko, S. and Krohn, K. (1994). Major histocompatibility complex class II and III in Addison's disease. MHC alleles do not predict autoantibody specificity and 21-hydroxylase gene polymorphism has no independent role in disease susceptibility. *Hum. Immunol. 41*, 135–140.

Patterson, C.C., Dahlquist, G., Soltesz, G., Green, A. and EURODIAB ACE Study Group. (2001). Europe and diabetes. Is childhood-onset type I diabetes a wealth-related disease? An ecological analysis of European incidence rates. *Diabetologia 44* (Suppl 3), B9–16.

Payne, F., Smyth, D.J., Pask, R., Barratt, B.J., Cooper, J.D., Twells, R.C., Walker, N.M., Lam, A.C., Smink, L.J., Nutland, S., Rance, H.E. and Todd, J.A. (2004). Haplotype tag single nucleotide polymorphism analysis of the human orthologues of the rat type 1 diabetes genes Ian4 (Lyp/IDDM1) and Cblb. *Diabetes 53,* 505–509.

Pearce, S.H., Cheetham, T., Imrie, H., Vaidya, B., Barnes, N.D., Bilous, R.W., Carr, D., Meeran, K., Shaw, N.J., Smith, C.S., Toft, A.D., Williams, G. and Kendall-Taylor, P. (1998). A common and recurrent 13-bp deletion in the autoimmune regulator gene in British kindreds with autoimmune polyendocrinopathy type 1. *Am. J. Hum. Genet. 63*, 1675–1684.

Perheentupa, J. (2002). APS-I/APECED: the clinical disease and therapy. *Endocrinol. Metab. Clin. North Am. 31,* 295–320.

Perniola, R., Falorni, A., Clemente, M.G., Forini, F., Accogli, E. and Lobreglio, G. (2000). Organ-specific and non-organ-specific autoantibodies in children and young adults with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *Eur. J. Endocrinol. 143*, 497–503.

Peterson, P., Partanen, J., Aavik, E., Salmi, H., Pelkonen, R. and Krohn, K.J. (1995). Steroid 21-hydroxylase gene polymorphism in Addison's disease patients. *Tissue Antigens 46*, 63–67.

Peterson, P., Pitkänen, J., Sillanpää, N. and Krohn, K. (2004). Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED): a model disease to study molecular aspects of endocrine autoimmunity. *Clin. Exp. Immunol. 135*, 348–357.

Pitkänen, J. and Peterson, P. (2003). Autoimmune regulator: from loss of function to autoimmunity. *Genes Immun. 4*, 12–21.

Ramsey, C., Winqvist, O., Puhakka, L., Halonen, M., Moro, A., Kämpe, O., Eskelin, P., Pelto-Huikko, M. and Peltonen, L. (2002). Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum. Mol. Genet. 11*, 397–409.

Redondo, M.J. and Eisenbarth, G.S. (2002). Genetic control of autoimmunity in Type I diabetes and associated disorders. *Diabetologia 45*, 605–622.

Riley, W.J., Maclaren, N.K. and Neufeld, M (1980). Adrenal autoantibodies and Addison's disease in insulin-dependent diabetes mellitus. *J. Pediat. 97*, 191–195.

Roep, B.O. (2002). Autoreactive T cells in endocrine/organ-specific autoimmunity: why has progress been so slow? *Springer Semin. Immunopathol. 24*, 261–271.

Sakaguchi, S., Sakaguchi, N., Shimizu, J., Yamazaki, S., Sakihama, T., Itoh, M., Kuniyasu, Y., Nomura, T., Toda, M. and Takahashi, T. (2001). Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells:

their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev. 182*, 18–32.

Schatz, D.A. and Winter, W.E. (2002). Autoimmune polyglandular syndrome II: clinical syndrome and treatment. *Endocrinol. Metab. Clin. North Am. 31*, 339–352.

Schmidt, M.B. (1926). Eine biglanduläre erkrankung (Nebennieren und schilddruse) bei morbus Addisonii. *Verh. Dtsch. Ges. Pathol. 21*, 212–221.

Scott, H.S., Heino, M., Peterson, P., Mittaz, L., Lalioti, M.D., Betterle, C., Cohen, A., Seri, M., Lerone, M., Romeo, G., Collin, P., Salo, M., Metcalfe, R., Weetman, A., Papasavvas, M.P., Rossier, C., Nagamine, K., Kudoh, J., Shimizu, N., Krohn, K.J. and Antonarakis, S.E. (1998). Common mutations in autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy patients of different origins. *Mol. Endocrinol. 12*, 1112–1119.

Sediva, A., Cihakova, D. and Lebl, J. (2002). Immunological findings in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) and their family members: are heterozygotes subclinically affected? *J. Pediatr. Endocrinol. Metab. 15,* 1491–1496.

Sköldberg, F., Portela-Gomes, G.M., Grimelius, L., Nilsson, G., Perheentupa, J., Betterle, C., Husebye, E.S, Gustafsson, J., Ronnblom, A., Rorsman, F. and Kämpe, O. (2003). Histidine decarboxylase, a pyridoxal phosphate-dependent enzyme, is an autoantigen of gastric enterochromaffin-like cells. *J. Clin. Endocrinol. Metab. 88*, 1445–1452.

Sköldberg, F., Rorsman, F., Perheentupa, J., Landin-Olsson, M., Husebye, E.S, Gustafsson, J. and Kämpe, O. (2004). Analysis of antibody reactivity against cysteine sulfinic acid decarboxylase, a pyridoxal phosphate-dependent enzyme, in endocrine autoimmune disease. *J. Clin. Endocrinol. Metab. 89*, 1636–1640.

Söderbergh, A., Rorsman, F., Halonen, M., Ekwall, O., Björses, P., Kämpe, O and Husebye, E.S. (2000). Autoantibodies against aromatic L-amino acid decarboxylase identifies a subgroup of patients with Addison's disease. *J. Clin. Endocrinol. Metab. 85*, 460–463.

Söderbergh, A., Myhre, A.G., Ekwall, O., Gebre-Medhin, G., Hedstrand, H., Landgren, E., Miettinen, A., Eskelin, P., Halonen, M., Tuomi, T., Gustafsson, J., Husebye, E.S, Perheentupa, J., Gylling, M., Manns, M.P., Rorsman, F., Kämpe, O and Nilsson, T. (2004). Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. J. Clin. Endocrinol. Metab. 89, 557–562.

Sotsiou, F., Bottazzo, G.F. and Doniach, D. (1980). Immunofluorescence studies on autoantibodies to steroid producing cells, and to germline cells in endocrine disease and fertility. Clin. Exp. Immunol. 39, 97–111.

Spinner, M.W., Blizzard, R.M, and Childs, B. (1968). Clinical and genetic heterogeneity in idiopathic Addison's disease and hypoparathyroidism. J. Clin. Endocrinol. Metab. 28, 795–804.

Ueda, H., Howson, J.M., Esposito, L., Heward, J., Snook, H., Chamberlain, G., Rainbow, D.B., Hunter, K.M., Smith, A.N., Di Genova, G., Herr, M.H., Dahlman, I., Payne, F., Smyth, D., Lowe, C., Twells, R.C., Howlett, S., Healy, B., Nutland, S., Rance, H.E., Everett, V., Smink, L.J., Lam, A.C., Cordell, H.J., Walker, N.M., Bordin, C., Hulme, J., Motzo, C., Cucca, F., Hess, J.F., Metzker, M.L., Rogers, J., Gregory, S., Allahabadia, A., Nithiyananthan, R., Tuomilehto-Wolf, E., Tuomilehto, J., Bingley, P., Gillespie, K.M., Undlien, D.E., Ronningen, K.S., Guja, C., Ionescu-TirgoviŸte, C., Savage, D.A., Maxwell, A.P., Carson, D.J., Patterson, C.C., Franklyn, J.A., Clayton, D.G., Peterson, L.B., Wicker, L.S., Todd, J.A. and Gough, S.C. (2003). Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature 423*, 506–511.

Uibo, R., Aavik, E., Peterson, P., Perheentupa, J., Aranko, S., Pelkonen, R. and Krohn, K.J. (1994). Autoantibodies to cytochrome P450 enzymes P450scc, P450c17, and P450c21 in autoimmune polyglandular disease types I and II and in isolated Addison's disease. *J. Clin. Endocrinol. Metab. 78,* 323–328.

Vaidya, B. and Pearce, S. (2004). The emerging role of the CTLA-4 gene in autoimmune endocrinopathies. *Eur. J. Endocrinol. 150*, 619–626.

Vaidya, B., Imrie, H., Geatch, D.R., Perros, P., Ball, S.G., Baylis, P.H., Carr, D., Hurel, S.J., James, R.A., Kelly, W.F., Kemp, E.H., Young, E.T., Weetman, A.P., Kendall-Taylor, P. and Pearce, S.H. (2000). Association analysis of the cytotoxic T lymphocyte antigen-4 (CTLA-4) and autoimmune regulator-1 (AIRE-1) genes in sporadic autoimmune Addison's disease. *J. Clin. Endocrinol. Metab. 85,* 688–691.

Velloso, L.A., Winqvist, O., Gustafsson, J., Kämpe, O. and Karlsson, F.A. (1994). Autoantibodies against a novel 51 kDa islet antigen and glutamate decarboxylase isoforms in autoimmune polyendocrine syndrome type I. *Diabetologia 37*, 61–69.

Velloso, L.A., Eizirik, D.L., Karlsson, F.A. and Kämpe, O. (1994). Absence of autoantibodies against glutamate decarboxylase (GAD) in the non-obese diabetic (NOD) mouse and low expression of the enzyme in mouse islets. *Clin. Exp. Immunol. 96*, 129–137.

Wang CY, Davoodi-Semiromi A, Huang W, Connor E, Shi JD and She JX. (1998). Characterization of mutations in patients with autoimmune polyglandular syndrome type 1 (APS-1). *Hum. Genet.* 103, 681–685.

Wilks, S. (1862). On diseases of the suprarenal capsule or morbus addisonii. *Guy's Hosp. Rep. 8*, 1.

Winqvist, O., Karlsson, F.A. and Kämpe, O. (1992). 21-Hydroxylase, a major autoantigen in idiopathic Addison's disease. *Lancet 339*, 1559–1562.

Winqvist, O., Gustafsson, J., Rorsman, F., Karlsson, F.A. and Kämpe, O. (1993). Two different cytochrome P450 enzymes are the adrenal antigens in autoimmune polyendocrine syndrome type I and Addison's disease. *J. Clin. Invest. 92*, 2377–2385.

Winqvist, O., Gebre-Medhin, G., Gustafsson, J., Martin Ritzen, E., Lundkvist, Ö., Karlsson, F.A. and Kämpe, O. (1995). Identification of the main gonadal autoantigens in patients with adrenal insufficiency and associated ovarian failure. *J. Clin. Endocrinol. Metab. 80*, 1717–1723.

Yang, Y. and Santamaria, P. (2003). Dissecting autoimmune diabetes through genetic manipulation of non-obese diabetic mice. *Diabetologia 46*, 1447–1464.

Yokoi, N., Kanazawa, M., Kitada, K., Tanaka, A., Kanazawa, Y., Suda, S., Ito, H., Serikawa, T. and Komeda, K. (1997). A non-MHC locus essential for autoimmune type I diabetes in the Komeda Diabetes-Prone rat. *J. Clin. Invest. 100*, 2015–2021.

Yokoi, N., Komeda, K., Wang, H.Y., Yano, H., Kitada, K., Saitoh, Y., Seino, Y., Yasuda, K., Serikawa, T. and Seino, S. (2002). Cblb is a major susceptibility gene for rat type 1 diabetes mellitus. *Nat. Genet. 31*, 391–394.

CHAPTER

39

Gastritis and Pernicious Anemia

BAN-HOCK TOH, SENGA WHITTINGHAM, AND FRANK ALDERUCCIO

Department of Immunology, Monash University Medical School, Prahran, Victoria 3181, Australia

Autoimmune gastritis is an asymptomatic and innocuous autoimmune disease. It claims significance among the autoimmune diseases because it is a forerunner of pernicious anemia.

At a South London Medical Society meeting in 1849, Thomas Addison reported the clinical features of a new disease that he described as a "very remarkable form of general anemia." In a lecture in 1860, Austin Flint linked the anemia to a "degenerative disease of the glandular tubuli of the stomach." Histologic evidence of the gastric atrophy was provided by Samuel Fenwick in 1870. A year later in 1871, a fatal anemia was termed *perniciosa* by Anton Biermer. Subacute combined degeneration was later applied to the posterolateral spinal cord lesions that can be associated with the anemia (Russell et al., 1900).

At first the causal connection between pernicious anemia and chronic gastritis was incomprehensible. In 1926 Minot and Murphy observed that feeding patients with large meals of cooked liver led to a reticulocyte response and cured the anemia (Minot and Murphy, 2001). Later Castle (1953) showed that the anemia was a result of a combined deficiency of an "extrinsic factor" subsequently identified as vitamin B12 and present in the liver (Lester-Smith, 1948; Rickes, 1948), and an "intrinsic factor" (IF) in gastric juice (Highley et al., 1967). Oral treatment with extracts of hog stomach (Sharp, 1929; Sturgis and Isaacs, 1929) resulted in remission for several years (Wilkinson, 1949). However, relapses tended to occur and some cases became refractory to increasing amounts of the extract (Berlin et al., 1958). This refractory state was due to a serum factor that inhibited the effectiveness of intrinsic factor (Schwartz, 1958). Subsequently, sera from patients with pernicious anemia were shown to contain autoantibodies to intrinsic factor (Jeffries et al., 1962; Jacob and Schilling, 1966; Bardhan et al., 1968) and to gastric parietal cells (Irvine et al., 1962; Markson and Moore, 1962; Taylor et al., 1962). However, little progress was made in understanding the pathology of the gastric lesion until a flexible biopsy tube was designed that permitted the taking of samples of the gastric mucosa for histologic examination (Wood et al., 1949).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Pernicious anemia was observed to cluster in families (McIntyre et al., 1959; Whittingham et al., 1969) and to coexist with autoimmune thyroid diseases (Tudhope and Wilson, 1960), to which the term "thyrogastric autoimmunity" was applied (Whittingham et al., 1975). These observations suggested a genetic component to the disorder, a suggestion further strengthened by an association with another endocrine autoimmune disease, type 1 diabetes mellitus (Ungar et al., 1968). The suggestion by Taylor (1959; 1961) that the inhibitory substance to gastric intrinsic factor in the serum of patients with pernicious anemia was antibody and possibly autoantibody and the recognition that pernicious anemia fulfilled the markers of autoimmune disease put forward by Mackay and Burnet (1963) led to its acceptance as an autoimmune disease of the stomach and to the central questions of concern to immunologists today: What is the mechanism of immunologic tolerance to self, how is it abrogated to induce autoimmune lesions in tissues and how can tolerance be reestablished to prevent and reverse disease?

DIAGNOSIS

Autoimmune gastritis is in the early stages of the disease clinically "silent" and demonstrable only by serologic detection of antibodies to gastric parietal cells. It is only when stores of vitamin B12 are depleted that pernicious anemia manifests itself clinically. Only a small proportion (10–15%) of patients with autoimmune gastritis develop pernicious anemia (Irvine et al., 1974; Strickland and Mackay, 1973) after a latent period of 20–30 years (Toh et al., 1997). Nonetheless, at an estimated prevalence of about 2% in Western adult populations at or over the age of 60 years, pernicious anemia represents the commonest cause of vitamin B12 deficiency (Carmel, 1996). Although "silent" until the end stage, the gastric lesion can be predicted years before clinical presentation by immunologic markers specific for gastric autoimmunity. Terminally, the gastritis results in deficiency of intrinsic factor, a protein that binds avidly to dietary vitamin B12 (Glass, 1963) and promotes its transport to the terminal ileum for absorption (Donaldson et al., 1967; Kapadia et al., 1983) by ileal cubulin receptors, a multifunctional endocytic receptor (Lindblom et al., 1999). Consequently, the gastritis is expressed clinically as vitamin B12 deficiency associated with megaloblastic anemia arising as a consequence of the requirement of the vitamin for DNA synthesis. The megaloblastic anemia is demonstrated by examination of blood and bone marrow (Chanarin, 1979). The anemia was indeed "pernicious" when first discovered but is now readily controlled by vitamin B12 treatment.

Clinical Features

Pernicious anemia is uncommon before the age of 30. Racial groups other than those from northern Europe are not exempt as the disease has been reported in blacks and in Latin-Americans, with an earlier age of onset in black women (Carmel and Johnson, 1978). Advanced stages of pernicious anemia are rarely seen these days but the prototype would be a person, usually a woman in late middle age, who appears pale, tired, and depressed and may complain of a sore tongue and abdominal discomfort. There may be accompanying neuropsychiatric syndromes in about 40% of patients arising from progressive demyelination in the spinal cord (subacute combined degeneration), peripheral nerves, and cerebrum (Savage and Lindenbaum, 1995). The first neurologic abnormality is usually sensory impairment, most often presenting as distal and symmetrical paresthesiae of the lower limbs and frequently associated with ataxia. Corticospinal tract involvement is common in more advanced cases, with abnormal reflexes, motor impairment and, ultimately, spastic paraparesis. A minority of patients exhibit mental or psychiatric disturbances or autonomic signs but these rarely if ever occur in the absence of other neurologic changes. Neuropsychiatric abnormalities occur in about 30% of patients with vitamin B12 deficiency in the absence of anemia or psychosis (Lindenbaum et al., 1995).

Pernicious anemia associates predominantly with the autoimmune endocrinopathies and the antireceptor autoimmune diseases. The associated diseases include Hashimoto's thyroiditis, insulin-dependent type 1 diabetes mellitus, primary Addison's disease, primary ovarian failure, primary hypoparathyroidism, premature graying of the hair, vitiligo, thyrotoxicosis, myasthenia gravis, and the Lambert-Eaton syndrome (Table 39.1). These organ-specific "thyrogastric"

TABLE 39.1 Diseases associated with pernicious anemia that cluster in "PA families"

Diseases	References
Thyrotoxicosis, Hashimoto's thyroiditis	Ardeman et al. (1966b)
Primary hypothyroidism	Irvine et al. (1965), Irvine (1975), Schiller et al. (1968), Tudhope and Wilson (1960)
Insulin-dependent diabetes mellitus	Irvine et al. (1970), Whittingham et al. (1971)
Primary Addison's disease	Blizzard et al. (1967), Irvine (1978)
Primary ovarian failure	Irvine and Barnes (1974)
Primary hypoparathyroidism	Blizzard et al. (1966)
Premature graying of hair	Whittingham et al. (1969)
Vitiligo	Bor et al. (1969)
Myasthenia gravis	Mackay (1971)
Lambert–Eaton syndrome	Guttmann et al. (1972)

autoimmune diseases not only may occur in the same patient with pernicious anemia but aggregate in "pernicious anemia families" (Ardeman et al., 1966a; Wangel et al., 1968; Whittingham et al., 1969).

Immunologic Diagnostic Markers

Two circulating autoantibodies, parietal cell antibodies directed to gastric H^+/K^+ ATPase and autoantibodies to intrinsic factor, are typically found in patients with autoimmune gastritis or with autoimmune gastritis and pernicious anemia.

Parietal cell antibodies to gastric H^+/K^+ ATPase are diagnostic of the underlying pathologic lesion of autoimmune gastritis as gastric biopsies carried out in asymptomatic patients have revealed the presence of type A gastritis (Uibo et al., 1984). However, it is not known whether the titer of these antibodies reflects the severity of the underlying gastric pathology. These antibodies are routinely detected by indirect immunofluorescence or, to gastric H^+/K^+ ATPase, by ELISA (Chuang et al., 1992). Antibodies to gastric parietal cells are demonstrated by serum reactivity with the cytoplasm of gastric parietal cells in unfixed, air-dried, and frozen sections of mouse stomach (see Figure 39.4). Mouse stomach is preferable to rat stomach because of a lower frequency of heterophile reactions (Muller et al., 1971) that could be misinterpreted as antibody to parietal cells. The antibodies are detected by immunofluorescence in ~90% of patients with pernicious anemia, with a prevalence of 2–5% in the general population, and in about 30% of patients with other thyrogastric autoimmune diseases, including type 1 diabetes mellitus (De Block et al., 2001; 2003). These antibodies also increase in prevalence with age and are associated with rising serum gastrin levels (Jassel et al., 1999; De Block et al., 2001). With progression of autoimmune gastritis and the total loss of parietal cell mass, the prevalence of these antibodies decreases, possibly due to loss of antigenic drive (Davidson et al., 1989), and this may also explain the lower antibody prevalence (55%) in one study of pernicious anemia (Carmel, 1992).

For the diagnosis of pernicious anemia, intrinsic factor antibodies have a much higher disease specificity, albeit lower sensitivity than antibodies to gastric parietal cells. Titers of intrinsic factor antibodies appear to be of no value in predicting the development of pernicious anemia (Irvine et al., 1974). Since the Schilling test is no longer used as a diagnostic procedure, there has been increased dependence on intrinsic factor antibodies for diagnosis. Antibodies to the vitamin B12 binding site of intrinsic factor are demonstrable in serum of ~70% of patients and to a second site in ~50%. These frequencies are greater if gastric juice is assayed. There may be coexisting autoantibodies specific for the various other autoimmune diseases in the thyrogastric cluster (Table 39.2). The epitope for anti-intrinsic factor autoantibodies to the vitamin B12 binding site is located between amino acids 251 and 265 of the protein (Gueant et al., 1997) and is homologous with peptides from herpes virus Saimiri and from pathogenic *E. coli*. The incidence of these antibodies rises with increasing duration of disease, almost doubling after 10 years (Ungar et al., 1967). To explain these specificities, it is tempting to speculate that the production of autoantibodies to intrinsic factor and to the gastric H^+/K^+ ATPase is a consequence of intermolecular determinant spreading (Sercarz, 2000) from a membrane-associated self-antigen (gastric H^+/K^+ ATPase) to a secretory protein (intrinsic factor) in the gastric parietal cell.

Other Laboratory Indices

At the stage of pernicious anemia, all patients have a serum vitamin B12 level of <200 pg/ml (Ungar et al., 1968); >95% will have a level of serum pepsinogen I <20 ng/ml (Samloff et al., 1982); and 75–80% will have a serum gastrin level >100 pm liter (Varis et al., 1979). In keeping with the sparing of the antrum from the inflammatory process, the level of pepsinogen II is normal and the low ratio (<1.0; normal 6.2) of pepsinogen I to pepsinogen II is predictive of the histologic status of the gastric mucosa (Samloff et al., 1982). At earlier stages of autoimmune gastritis when the destructive lesion is "silent," there may be a reduction in levels of these various laboratory indices suggestive of the disease process that can eventually lead to pernicious anemia (Irvine et al., 1974). A biopsy of the stomach shows marked reduction of the secretory glands of the stomach or frank gastric atrophy (Figures 39.1 and 39.3), varying degrees of intestinalization (metaplasia) of the gastric glands (Strickland and Mackay, 1973) (Figure 39.3), and in the earlier lesions (Figure 39.3), prominence of lymphocytes and plasma cells in the inflammatory infiltrate in the submucosa and extending along the lamina propria into the mucosa. The acidity of gastric juice is lost due to functional impairment of gastric parietal cells. Absence of hydrochloric acid from the gastric juice is considered an essential prerequisite for the diagnosis of pernicious anemia (Chanarin, 1979). Confirmation of the presence of megaloblastic anemia is by the examination of the blood film and bone marrow and until recently, vitamin B12 absorption was measured by the Schilling test.

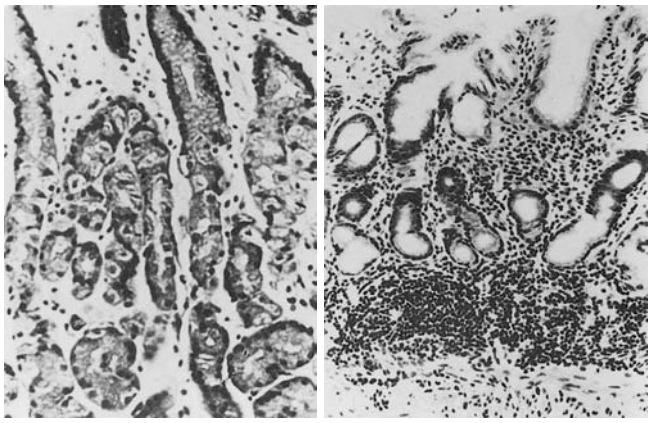
PATHOLOGY

The Type A-Type B Classification of Strickland and Mackay

Strickland and Mackay (1973) proposed a classification of gastritis based on histologic findings of the gastric

TABLE 39.2 Frequency of gastric autoantibodies in diseases in the autoimmune cluster compared with control populations

	Frequency (%) of autoantibodies to		
	Gastric parietal cells	**Gastric intrinsic factor**	**References**
Disease group (number tested)			
Thyrotoxicosis (302)	24	3.0	Irvine (1965)
Hashimoto's thyroiditis (120)	NT*	5.0	Irvine (1975)
Primary hypothyroidism (297)	NT	6.7	Irvine (1975)
Insulin-dependent diabetes mellitus			
Aged <30 years (771)	9.0	NT	Riley et al. (1982)
Aged 10–90 years (200)	28.0	40.0	Ungar et al. (1968)
Primary Addison's disease (261)	31.0	8.4	Irvine and Barnes (1975)
Primary ovarian failure (5)	40.0	40.0	Irvine et al. (1968)
Primary hypoparathyroidism (68)	22.0	NT	Blizzard et al. (1966)
Vitiligo (80)	21.0	NT	Brostoff et al. (1969)
Lambert–Eaton syndrome (46)	26.0	11.4	Lennon et al. (1982)
Control (number tested)			
Chronic atrophic gastritis, type B (nonpernicious anemia) (13)	0	0	Whittingham et al. (1969)
Duodenal ulcer (200)	5.0	0	Ungar et al. (1976)
Australian population (34,922)	4.8	0	Hooper et al. (1972)
Aged 21–30 years (551)	2.2	NT	
Aged 61–65 years (317)	6.3	NT	
Australian blood donors (500)	NT	0	Ungar et al. (1968)
Scottish blood donors, females aged 40–60 years (141)	9.0	0	Irvine (1965)



A B

FIGURE 39.1 The early lesion of autoimmune gastritis. *A,* The normal mucosa of the body of the stomach showing gastric glands and the absence of a chronic inflammatory infiltrate in the lamina propria. *B,* An early lesion of autoimmune gastritis showing a dense chronic inflammatory infiltrate in the gastric submucosa that extends into the lamina propria with the accompanying loss of gastric parietal and zymogenic cells. ×300.

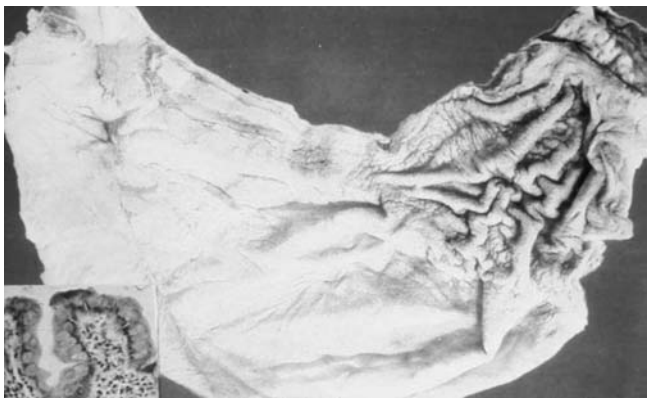


FIGURE 39.2 Macroscopic appearance of advanced autoimmune gastritis in a patient with pernicious anemia showing the extreme atrophy of the mucosa of the fundus and body of the stomach with loss of rugal folds contrasted with the healthy mucosa of the gastric antrum. Inset is the microscopic appearance of the mucosa.

mucosa, the presence of gastric parietal cell antibody, and serum levels of gastrin. Type A gastritis, the "pernicious anemia type," is restricted to the fundus and body of the stomach. Early lesions are characterized by chronic inflammation in the submucosa that extends into the lamina propria of the mucosa between gastric glands (Figure 39.1) with accompanying loss of gastric and zymogenic cells. In advanced disease, gastric atrophy is readily recognized macroscopically and microscopically (Figure 39.2). The wall of the fundus and body of the stomach becomes paper

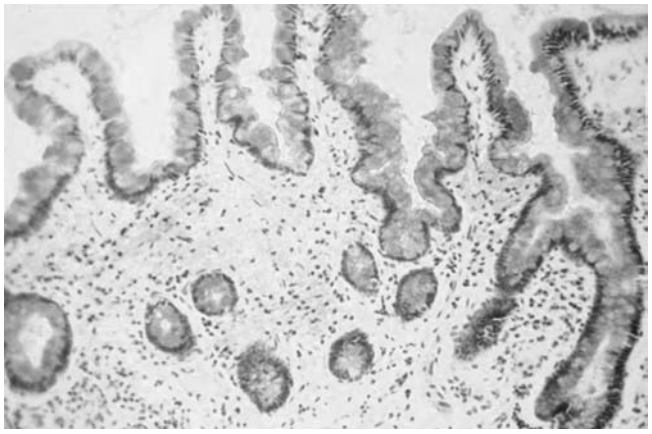


FIGURE 39.3 Microscopic appearance of the gastric mucosa of advanced autoimmune gastritis showing a chronic inflammatory infiltrate in the gastric mucosa and the loss of parietal and zymogenic cells and replacement with mucus-containing cells (brown stain).

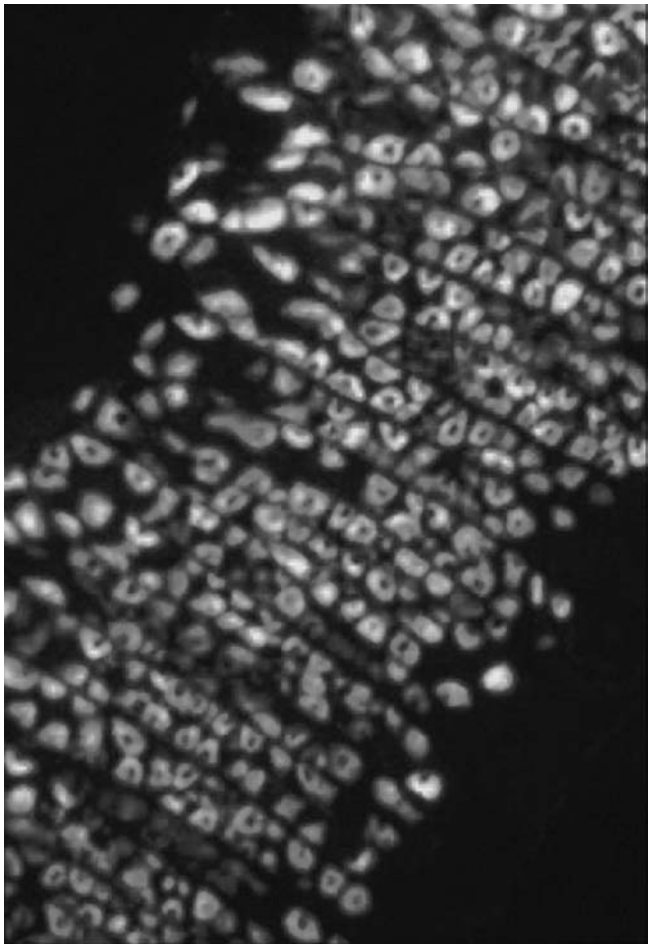


FIGURE 39.4 Indirect immunofluorescence staining of gastric parietal cells in a mouse stomach reactive with serum from a patient with autoimmune gastritis. ×400.

thin because the gastric glands are markedly reduced or absent. In particular, parietal cells and zymogenic (chief) cells are absent from the gastric mucosa and replaced by mucus-containing cells resembling those of the intestine (intestinal metaplasia, Figure 39.3). It is unclear why zymogenic cells are lost because there is no convincing evidence of an immune response directed against them. Studies of mouse models of autoimmune gastritis (see below) suggest that the Fas-mediated loss of gastric parietal cells results in a maturation arrest and the failure of precursor stem cells to differentiate to zymogenic cells.

Type A gastritis is characterized by circulating antibodies to gastric parietal cells (Figure 39.4) that have subsequently been shown to be directed to the gastric H^+/K^+ ATPase (Toh et al., 1997), achlorhydria, and high levels of serum gastrin secreted by the intact antral glands. Strickland and Mackay observed that five out of 30 patients with type A gastritis (16%) developed overt or latent pernicious anemia during a follow-up period of 3–24 years. Type A gastritis is also the gastritis characteristic of families in whom pernicious anemia predominates (Varis, 1981; Kekki et al., 1983). The gastric parietal cell must be the target of the autoimmune process because the histologic findings in pernicious anemia are restricted to the parietal cell-containing fundus and body of the stomach and autoantibodies are directed against the gastric parietal cell, and to intrinsic factor, its secretory product.

Type B gastritis, the nonpernicious anemia type, involves the antrum initially but can extend to the fundus and body of the stomach and shows incomplete failure of acid secretion and low levels of serum gastrin because of the antral gastritis. Type B gastritis is usually associated with *Helicobacter pylori* infection; type A is not (Fong et al., 1991). Cloning of the *H. pylori* vacuolar cytotoxin gene and demonstration that the cytotoxin VacA can induce gastric lesions suggested a role for the cytotoxin in the induction of type B gastritis (Telford et al., 1994). The bacterium uses adhesins such as BabA to bind to fucosylated Lewis blood group B antigen on gastric epithelial cell surfaces but is noninvasive. The gastritis is the consequence of the host response to the organism (Suerbaum and Michetti, 2002). Most strains harbor the *cag* pathogenicity island that encodes proteins that translocate the Cag A protein into the cell, leading to cytokine production by the infected cell. *H. pylori*-infected patients with gastric autoimmunity harbor *in vivo*-activated gastric $CD4^+$ T cells that recognize both H^+/K^+ ATPase and *H. pylori* antigens. Characterization of the submolecular specificity of such gastric T-cells identified cross-reactive epitopes from nine *H. pylori* proteins. Cross-reactive *H. pylori* peptides induced T cell prolifera-

tion and expression of T helper type 1 functions. The data suggest that *H. pylori* infection can activate cross-reactive gastric T cells leading to gastric autoimmunity via molecular mimicry (Amedei et al., 2003), providing a plausible explanation for the extension of type B antral gastritis to the corpus of the stomach. Also, although the antral gastritis is not associated with circulating antibodies to gastric parietal cells, extension of pathology to the corpus has been reported to be associated with an anticanalicular antibody directed towards gastric H^+/K^+ ATPase (Appelmelk et al., 1998).

Pernicious anemia in patients with the common variable type of immunodeficiency associated with low levels of serum immunoglobulins can be distinguished from classic pernicious anemia on this classification. This former type of pernicious anemia usually occurs in a younger age group, is histologically type B, is associated with a negative test for antibodies to gastric parietal cells and intrinsic factor, and shows a low level of serum gastrin (Twomey et al., 1969; 1970; Hughes et al., 1972; Cowling et al., 1974). Pernicious anemia in childhood is not associated with gastritis or achlorhydria and is the result of inadequate intrinsic factor production. A 4-base deletion spanning positions 104 to 107 in exon 2, resulting in premature termination of translation, has been reported. This mutation also eliminates a site for Bst XI endonuclease and introduces a site for BsaBI for identifying this deletion in hereditary IF deficiency.

Evolution of Gastric Atrophy

The evolution of gastric atrophy in most cases of pernicious anemia probably spans 20–30 years but this is difficult to assess in individual cases. Nearly all patients with gastric parietal cell antibody whose gastric mucosae have been examined histologically have shown some evidence of gastritis (Serafini et al., 1970). Thus, the presence of gastric parietal cell antibody in the serum is predictive of autoimmune gastritis (Irvine et al., 1965). Conversely, gastric parietal cell antibody is not observed when gastritis is due to diseases affecting the body of the stomach that are not autoimmune (see Table 39.2). Why some patients with autoimmune gastritis progress to pernicious anemia while others maintain sufficient vitamin B12 absorption for long periods is not known. However, this point is interesting, because pernicious anemia is merely the terminal stage of the process. The youngest person studied with gastric parietal cell antibody was aged 13 years, and her gastric mucosa biopsied at age 17 showed mild gastritis (Whittingham et al., unpublished observation).

Reversibility of the Lesion

There are many reports of regeneration of gastric parietal cells, improvement in gastric function, and hematologic remission after treatment with corticosteroids (Figure 39.5; Doig et al., 1957; Gordin, 1959; Ardeman and Chanarin, 1965; Jeffries et al., 1966; Rodbro et al., 1967; Wall et al., 1968; Strickland et al., 1969; Baggett and Welsh, 1970) or azathioprine (Jorge and Sanchez, 1973). This suggests that the gastric mucosa is the direct target of an autoimmune process that can be checked by immunosuppressive drugs. The observations also suggest that precursor stem cells capable of differentiating into parietal cells and zymogenic cells are present in stomachs of patients with autoimmune gastritis and that these are responsible for the regeneration of the differentiated cells when the destructive autoimmune process is controlled. The suggestion is supported by observations in experimental models of autoimmune gastritis where there is evidence of the persistence, and even expansion, of these precursor stem cells in gastritic stomachs (see below). It is also of interest and relevance that reversal of recovery with degeneration of the gastric mucosa recurred once immunosuppressive treatment was halted (Wall et al., 1968). This highlights the ever-present destructive nature of the immune system in these patients.

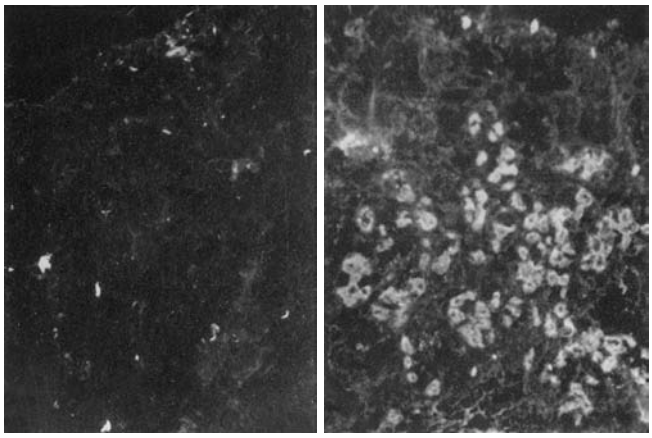


FIGURE 39.5 Indirect immunofluorescence staining showing (*A*) absence of gastric parietal cells in the stomach of a patient with pernicious anemia and (*B*) re-emergence of gastric parietal cells after treatment with 20 mg prednisolone daily for 6 weeks. Parietal cells were demonstrated with serum from a patient with pernicious anemia. ×400.

Gastric Neoplasia

There is historical evidence for a link between type A atrophic gastritis and its sequel, gastric atrophy, and adenocarcinoma of the stomach, but the association may not be strong. Only 2% of all gastric cancers were associated with pernicious anemia in a large autopsy survey in Malmö, Sweden (Eriksson et al., 1981), even when pernicious

anemia had been present for >15 years. In a study by Walker et al. (1971) of cases of type A and type B gastritis, gastric cancer supervened only in patients with type B gastritis. None was observed among patients with type A gastritis. These findings were corroborated by Irvine et al. (1974). The studies of earlier years, which reported an increased risk (Kaplan and Riglar, 1945; Mosbech and Videbaek, 1950; Elsborg and Mosbech, 1979), would not have taken account of the heterogeneity of atrophic gastritis. More recent studies, however, suggest that the risk of gastric cancer is increased 3–5 times in patients with pernicious anemia (Sjoblom et al., 1993; Hsing et al., 1993; Fuchs and Mayer, 1995; Kokkola et al., 1998). Intestinal metaplasia is a risk factor for gastric adenocarcinoma. Also, achlorhydria and bacterial overgrowth may contribute to the formation of carcinogenic nitrosoamines.

Patients with pernicious anemia have a much higher risk (13 times) of developing gastric carcinoids (Kokkola et al., 1998). The carcinoids are likely a consequence of the trophic action of the elevated serum gastrin levels consequent to the achlorhydria that stimulates the proliferation of endocrine cells in the stomach (Rode et al., 1986; Kulke and Mayer, 1999).

AUTOIMMUNE FEATURES

Autoantibodies to Gastric Parietal Cells and to Intrinsic Factor

Autoimmune gastritis is associated with autoantibodies to gastric parietal cells and to its secreted product, intrinsic factor. Like most autoantibodies, these autoantibodies are polyvalent but are predominantly of the IgG isotype (Serafini et al., 1970). IgA antibodies to gastric intrinsic factor have been demonstrated in gastric juice (Goldberg and Bluestone, 1970). Autoantibodies to gastric parietal cells are routinely detected by immunofluorescence (see Figure 39.4). These autoantibodies are directed toward both the catalytic 100 kDa α-subunit and the 60–90 kDa glycoprotein β-subunit of the gastric H^+/K^+ ATPase (Figure 39.6; Karlsson et al., 1988; Toh et al., 1990; Callaghan et al., 1993; Ma et al., 1994). The gastric H^+/K^+ ATPase is the enzyme responsible for acidification of gastric juice (Rabon and Reuben, 1990). It belongs to a family of ion-motive P-type ATPases including other H^+ ATPases as well as the Na^+/K^+ ATPase and the Ca^{2+} ATPase. P-type ATPases have highly conserved catalytic α-subunits that are phosphorylated during their reaction cycles (Pedersen and Carofoli, 1987; van Driel and Callaghan, 1995). Only the H^+/K^+ ATPase and the Na^+/K^+ ATPase have an associated β-subunit comprising a heavily glycosylated 35 kDa core protein. The gastric enzyme is located on specialized secretory membranes of

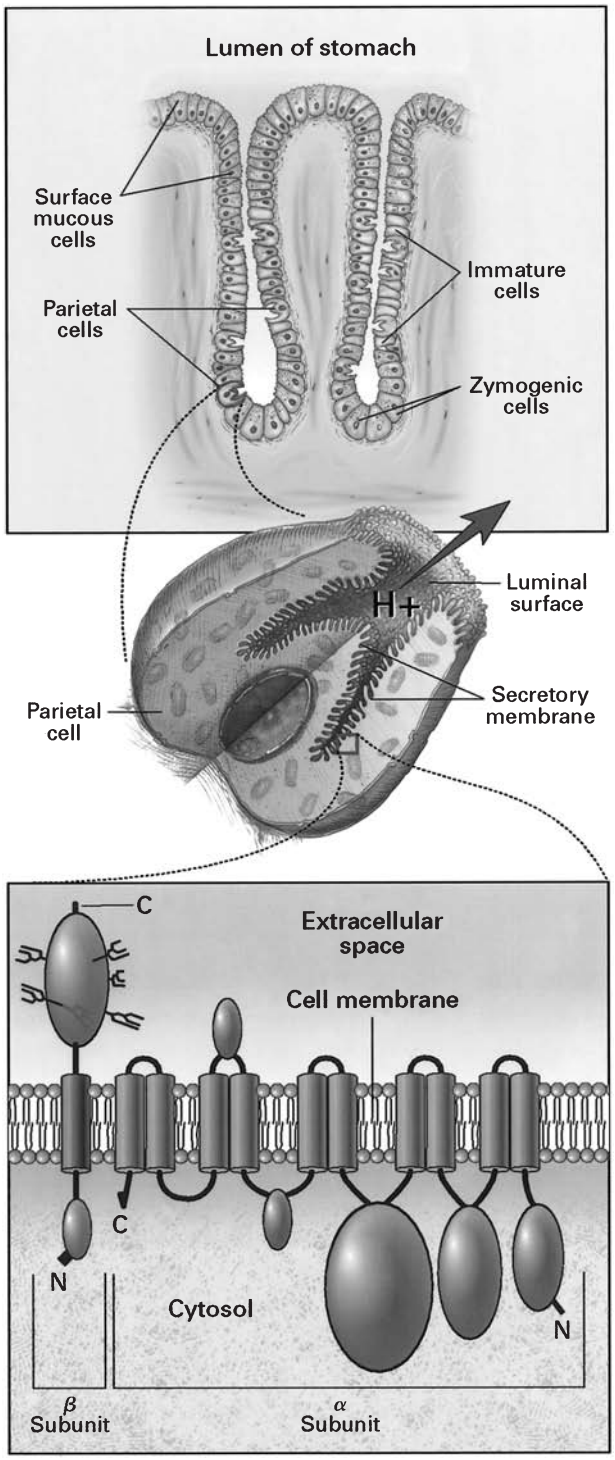


FIGURE 39.6 Gastric parietal cell H^+/K^+ ATPase as the molecular target in autoimmune gastritis associated with pernicious anemia. Top panel represents a gastric gland showing location of parietal cells in relation to zymogenic cells, immature cells, and surface mucus cells. Middle panel represents a stimulated gastric parietal cell showing the lining membrane of the secretory canaliculus on which gastric H^+/K^+ ATPase is located. Bottom panel represents the catalytic α- and the glycoprotein β-subunits of gastric H^+/K^+ ATPase showing their orientation in the lining membrane of the secretory canaliculus of the parietal cells. N denotes the N-terminal of protein and C the C-terminal of protein. Reproduced with kind permission of the *New England Journal of Medicine*.

gastric parietal cells (Hoedemaeker and Ito, 1970; Pettitt et al., 1995; 1996). Parietal cell antibodies have been shown to deplete H^+/K^+ ATPase activity from parietal cell membranes *in vitro* (Burman et al., 1989). Antibody reactivity with the α-subunit of the H^+/K^+ ATPase includes an epitope on the cytosolic side of the secretory membrane (Song et al., 1994). Antibody reactivity with the β-subunit requires the antigen to be disulfide bonded and glycosylated, suggesting that the β-subunit autoepitopes are located in the luminal domain of the glycoprotein (Goldkorn et al., 1989). The N-glycans of the β-subunit bear polylactosamine sequences permitting the purification of gastric H^+/K^+ ATPase by tomato lectin affinity chromatography (Callaghan et al., 1992).

Parietal cell autoantibodies also react with the surface membrane of dissociated parietal cells by immunofluorescence (Masala et al., 1980; de Aizpurua et al., 1983a) and are cytolytic to parietal cells *in vitro* (de Aizpurua et al., 1983b). The suggestion that the surface membrane antigen may be the gastrin receptor (de Aizpurua et al., 1985) has not been confirmed (Burman et al., 1989). Further, immunoblots and immunoprecipitates of gastric membrane extracts with parietal cell autoantibody have demonstrated reactivity only with the two subunits of the gastric H^+/K^+ ATPase. Autoantibody binding with the surface membrane of parietal cells is most likely due to reactivity with the gastric H^+/K^+ ATPase exposed on the plasma membrane of parietal cells that have lost their polarity following dissociation by collagenase.

In rats, hypochlorhydria and atrophy of gastric parietal cells were induced by infusion of an IgG fraction of human serum containing gastric parietal cell antibodies (Tanaka and Glass, 1970). However, it is unlikely that the gastric lesion is mediated by antibody because these parietal cell autoantibodies are directed solely against the gastric H^+/K^+ ATPase, an autoantigen sequestered in the internal and apical secretory membranes of the parietal cell and absent from the basolateral membranes and the autoantigen is inaccessible to circulating autoantibodies.

Human intrinsic factor is a glycoprotein with a molecular weight of ~44,000. Each molecule of intrinsic factor has the capacity to bind to one molecule of vitamin B12 (Chanarin, 1979). Two distinct antibodies are detected by radioimmunoassay; one reacts with the binding site for vitamin B12 and blocks subsequent binding of intrinsic factor with free vitamin, and the other reacts with an antigenic determinant remote from this site (Samloff et al., 1968; Rothenberg et al., 1971).

Cell-Mediated Immunity to Gastric Antigens

Studies of animal models suggest that $CD4^+$ T cells mediate the gastritic lesion (see below). In humans, a significant increase in $CD4^+$ and $CD8^+$ T cells and a six-fold increase in non–T cells (probably B cells) have been observed in the cellular infiltrate of stomachs of patients with pernicious anemia (Kaye et al., 1983). Irvine et al. (1965) have shown by electron microscopy that lymphocytes line up against the membranes of gastric parietal cells and zymogenic cells in the gastric mucosa. A similar observation has been reported in a mouse model of autoimmune gastritis induced by neonatal thymectomy (Toh et al., 1993). Phenotypic analysis of the peripheral blood T cells from patients with pernicious anemia have not shown any significant differences compared to age-matched controls (Vargas et al., 1995). To our knowledge, no studies have been made on the sentinel gastric lymph nodes which are the sites appropriate for such studies.

Interpretation of the early experiments on the contribution of cell-mediated immunity to the gastric lesion was difficult because of the paucity of knowledge about the cellular immune system. Cell-mediated immunity *in vitro* was demonstrated by the proliferation of peripheral blood lymphocytes in the presence of human intrinsic factor, gastric juice or an homogenate of gastric mucosa (Tai and McGuigan, 1969) or by inhibition of migration of peripheral blood leukocytes in the presence of human gastric juice or intrinsic factor (Fisher et al., 1966; Rose et al., 1970; Fixa et al., 1972; Goldstone et al., 1973). Glass (1977) reported positive cutaneous delayed-type hypersensitivity (DTH) reactions in autoimmune gastritis to gastric intrinsic factor and extracts of gastric mucosa; the number of positive responses was low and in one study inhibition of leukocyte migration was as effective with liver mitochondria as it was with gastric antigens (Goldstone et al., 1973). Later, assays by one of us using gastric parietal cell microsomes as antigen gave us clearer results in a leukocyte migration inhibition assay (Whittingham et al., 1975).

With identification of the gastric H^+/K^+ ATPase as the major gastric antigen, $CD4^+$ T-cell clones have been isolated from the infiltrates of the gastric mucosa of patients with autoimmune gastritis and shown to proliferate in response to porcine H^+/K^+ ATPase and display a Th1 profile. Virtually all of the H^+/K^+ ATPase-specific clones produced TNF-α and provided help for B-cell immunoglobulin production, and most expressed perforin-mediated cytotoxicity against antigen-presenting cells and induced Fas-Fas ligand-mediated apoptosis in target cells (D'Elios et al., 2001). The human $CD4^+$ T-cell clones recognized six epitopes in the α-chain and five in the β-chain (Bergman et al., 2003), of which four overlapped with epitopes relevant to experimental autoimmune gastritis, including a previously described gastric epitope (Alderuccio et al., 2000). Gastric T-cell recognition of the peptide epitopes resulted in secretion of Th1 cytokines. The data suggest a striking similarity between human and mouse autoimmune gastritis, at the epitope level, with regard to cytokine secretion and likely also with regard to pathogenic mechanisms.

TABLE 39.3 Frequency of coexisting autoantibodies in 90 patients with pernicious anemia

Autoantibodies to	Number positive (%)
Thyroid microsomes	41 (46)
Thyroglobulin	9 (10)
Pancreatic islet cells	5 (6)
Adrenal cortical cells	4 (4)
Ovarian cells	2 (2)

Clustering of Autoantibodies and Thyrogastric Autoimmunity

The detection in patients with pernicious anemia of a high frequency of thyroid autoantibodies and a lower but clearly increased frequency of autoantibodies to pancreatic islet β cells, adrenal cortical cells, and ovary (Table 39.3) is in keeping with the clinical association of pernicious anemia with the autoimmune diseases that these autoantibodies specify. Also in accord with this clustering of disease is the increased frequency of gastric parietal cell and intrinsic factor antibodies observed in these diseases (see Table 39.2). Approximately 15–20% of type 1 diabetic patients exhibit parietal cell antibodies targeting gastric H^+/K^+ ATPase. It has been suggested that these patients, and in particular those who are hypergastrinemic, should be screened for autoimmune gastritis, iron deficiency, and pernicious anemia. The clustering of these autoimmune diseases points to a shared genetic predisposition, although the nature of the responsible gene(s) remains unknown.

Immunologic Mechanisms of Vitamin B12 Malabsorption

Two immunologically mediated processes lead to malabsorption of vitamin B12. The first is depletion of gastric parietal cells, which secrete gastric intrinsic factor. Normally, intrinsic factor is secreted far in excess of that required for absorption of vitamin B12, but as immunologic destruction of gastric parietal cells proceeds to gastric atrophy, the level of secretion falls below that required for complexing with dietary vitamin B12. Over and above this effect, there are autoantibodies reactive with the receptor site for vitamin B12 on intrinsic factor. These are demonstrable in serum and gastric juice (Fisher et al., 1966; Schade et al., 1966), and interfere with formation of the stable complex required for transport of vitamin B12 from the stomach to the absorption site on epithelial cells in the distal ileum (Donaldson et al., 1967; Kapadia et al., 1983). This complex may be present exclusively with no free intrinsic factor detectable (Goldberg and Bluestone, 1970; Rose et al., 1970). Serum from rabbits injected with intrinsic factor contained similar inhibitory properties (Taylor and Morton, 1959). In pernicious anemia, megaloblastic anemia and neuropathy are the direct effects of the ensuing deficiency of vitamin B12 (Chanarin et al., 1981).

GENETIC FEATURES

Predisposition to pernicious anemia appears to be genetically determined, at least in part, although the mode of inheritance is unknown. Evidence for genetic factors influencing the expression of pernicious anemia includes clustering of this disease in families and with other autoimmune diseases (see Table 39.1), as well as a racial predilection for northern Europeans. Pernicious anemia is rare among southern Europeans and earlier reports suggest that it is almost nonexistent among black and Asian people (Jayaratnam et al., 1967; Irvine et al., 1969). In keeping with racial differences, pernicious anemia is associated with phenotypic markers that are absent or occur with low frequency in these racial groups. These markers include blue eyes, fair skin, and blood group A (Callender and Denborough, 1957). However, a more recent study by Carmel (1996) reported a higher prevalence in black people, particularly among black women.

There have been reports of a number of white families with a high frequency of pernicious anemia over several generations (Callender and Denborough, 1957; McIntyre et al., 1959; Doniach et al., 1965; Ardeman et al., 1966b; Wangel et al., 1968; Whittingham et al., 1969). A raised but not absolute concordance of pernicious anemia has been observed in monozygotic twins (Delva et al., 1965; Irvine et al., 1965; Balcerzak et al., 1968). HLA markers B7, B12, DR2, and DR4 have been reported with increased frequency in patients with pernicious anemia, and B8, B18, and Bw15 in patients with pernicious anemia and autoimmune endocrinopathies (Ungar et al., 1977). The data of Ungar et al. (1981) suggest that DR2 may protect against the development of autoimmune endocrinopathies in patients with pernicious anemia. However, an association of HLA markers with pernicious anemia has not been substantiated in other studies (reviewed by Whittingham, 1991).

ENVIRONMENTAL INFLUENCES

An environmental trigger for the initiation of autoimmune gastritis remains uncertain. A case has been made for *H. pylori* as the principal environmental trigger for this disease (Appelmelk et al., 1998; Bergman et al., 2005). This rests primarily on reports that while *H. pylori* initially colonizes the antrum, resulting in antral gastritis, extension of the chronic inflammatory process to the body of the stomach is associated with an autoimmune reaction to gastric H^+/K^+

ATPase. In support of this hypothesis, $CD4^+$ T-cell clones isolated from stomachs of patients with autoimmune gastritis have been reported to cross-react with nine epitopes shared between *H. pylori* and gastric H^+/K^+ ATPase (Amedei et al., 2003). The *H. pylori* peptides induced T-cell proliferation and expression of Th1 function. However, whether *H. pylori* is the environmental trigger for autoimmune gastritis with sparing of the gastric antrum arising from the conversion of type B pangastritis to type A gastritis remains unproven. The epidemiologic data are suggestive but not conclusive (Uibo, 2005). In the mouse, autoimmune gastritis initiated by neonatal thymectomy regressed after infection with *H. pylori* (Okazaki et al., 2003).

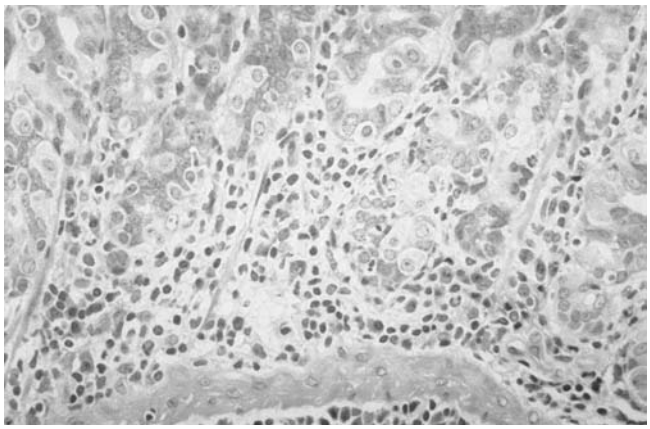


FIGURE 39.7 Microscopic appearance of the gastric mucosa of a mouse with autoimmune gastritis showing a chronic inflammatory infiltrate in the gastric submucosa with extension into the lamina propria between gastric glands. ×300.

ANIMAL MODELS

While studies of autoimmune gastritis and pernicious anemia have suggested that the parietal cell is targeted in the gastric lesion and that the associated parietal cell antibodies are directed against the gastric H^+/K^+ ATPase, they do not directly address the critical question of the role of the gastric H^+/K^+ ATPase in disease induction. Most investigations of organ-specific autoimmunity in humans have identified autoantigens associated with advanced disease. Much less is known of the early events that initiate an autoimmune response to self-antigens and, in particular, the way in which self-antigens are presented by antigen-presenting cells for recognition by T cells to activate the self-destructive autoimmune process. These molecular and cellular events associated with the onset of disease can most readily be studied in animal models of autoimmunity. Using animal models, considerable effort is now directed at identifying the relevant autoantigens responsible for the initiation of the disease, as these represent the key targets for intervention therapies. These issues are especially relevant to autoimmune gastritis and pernicious anemia where the clinical signs of disease can occur decades after the event which initiated the primary autoimmune response.

Experimental autoimmune gastritis is an excellent animal model of the human disease in that its pathology and its autoimmune response to the gastric H^+/K^+ ATPase are very similar to those of the human disease (Jones et al., 1991). As in humans, the gastric lesion is characterized by a chronic inflammatory infiltrate in the gastric mucosa that extends into the lamina propria between gastric glands (Figure 39.7) and is accompanied by loss of gastric parietal and zymogenic cells that is best revealed by the modified Maxwell's histochemical stain (Figure 39.8). Murine gastritis differs from the human disease in the presence of hypertrophy arising from expansion of a mucosal precursor population. While there is some evidence of progression to macrocytic anemia, the question of whether the mice develop autoantibodies to intrinsic factor remains unresolved. Gastritis is readily induced by neonatal thymectomy and by a range of other procedures (Figure 39.9); its strain dependence allows for analysis of genetic factors and as a nonlethal disease it allows for the investigation of treatment strategies. Indeed, the mouse model has proven to be a powerful tool for the identification of the initiating autoantigens, the role of dendritic cells and macrophages in the initiation of the autoimmune response, the role of regulatory T cells, the mechanisms for the loss of tolerance to a peripheral tissue-specific autoantigen, and for devising novel strategies to

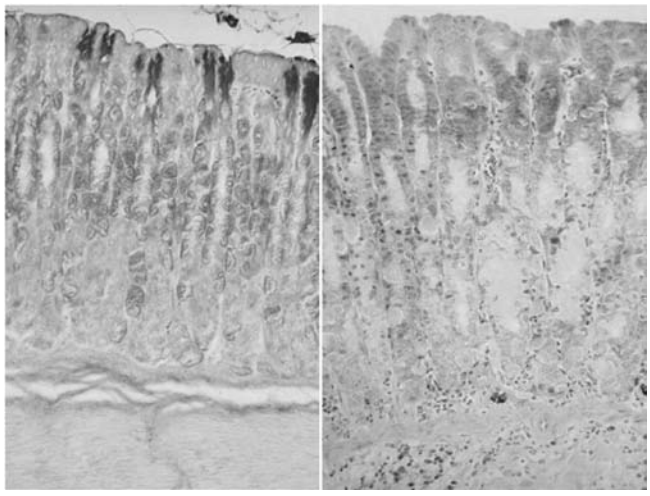


FIGURE 39.8 Microscopic appearance of the normal gastric mucosa of a mouse (left panel) and of the mucosa of a mouse with autoimmune gastritis (right panel). With modified Maxwell's stain, gastric parietal cells in the normal gastric mucosa located towards the base of the glands stain blue while zymogenic cells in the mid-portions of the gland stain purple. In the mouse with autoimmune gastritis, the mucosa is hypertrophied and the gastric parietal and zymogenic cells have been lost and replaced by mucus-containing cells that stain yellow.

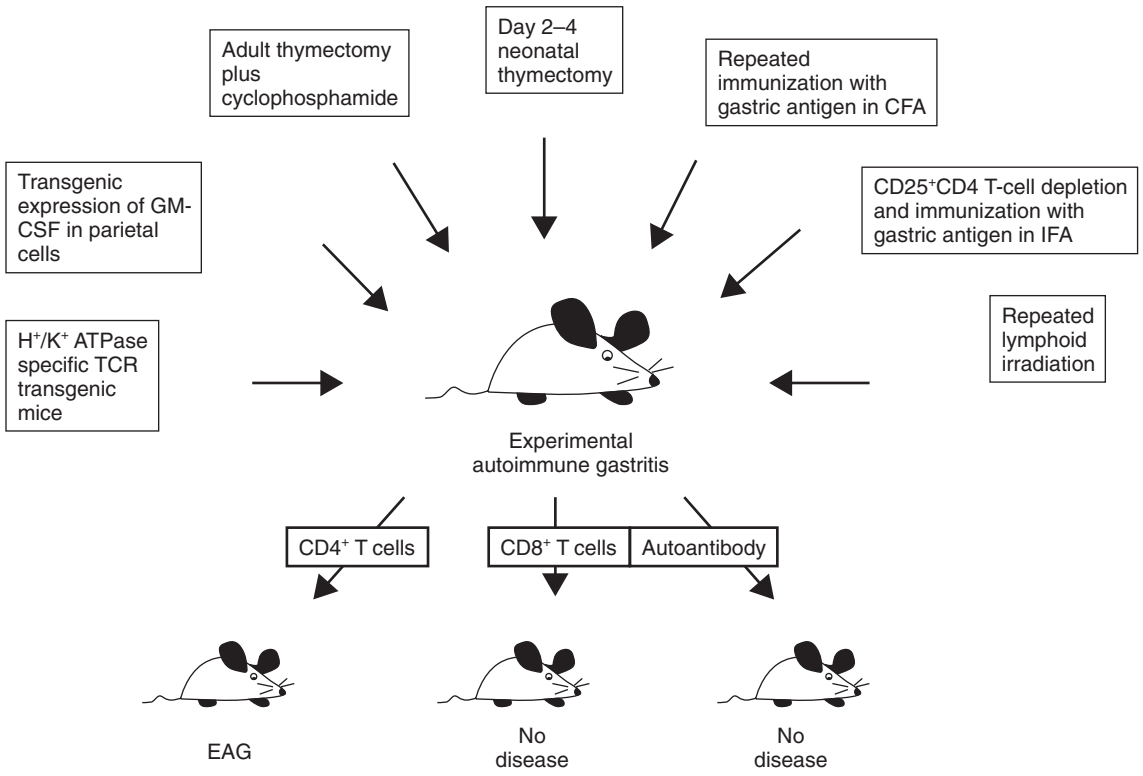


FIGURE 39.9 The range of procedures that can induce experimental autoimmune gastritis (EAG) in BALB/c mice. The readout of EAG includes the generation of autoantibodies to the H^+/K^+ ATPase of gastric parietal cells and a chronic inflammatory infiltrate with tissue destruction within the gastric mucosa. Transfer experiments have shown that $CD4^+$ T cells are the critical pathogenic cells in this model, with no demonstrable role for $CD8^+$ T cells or autoantibody.

reestablish tolerance and reverse pathology (Alderuccio and Toh, 2000; Alderuccio et al, 2002).

Spontaneous Autoimmune Gastritis

Spontaneous autoimmune gastritis has been reported in animals. For instance, autoantibodies to rat gastric parietal cells were demonstrated in 68% of Bio-Breeding (BB)/W rats with spontaneous insulin-dependent diabetes mellitus (Elder et al., 1982). These antibodies were associated with mild to moderate gastritis and loss of specialized cells, fibrosis, and squamous metaplasia, although no significant reduction in acid secretion or fall in serum level of vitamin B12 was detected in the 90–300-day-old rats studied. Antibodies to gastric parietal cells have also been shown to coexist serologically with thyroid antibodies in obese strain chickens with thyroiditis (Khoury et al., 1982) but gastritis in the chicken is difficult to interpret and considered to be an unsatisfactory model of human autoimmune gastritis. Spontaneous gastritis has more recently been reported in C3H/He mice with an incidence of about 20% (Alderuccio and Toh, 1998) and may prove to be an informative model of human autoimmune gastritis.

Post-thymectomy Autoimmune Gastritis

The first report of autoimmunity following neonatal thymectomy was the induction of autoimmune thyroiditis (Nishizuka et al., 1973). Since then organ-specific autoimmunity closely mirroring the spectrum of human thyrogastric autoimmunity has been observed following neonatal thymectomy in a variety of mouse strains (Kojima and Prehn, 1981). Removal of the neonatal thymus 2–4 days after birth has been shown to prevent the emigration of $CD4^+CD25^+$ regulatory T cells from the thymus to the periphery (Fehervari and Sakaguchi, 2004). The removal of this regulatory population is held to be primarily responsible for the subsequent unchecked expansion of pathogenic self-reactive T cells that leads to autoimmune disease. However, manipulation of mice during the neonatal period is not an essential prerequisite for the development of autoimmunity. Adult thymectomy combined with repeated low-dose radiation (Penhale et al., 1975) or with a single dose of cyclophosphamide can also initiate autoimmunity (see Figure 39.9; Barrett et al., 1995a). In adults, neither adult thymectomy alone nor cyclophosphamide-induced transient lymphopenia alone can initiate gastritis, yet the

degree of the transient lymphopenia induced by combined adult thymectomy and cyclophosphamide treatment, compared to that induced by cyclophosphamide treatment alone, is indistinguishable. These observations underpin the essential role of the thymus as the source of $CD4^+CD25^+$ regulatory T cells that maintain tolerance to self-antigens in neonatal and adult life (Sakaguchi et al., 1995; Sakaguchi, 2004; Field et al., 2005). The importance of this population for tolerance is further supported by the need to remove these T cells using depleting anti-CD25 antibody to establish sustained autoimmune gastritis following immunization with gastric antigen (McHugh and Shevach, 2002). These regulatory T cells constitute 2–5% of CD4 single positive thymocytes and 5–10% of peripheral $CD4^+$ T cells, are of high avidity, are positively selected in the thymus, require expression of the transcriptional factor Fox3p for suppressor activity and are thought to prevent expansion of pathogenic T cells by a cytokine-independent, cell-to-cell contact mechanism. However, the precise mechanism of suppression remains unknown (Sakaguchi, 2004). It seems likely that, in addition to the removal of this T regulatory population, the homeostatic expansion of the pathogenic T cells also plays a role in the initiation of autoimmunity (King et al., 2004).

Following neonatal thymectomy, the particular organ affected by autoimmune disease is dependent on the mouse strain, indicating genetic factors determining tissue tropism. For autoimmune gastritis, BALB/c mice are most susceptible to disease, in both the neonate (see Figure 39.9; Kojima and Prehn, 1981; Tung et al., 1987) and the adult. Autoimmune gastritis is not dependent on the presence of a particular H2 haplotype as mice with several different major histocompatibility complex (MHC) haplotypes develop gastritis (Kojima and Prehn, 1981). Four linkage regions, two on distal chromosome 4 (*Gasa1* and *Gasa2*), one on chromosome 6 (*Gasa3*) and one in the H2 (*Gasa4*), have been identified (Silveira et al., 1999; 2001; Baxter et al., 2005). Three of these four genes colocalize with NOD mouse diabetes susceptibility genes, the strongest concordance identified to date between any two autoimmune diseases. The incidence of gastritis in BALB/c mice is 60–90%, although other strains develop gastritis with lower incidences. These glands show destructive changes in parietal cells and zymogenic cells (Martinelli et al., 1996).

Autoimmune Gastritis Induced by Immunization

Early attempts to induce autoimmune gastritis used crude gastric extracts as the immunogen. For example, immunization of rhesus monkeys with extracts of gastric mucosa in complete Freund's adjuvant (Andrada et al., 1969) resulted in gastritis, gastric parietal cell antibodies, and cutaneous delayed-type hypersensitivity reactions to gastric antigens. Immunization of dogs with gastric juice and extracts of gastric mucosa also resulted in gastritis and equivalent humoral and cellular reactions to gastric antigens but in this model, unlike the human disease, there was severe atrophy and only a minimal inflammatory response in the gastric mucosa (Hennes et al., 1962; Fixa et al., 1964; 1972; Krohn and Finlayson, 1973). The cellular response was thought to be more important in the production of gastritis in dogs (Krohn and Finlayson, 1973), although a combined humoral and cellular response to immunization has been demonstrated. Autoimmune gastritis has also been induced in mice by immunization with gastric parietal cells (Kontani et al., 1992).

The discovery of the gastric H^+/K^+ ATPase as the molecular target of parietal cell antibodies in both human autoimmune gastritis with pernicious anemia and in thymectomy-induced models of gastric autoimmunity prompted the use of purified gastric H^+/K^+ ATPase containing both the α- and β-subunits for the induction of autoimmune gastritis by immunization. Gastritis and circulating parietal cell autoantibodies to gastric H^+/K^+ ATPase developed maximally in BALB/c mice after four immunizations of the mouse gastric enzyme emulsified in Freund's complete adjuvant. The gastritis was accompanied by destruction of both gastric parietal cells and zymogenic cells. This suggests that the loss of zymogenic cells is secondary to an immune response to the gastric parietal cells H^+/K^+ ATPase. The mucosal lesion was accompanied by expansion in the population of precursor stem cells similar to that seen in the gastritis induced by neonatal thymectomy. The gastritis was transferred to BALB/c mice by splenic T cells from gastritic mice to immunocompromised hosts. It was reversible following cessation of immunization which resulted in regeneration of parietal cells and zymogenic cells, derived presumably by differentiation of the expanded stem cell production (Scarff et al., 1997). Reversibility of the lesion appeared to be the consequence of the presence of regulatory $CD4^+CD25^+$ T cells in these mice because the antibody-mediated removal of this population together with a single immunization with gastric antigen was sufficient to initiate an irreversible gastritis (McHugh and Shevach, 2002).

Transgenic Mouse Models of Autoimmune Gastritis

Autoimmune gastritis has been reported in transgenic mice that express the proinflammatory cytokine GM-CSF in the stomach (Biondo et al., 2001) and in mice that express rearranged TCR genes with specificity for the α- or β-subunits of the gastric H^+/K^+ ATPase (Alderuccio et al., 2000; McHugh et al., 2001).

In mice that express GM-CSF in the stomach, infiltration of macrophages and dendritic cells into the stomach precedes $CD4^+$ T cells. It seems likely that the local presence

of GM-CSF leads to recruitment of macrophages and dendritic cells followed by their activation and migration to the draining lymph node, where they break tolerance by activating potentially pathogenic $CD4^+$ T cells that migrate to the stomach to initiate gastritis. The development of autoimmune gastritis in BALB/c mice following transgenic expression of GM-CSF in parietal cells in the stomach indicates that the actions of these $CD4^+CD25^+$ regulatory T cells can be overridden by the presence of a local proinflammatory cytokine in the stomach (Biondo et al., 2001). Pathogenic $CD4^+$ T cells isolated from PC-GMCSF transgenic mice transferred disease to naïve syngeneic recipients.

T-cell receptor (TCR) transgenic mice bred to the gastritis-susceptible BALB/c genetic background and expressing a TCR specific for the gastric H^+/K^+ ATPase α-subunit develop gastritis (100%) at a much greater frequency than do TCR β-subunit transgenic mice (20%). This difference in disease incidence in these two TCR transgenic models remains unexplained. It is possible that the lower incidence of gastritis in the TCR β-subunit transgenic mice depends on the influence of a $CD4^+CD25^+$ regulatory T-cell population that is absent in the TCR α-subunit transgenic mice.

PATHOGENESIS

For most autoimmune diseases, it is not known whether the molecules recognized by autoantibodies are also T-cell targets or whether the immune response to these antigens is involved in initiation of disease. The gastric H^+/K^+ ATPase targeted by parietal cell autoantibodies in human and in murine autoimmune gastritis is a peripheral autoantigen. Although peripheral antigens are not expected to be present in the thymus, there are increasing reports of promiscuous expression of these antigens, particularly in thymic medullary epithelial cells (Gotter et al., 2004) where ectopic presence of these antigens has been suggested to shape the T-cell repertoire (Anderson and Kuchroo, 2003). This suggestion has gained added relevance with reports of the AIRE transcriptional factor in a small subset of thymic medullary epithelial cells and its postulated role in tolerance to organ-specific autoantigens, presumably through clonal deletion (Vernanzi et al., 2004). However the role of AIRE in tolerance to gastric autoantigens remains uncertain. The α- but not the β-subunit of the gastric H^+/K^+ ATPase has been identified in thymic dendritic cells, suggesting that self-antigen expression by dendritic cells may have a role in maintenance of tolerance. Nonetheless, the capacity to initiate experimental autoimmune gastritis indicates that potentially pathogenic T cells are still present in the periphery.

To ascertain whether the presentation of self-antigen by MHC class II positive cells in the thymus has a role in tolerance induction, transgenic mice were generated that express the α- or β-subunit of the gastric H^+/K^+ ATPase in the thymus driven by an MHC class II promoter (see Figure 39.8). Transgenic mice that expressed the β-subunit in the thymus failed to develop gastritis initiated by a variety of methods as judged by histologic evidence of gastritis and production of circulating antibodies to gastric H^+/K^+ ATPase (Alderuccio et al., 1995; Barrett et al., 1995b; Alderuccio and Toh, 1998). In contrast, transgenic mice that expressed the α-subunit in the thymus driven by the same MHC class II promoter remained susceptible to gastritis initiated by neonatal thymectomy (Figure 39.10). These observations suggest that the β-subunit of the gastric H^+/K^+ ATPase contains the causative autoepitope (Toh et al., 2000).

Studies in mice have clearly indicated that autoimmune gastritis is initiated by $CD4^+$ T cells. First, gastritis can be transferred to immunocompromised hosts by $CD4^+$ T lymphocytes but not by sera from animals with autoimmune gastritis (see Figure 39.9; reviewed by Gleeson and Toh, 1991; Toh et al., 1992; 1997). In addition, histopathologic features of gastritis occur in mice before the appearance of autoantibodies. The early gastric lesion is composed predominantly of $CD4^+$ T cells and macrophages with production of a mix of Th1 and Th2 type cytokines but not interleukin-4 (IL-4), suggesting a key role for these cells and their cytokines in initiation of the disease (Martinelli et al., 1996; Katakai et al., 1998). Interferon-γ, probably produced by the Th1 cells, appears to be crucial for the induction of gastritic lesion as a single injection of antibodies to interferon-γ immediately following neonatal thymectomy prevents autoimmune gastritis (Barrett et al., 1996). $CD8^+$ T cells do not seem to have a role in this disease since depletion of this T-cell population by treatment with anti-CD8 antibody did not reduce the capacity of the remaining T cells to transfer disease (De Silva et al., 1998). B lymphocytes are late migrants to the inflamed gastric mucosa and aggregate in follicular-like structures (Martinelli et al., 1996), likely mediated by the expression of a B-cell homing chemokine, CXC chemokine ligand 13, by dendritic cells within the follicles (Katakai et al., 2003).

The identification of the β-subunit of the gastric H^+/K^+ ATPase as the causative antigen and of $CD4^+$ T cells as the effectors of gastric injury suggests the following model for the genesis of autoimmune gastritis. Gastric antigen released following cell death is taken up locally in the stomach by dendritic cells that migrate to the draining lymph node, resulting in the activation of naïve pathogenic $CD4^+$ T cells. The activated T cells initiate tissue damage, possibly by binding to MHC class II molecules that are upregulated in the inflamed gastric mucosa. Support for this model is provided by the observation by immunofluorescence microscopy of a physical association of $CD11c^+$ dendritic cells with parietal cells in the gastric mucosa. H^+/K^+ ATPase protein was found within vesicular compartments of a few $CD11c^+$ dendritic cells only in the draining gastric lymph node and these antigen-containing dendritic cells increased

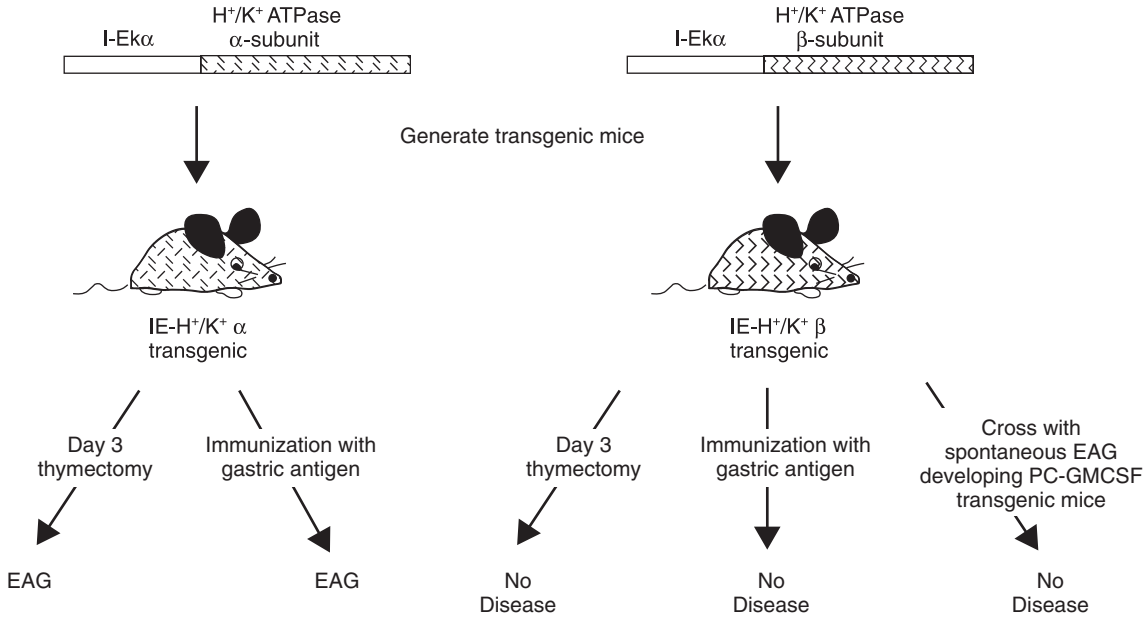


FIGURE 39.10 Experimental evidence suggesting the autoimmune response to gastric H^+/K^+ ATPase β-subunit is critical for EAG induction. Transgenic mice were generated expressing gastric H^+/K^+ ATPase α- or β-subunit under the control of the E-I MHC class II promoter. IE- H^+/K^+ α transgenic mice remained susceptible to EAG induction following day 3 neonatal thymectomy or immunization with gastric autoantigen. In contrast, IE- H^+/K^+ β mice were EAG resistant following neonatal thymectomy or immunization or when crossed to PC-GMCSF transgenic mice that spontaneously develop EAG.

markedly in number with the onset of tissue destruction in autoimmune animals. Both CD8α(hi) and CD8α(lo) gastric dendritic cells showed evidence of constitutive *in vivo* processing and presentation of H^+/K^+ ATPase. These data also demonstrate that dendritic cells in the draining lymph node can present a tissue-specific self-antigen under noninflammatory conditions without fully deleting autoreactive T cells or inducing active autoimmunity (Scheinecker et al., 2002). The homing of the activated $CD4^+$ T cells to the gastric mucosa appears to be mediated by the interaction of α4 β7 on the lymphocytes with mucosal MAdCAM-1, given that a significant increase in the area of mucosal endothelium expressing MAdCAM-1 in gastritic mice was observed and that treatment of mice with either a MAdCAM-1-specific or α4 β7-specific monoclonal antibody reduced the incidence of gastritis (Barrett et al., 2000; Katakai et al., 2002). While neither the chemokine receptor CCR5 nor TNF-α appears to have a role in the inflammatory process (Field et al., 2003; Marshall et al., 2004), the expression pattern of the cytokines and chemokines expressed in the stomach includes lymphotoxin-β and is consistent with a Th1-biased, memory T-cell-dependent immunomicroenvironment (Katakai et al., 2003). Fas/FasL interaction appears to be required for the initiation of parietal cell death in the gastric mucosa (Nishio et al., 1996; Marshall et al., 2002).

TREATMENT AND OUTCOME

The standard treatment is to correct the deficiency of vitamin B12 by the administration of vitamin B12. While the time-honored route of vitamin delivery is by parenteral injection, there is increasing evidence that the oral route is equally effective, particularly with the vitamin given at a high dosage (1000 μg) (Kaltenbach et al., 2002; Lane and Rojas-Fernandez, 2002; Nyholm et al., 2003). This corrects the anemia but there are no adequate data at present to indicate whether oral vitamin B12 can correct the neuropathy, particularly when it is severe. Erroneous treatment of patients with folic acid may not only mask the anemia caused by vitamin B12 deficiency but lead to irreversible neurologic damage (Dhar et al., 2003).

The identification in mouse models of autoimmune gastritis of proinflammatory Th1 $CD4^+$ T cells directed against the β-subunit of the gastric H^+/K^+ ATPase has suggested a novel therapeutic approach to reestablish tolerance to self-antigen and the prevention and/or reversal of autoimmune disease. The approach uses transplantation with bone marrow stem cells that have been genetically engineered to direct expression of culprit antigen in bone marrow-derived antigen-presenting cells in the thymus (Alderuccio et al., 2003). Initial reports have shown that transplantation with

bone marrow from transgenic mice that have been engineered to express the gastric H^+/K^+ ATPase β-subunit antigen (Murphy et al., 2003) or proinsulin II in the thymus (Steptoe et al., 2003) has succeeded in establishing tolerance to the antigen in the case of autoimmune gastritis and of preventing disease in the case of diabetes in NOD mice. These highly promising results suggest that this strategy may have application for autoimmune diseases that respond poorly to replacement therapy or immunosuppressive drugs.

CONCLUDING REMARKS—FUTURE PROSPECTS

Pernicious anemia and gastric atrophy are terminal events in a protracted chronic autoimmune gastritis that affects the fundus and body of the stomach. They are usually expressed clinically in late middle age. The vitamin B12 deficiency and ensuing megaloblastic anemia are the result of gastric parietal cell loss with failure of production of intrinsic factor, an obligatory factor for absorption of the vitamin from the terminal ileum.

Autoimmune gastritis fulfills the criteria for an organ-specific autoimmune disease: autoantibodies to gastric antigens, infiltration of mononuclear cells into the target organ with evidence of destruction, a regenerative response of the affected tissue to corticosteroid drugs, familial predisposition, and association with other autoimmune diseases, mostly the autoimmune endocrinopathies. The molecular target of parietal cell autoantibodies is the gastric H^+/K^+ ATPase located on secretory membranes of gastric parietal cells and autoantibodies to gastric intrinsic factor.

Animal models have provided insights into the pathogenesis of autoimmune gastritis. The BB/W rat, obese chicken, and C3H/He mouse represent spontaneous animal models of autoimmune gastritis. It can be induced in susceptible mouse strains by removal of $CD4^+CD25^+$ regulatory T cells (e.g., neonatal thymectomy), by immunization with gastric H^+/K^+ ATPase emulsified in Freund's complete adjuvant, and by the generation of transgenic mice that express either the proinflammatory cytokine GM-CSF in the stomach or rearranged TCRs directed against gastritogenic epitopes of the α- or β-subunits of the gastric H^+/K^+ ATPase. The murine disease is initiated by proinflammatory Th1 $CD4^+$ T cells that are activated in the draining gastric lymph node by dendritic cells, loaded with the gastric antigen, that have migrated to this location from the stomach. These T cells are directed initially against the β-subunit of the gastric H^+/K^+ ATPase since active T-cell tolerance to this subunit alone prevents the development of autoimmune gastritis induced by a variety of methods such as thymectomy or immunization with the ATPase. Mucosal damage is initiated through engagement of Fas expressed by parietal cells in the inflamed gastric mucosa. Genetic engineering of bone marrow stem cells to target the gastric autoantigen to the thymus points the direction towards testing strategies directed towards reestablishment of tolerance and the prevention and reversal of pathology in intractable autoimmune diseases in which causative antigens are known.

Acknowledgments

We thank the National Health and Medical Research Council of Australia for its support.

References

Alderuccio, F. and Toh, B.H. (1998). Spontaneous autoimmune gastritis in C3H/He mice: a new mouse model for gastric autoimmunity. *Am. J. Pathol. 153*(4), 1311–1318.

Alderuccio, F. and Toh, B.H. (2000). Immunopathology of autoimmune gastritis: lessons from mouse models. *Histol. Histopathol. 15*(3), 869–879.

Alderuccio, F., Toh, B.H., Gleeson, P.A. and van Driel, I.R. (1995). A novel method for isolating mononuclear cells from the stomachs of mice with experimental autoimmune gastritis. *Autoimmunity 21*(3), 215–221.

Alderuccio, F., Cataldo, V., van Driel, I.R., Gleeson, P.A. and Toh, B.H.. (2000). Tolerance and autoimmunity to a gastritogenic peptide in TCR transgenic mice. *Int. Immunol. 12*(3), 343–352.

Alderuccio, F., Sentry, J.W., Marshall, A.C., Biondo, M. and Toh, B.H. (2002). Animal models of human disease: experimental autoimmune gastritis—a model for autoimmune gastritis and pernicious anemia. *Clin. Immunol. 102*(1), 48–58.

Alderuccio, F., Murphy, K. and Toh, B.H. (2003). Stem cells engineered to express self-antigen to treat autoimmunity. *Trends Immunol. 24*(4), 176–180.

Amedei, A., Bergman, M.P., Appelmelk, B.J., Azzurri, A., Benagiano, M., Tamburini, C., van der Zee, R., Telford, J.L., Vandenbroucke-Grauls, C.M., D'Elios, M.M. and Del Prete, G. (2003). Molecular mimicry between *Helicobacter pylori* antigens and H+, K+ adenosine triphosphatase in human gastric autoimmunity. *J. Exp. Med. 198*(8), 1147–1156.

Anderson, A.C. and Kuchroo, V.K. (2003). Expression of self-antigen in the thymus: a little goes a long way. *J. Exp. Med. 198*(11), 1627–1629.

Andrada, J., Rose, N.R. and Andrada, E.C. (1969). Experimental autoimmune gastritis in the Rhesus monkey. *Clin. Exp. Immunol. 4*(3), 293–310.

Appelmelk, B.J., Faller, G., Claeys, D., Kirchner, T. and Vandenbroucke-Grauls, C.M. (1998). Bugs on trial: the case of *Helicobacter pylori* and autoimmunity. *Immunol. Today 19*(7), 296–299.

Ardeman, S. and Chanarin, I. (1965). Steroids and addisonian pernicious anemia. *N. Engl. J. Med. 273*(25), 1352–1355.

Ardeman, S., Chanarin, I., Jacobs, A. and Griffiths, L. (1966a). Family study in Addisonian pernicious anemia. *Blood 27*(5), 599–610.

Ardeman, S., Chanarin, I., Krafchik, B. and Singer, W. (1966). Addisonian pernicious anaemia and intrinsic factor antibodies in thyroid disorders. *Q. J. Med. 35*(139), 421–431.

Baggett, R.T. and Welsh, J.D. (1970). Observations on the effects of glucocorticoid administration in pernicious anemia. *Am. J. Dig. Dis. 15*(10), 871–881.

Balcerzak, S.P., Westerman, M.P. and Heinle, E.W. (1968). Discordant occurrence of pernicious anemia in identical twins. *Blood 32*(5), 701–710.

Bardhan, K.D., Hall, J.R., Spray, G.H. and Callender, S.T. (1968). Blocking and binding autoantibody to intrinsic factor. *Lancet 1*(7533), 62–64.

Barrett, S.P., Toh, B.H., Alderuccio, F., van Driel, I.R. and Gleeson, P.A. (1995a). Organ-specific autoimmunity induced by adult thymectomy and cyclophosphamide-induced lymphopenia. *Eur. J. Immunol. 25*(1), 238–244.

Barrett, S.P., van Driel, I.R., Tan, S.S., Alderuccio, F., Toh, B.H. and Gleeson, P.A. (1995b). Expression of a gastric autoantigen in pancreatic islets results in non-destructive insulitis after neonatal thymectomy. *Eur. J. Immunol. 25*(9), 2686–2694.

Barrett, S.P., Gleeson, P.A., de Silva, H., Toh, B.H. and van Driel, I.R. (1996). Interferon-gamma is required during the initiation of an organ-specific autoimmune disease. *Eur. J. Immunol. 26*(7), 1652–1655.

Barrett, S.P., Riordon, A., Toh, B.H., Gleeson, P.A. and van Driel, I.R. (2000). Homing and adhesion molecules in autoimmune gastritis. *J. Leukoc. Biol. 67*(2), 169–173.

Baxter, A.G., Jordan, M.A., Silveira, P.A., Wilson, W.E. and van Driel, I.R. (2005). Genetic control of susceptibility to autoimmune gastritis. *Int. Rev. Immunol. 24*(1–2), 55–62.

Bergman, M.P., Amedei, A., D'Elios, M.M., Azzurri, A., Benagiano, M., Tamburini, C., van der Zee, R., Vandenbroucke-Grauls, C.M., Appelmelk, B.J. and Del Prete, G. (2003). Characterization of H+,K+-ATPase T-cell epitopes in human autoimmune gastritis. *Eur. J. Immunol. 33*(2), 539–545.

Bergman, M.P., Vandenbroucke-Grauls, C.M., Appelmelk, B.J., D'Elios, M.M., Amedei, A., Azzurri, A., Benagiano, M. and Del Prete, G. (2005). The story so far: *Helicobacter pylori* and gastric autoimmunity. *Int. Rev. Immunol. 24*(1–2), 63–91.

Berlin, R., Berlin, H., Brante, G. and Sjoberg, S.G. (1958). Refractoriness to intrinsic factor-B12 preparations abolished by massive doses of intrinsic factor; preliminary report. *Acta Med. Scand. 162*(4), 317–319.

Biondo, M., Nasa, Z., Marshall, A., Toh, B.H. and Alderuccio, F. (2001). Local transgenic expression of granulocyte macrophage-colony stimulating factor initiates autoimmunity. *J. Immunol. 166*(3), 2090–2099.

Blizzard, R.M., Chee, D. and Davis, W. (1966). The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism. *Clin. Exp. Immunol. 1*(2), 119–128.

Blizzard, R.M., Chee, D. and Davis, W. (1967). The incidence of adrenal and other antibodies in the sera of patients with idiopathic adrenal insufficiency (Addison's disease). *Clin. Exp. Immunol. 2*(1), 19–30.

Bor, S., Feiwel, M. and Chanarin, I. (1969). Vitiligo and its aetiological relationship to organ-specific autoimmune disease. *Br. J. Dermatol. 81*(2), 83–88.

Brostoff, J. (1969). Autoantibodies in patients with vitiligo. *Lancet 2*(7613), 177–178.

Burman, P., Mardh, S., Norberg, L. and Karlsson, F.A. (1989). Parietal cell antibodies in pernicious anemia inhibit H+, K+-adenosine triphosphatase, the proton pump of the stomach. *Gastroenterology 96*(6), 1434–1438.

Callaghan, J.M., Toh, B.H., Simpson, R.J., Baldwin, G.S. and Gleeson, P.A. (1992). Rapid purification of the gastric H+/K(+)-ATPase complex by tomato-lectin affinity chromatography. *Biochem. J. 283* (Pt 1), 63–68.

Callaghan, J.M., Khan, M.A., Alderuccio, F., van Driel, I.R., Gleeson, P.A. and Toh, B.H. (1993). Alpha and beta subunits of the gastric H+/K(+)-ATPase are concordantly targeted by parietal cell autoantibodies associated with autoimmune gastritis. *Autoimmunity 16*(4), 289–295.

Callender, S.T. and Denborough, M.A. (1957). A family study of pernicious anaemia. *Br. J. Haematol. 3*(1), 88–106.

Carmel, R. (1992). Reassessment of the relative prevalences of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anaemia: influence of patient age and race. *Clin. Exp. Immunol. 89*(1), 74–77.

Carmel, R. (1996). Prevalence of undiagnosed pernicious anemia in the elderly. *Arch. Intern. Med. 156*(10), 1097–1100.

Carmel, R. and Johnson, C.S. (1978). Racial patterns in pernicious anemia. Early age at onset and increased frequency of intrinsic-factor antibody in black women. *N. Engl. J. Med. 298*(12), 647–650.

Castle, W.B. (1953). Development of knowledge concerning the gastric intrinsic factor and its relation to pernicious anemia. *N. Engl. J. Med. 249*(15), 603–614.

Chanarin, I. (1979). *The Megaloblastic Anaemias*. Oxford: Blackwell.

Chanarin, I., Deacon, R., Perry, J. and Lumb, M. (1981). How vitamin B12 acts. *Br. J. Haematol. 47*(4), 487–491.

Chuang, J.S., Callaghan, J.M., Gleeson, P.A. and Toh, B.H. (1992). Diagnostic ELISA for parietal cell autoantibody using tomato lectin-purified gastric H+/K(+)-ATPase (proton pump). *Autoimmunity 12*(1), 1–7.

Cowling, D.C., Strickland, R.G., Ungar, B., Whittingham, S. and Rose, W.M. (1974). Pernicious-anaemia-like syndrome with immunoglobulin deficiency. *Med. J. Aust. 1*(1), 15–17.

Davidson, R.J., Atrah, H.I. and Sewell, H.F. (1989). Longitudinal study of circulating gastric antibodies in pernicious anaemia. *J. Clin. Pathol. 42*(10), 1092–1095.

de Aizpurua, H.J., Toh, B.H. and Ungar, B. (1983a). Parietal cell surface reactive autoantibody in pernicious anaemia demonstrated by indirect membrane immunofluorescence. *Clin. Exp. Immunol. 52*(2), 341–349.

de Aizpurua, H.J., Cosgrove, L.J., Ungar, B. and Toh, B.H. (1983b). Autoantibodies cytotoxic to gastric parietal cells in serum of patients with pernicious anemia. *N. Engl. J. Med. 309*(11), 625–629.

de Aizpurua, H.J., Ungar, B. and Toh, B.H. (1985). Autoantibody to the gastrin receptor in pernicious anemia. *N. Engl. J. Med. 313*(8), 479–483.

De Block, C.E., De Leeuw, I.H., Decochez, K., Winnock, F., Van Autreve, J., Van Campenhout, C.M., Martin, M., Gorus, F.K. and Belgian Diabetes Registry. (2001). The presence of thyrogastric antibodies in first degree relatives of type 1 diabetic patients is associated with age and proband antibody status. *J. Clin. Endocrinol. Metab. 86*(9), 4358–4363.

De Block, C.E., De Leeuw, I.H., Bogers, J.J., Pelckmans, P.A., Ieven, M.M., Van Marck, E.A, Van Acker, K.L. and Van Gaal, L.F. (2003). Autoimmune gastropathy in type 1 diabetic patients with parietal cell antibodies: histologic and clinical findings. *Diabetes Care 26*(1), 82–88.

De Silva, H., van Driel, I.R., La Grunta, N., Toh, B.H. and Gleeson, P.A. (1998). CD4+ T-cells, but not CD8+ T-cells, are required for the development of experimental autoimmune gastritis. *Immunology 93*(3), 405–408.

D'Elios, M.M., Bergman, M.P., Azzurri, A., Amedei, A., Benagiano, M., De Pont, J.J., Cianchi, F., Vandenbroucke-Grauls, C.M., Romagnani, S., Appelmelk, B.J. and Del Prete, G. (2001). H(+),K(+)-atpase (proton pump) is the target autoantigen of Th1-type cytotoxic T-cells in autoimmune gastritis. *Gastroenterology 120*(2), 377–386.

Delva, P.L., Macdonell, J.E. and Macintosh, O.C. (1965). Medical Journalism. VII. Sludging in the arteries of communication. *Can. Med. Assoc. J. 92*, 1132–1133.

Dhar, M., Bellevue, R. and Carmel, R. (2003). Pernicious anemia with neuropsychiatric dysfunction in a patient with sickle cell anemia treated with folate supplementation. *N. Engl. J. Med. 348*(22), 2204–2207.

Doig, A., Girdwood, R.H., Duthie, J.J. and Knox, J.D. (1957). Response of megaloblastic anaemia of prednisolone. *Lancet 273*(7003), 966–972.

Donaldson, R.M. Jr, Mackenzie, I.L. and Trier, J.S. (1967). Intrinsic factor-mediated attachment of vitamin B12 to brush borders and microvillous membranes of hamster intestine. *J. Clin. Invest. 46*(7), 1215–1228.

Doniach, D., Roitt, I.M. and Taylor, K.B. (1965). Autoimmunity in pernicious anemia and thyroiditis: a family study. *Ann. NY Acad. Sci. 124*(2), 605–625.

Elder, M., Maclaren, N., Riley, W. and McConnell, T. (1982). Gastric parietal cell and other autoantibodies in the BB rat. *Diabetes 31*(4 Pt 1), 313–318.

Elsborg, L. and Mosbech, J. (1979). Pernicious anaemia as a risk factor in gastric cancer. *Acta Med. Scand. 206*(4), 315–318.

Eriksson, S., Clase, L. and Moquist-Olsson, I. (1981). Pernicious anemia as a risk factor in gastric cancer. The extent of the problem. *Acta Med. Scand. 210*(6), 481–484.

Fehervari, Z. and Sakaguchi, S. (2004). Development and function of CD25+CD4+ regulatory T-cells. *Curr. Opin. Immunol. 16*(2), 203–208.

Field, J., Marshall, A.C., Hertzog, P., Wells, T.N., Alderuccio, F. and Toh, B.H. (2003). Chemokine receptor CCR5 is not required for development of experimental autoimmune gastritis. *Clin. Immunol. 109*(2), 238–247.

Field, J., Biondo, M., Murphy, K., Alderuccio, F. and Toh, B.H. (2005). Experimental autoimmune gastritis: mouse models of human organ-specific autoimmune disease. *Int. Rev. Immunol. 24*(1–2), 93–110.

Fisher, J.M., Rees, C. and Taylor, K.B. (1966). Intrinsic-factor antibodies in gastric juice of pernicious-anemia patients. *Lancet 2*(7454), 88–89.

Fixa, B., Vejbora, O., Komarkova, O., Langr, F. and Par'Izek, J. (1964). On immunologically induced gastric atrophy in dogs. *Gastroenterologia 102*, 331–338.

Fixa, B., Thiele, H.G., Komarkova, O. and Nozicka, Z. (1972). Gastric autoantibodies and cell-mediated immunity in pernicious anaemia—a comparative study. *Scand. J. Gastroenterol. 7*(3), 237–240.

Fong, T.L., Dooley, C.P., Dehesa, M., Cohen, H., Carmel, R., Fitzgibbons, P.L., Perez-Perez, G.I. and Blaser, M.J. (1991). *Helicobacter pylori* infection in pernicious anemia: a prospective controlled study. *Gastroenterology 100*(2), 328–332.

Fuchs, C.S. and Mayer, R.J. (1995). Gastric carcinoma. *N. Engl. J. Med. 333*(1), 32–41.

Glass, G.B. (1963). Gastric intrinsic factor and its function in the metabolism of vitamin B12. *Physiol. Rev. 43*, 529–549.

Glass, G.B. (1977). Immunology of atrophic gastritis. *NY State J. Med. 77*(11), 1697–1706.

Gleeson, P.A. and Toh, B.H. (1991). Molecular targets in pernicious anaemia. *Immunol. Today 12*(7), 233–238.

Goldberg, L.S. and Bluestone, R. (1970). Hidden gastric autoantibodies to intrinsic factor in pernicious anemia. *J. Lab. Clin. Med. 75*(3), 449–456.

Goldkorn, I., Gleeson, P.A. and Toh, B.H. (1989). Gastric parietal cell antigens of 60–90, 92, and 100–120 kDa associated with autoimmune gastritis and pernicious anemia. Role of N-glycans in the structure and antigenicity of the 60–90-kDa component. *J. Biol. Chem. 264*(31), 18768–18774.

Goldstone, A.H., Calder, E.A., Barnes, E.W. and Irvine, W.J. (1973). The effect of gastric antigens on the *in vitro* migration of leucocytes from patients with atrophic gastritis and pernicious anaemia. *Clin. Exp. Immunol. 14*(4), 501–508.

Gordin, R. (1959). Vitamin B12 absorption in corticosteroid-treated pernicious anaemia. *Acta Med. Scand. 164*(2), 159–165.

Gotter, J., Brors, B., Hergenhahn, M. and Kyewski, B. (2004). Medullary epithelial cells of the human thymus express a highly diverse selection of tissue-specific genes colocalized in chromosomal clusters. *J. Exp. Med. 199*(2), 155–166.

Gueant, J.L., Safi, A., Aimone-Gastin, I., Rabesona, H., Bronowicki, J.P., Plenat, F., Bigard, M.A. and Haertle, T. (1997). Autoantibodies in pernicious anemia type I patients recognize sequence 251–256 in human intrinsic factor. *Proc. Assoc. Am. Physicians 109*(5), 462–469.

Gutmann, L., Crosby, T.W., Takamori, M. and Martin, J.D. (1972). The Eaton-Lambert syndrome and autoimmune disorders. *Am. J. Med. 53*(3), 354–356.

Hennes, A.R., Sevelius, H., Lewellyn, T., Joel, W., Woods, A.H. and Wolf, S. (1962). Atrophic gastritis in dogs. Production by intradermal injection of gastric juice in Freund's adjuvant. *Arch. Pathol. 73*, 281–287.

Highley, D.R., Davies, M.C. and Ellenbogen, L. (1967). Hog intrinsic factor. II. Some physicochemical properties of vitamin B12-binding fractions from hog pylorus. *J. Biol. Chem. 242*(5), 1010–1015.

Hoedemaeker, P.J. and Ito, S. (1970). Ultrastructural localization of gastric parietal cell antigen with peroxidase-coupled antibody. *Lab. Invest. 22*(2), 184–188.

Hooper, B., Whittingham, S., Mathews, J.D., Mackay, I.R. and Curnow, D.H. (1972). Autoimmunity in a rural community. *Clin. Exp. Immunol. 12*(1), 79–87.

Hsing, A.W., Hansson, L.E., McLaughlin, J.K., Nyren, O., Blot, W.J., Ekbom, A. and Fraumeni, J.F. Jr. (1993). Pernicious anemia and subsequent cancer. A population-based cohort study. *Cancer 71*(3), 745–750.

Hughes, W.S., Brooks, F.P. and Conn, H.O. (1972). Serum gastrin levels in primary hypogammaglobulinemia and pernicious anemia. Studies in adults. *Ann. Intern. Med. 77*(5), 746–750.

Irvine, W.J. (1965). Immunologic aspects of pernicious anemia. *N. Engl. J. Med. 273,* 432–438.

Irvine, W.J. (1975). The association of autoimmune gastritis with autoimmune thyroid disease. *Clin. Endocrinol. Metab. 4*, 351–377.

Irvine, W.J. and Barnes, E.W. (1975). Addison's disease, ovarian failure and hypoparathyroidism. *Clin. Endocrinol. Metab. 4*, 379–434.

Irvine, W.J., Davies, S.H., Teitelbaum, S., Delamore, I.W. and Williams, A.W. (1962). Immunological relationship between pernicious anaemia and thyroid disease. *Br. Med. J. 2*, 454–456.

Irvine, W.J., Davies, S.H., Teitelbaum, S., Delamore, I.W. and Williams, A.W. (1965). The clinical and pathologic significance of gastric parietal cell antibody. *Ann. NY Acad. Sci. 124*(2), 657–691.

Irvine, W.J., McFadzean, A.J., Todd, D., Tso, S.C. and Yeung, R.T. (1969). Pernicious anaemia in the Chinese: a clinical and immunological study. *Clin. Exp. Immunol. 4*(4), 375–386.

Irvine, W.J., Clarke, B.F., Scarth, L., Cullen, D.R. and Duncan, L.J. (1970). Thyroid and gastric autoimmunity in patients with diabetes mellitus. *Lancet 2*(7665), 163–168.

Irvine, W.J., Cullen, D.R. and Mawhinney, H. (1974). Natural history of autoimmune achlorhydric atrophic gastritis. A 1–15-year follow-up study. *Lancet 2*(7879), 482–485.

Irvine, W.J., Gray, R.S., Morris, P.J. and Ting, A. (1978). HLA in primary atrophic hypothyroidism and Hashimoto goitre. *J. Clin. Lab. Immunol. 1*(3), 193–195.

Jacob, E. and Schilling, R.F. (1966). An *in vitro* test for the detection of serum antibody to intrinsic factor-vitamin B12 complex. *J. Lab. Clin. Med. 67*(3), 510–515.

Jassel, S.V., Ardill, J.E., Fillmore, D., Bamford, K.B., O'Connor, F.A. and Buchanan, K.D. (1999). The rise in circulating gastrin with age is due to increases in gastric autoimmunity and *Helicobacter pylori* infection. *Q. J. Med. 92*(7), 373–377.

Jayaratnam, F.J., Seah, C.S., Da Costa, J.L., Tan, K.K. and O'Brien, W. (1967). Pernicious anaemia among Asians in Singapore. *Br. Med. J. 3*(556), 18–20.

Jeffries, G.H., Hoskins, D.W. and Sleisenger, M.H. (1962). Antibody to intrinsic factor in serum from patients with pernicious anemia. *J. Clin. Invest. 41*, 1106–1115.

Jeffries, G.H., Todd, J.E. and Sleisenger, M.H. (1966). The effect of prednisolone on gastric mucosal histology, gastric secretion, and vitamin B 12 absorption in patients with pernicious anemia. *J. Clin. Invest. 45*(5), 803–812.

Jones, C.M., Callaghan, J.M., Gleeson, P.A., Mori, Y., Masuda, T. and Toh, B.H. (1991). The parietal cell autoantigens recognized in neonatal thymectomy-induced murine gastritis are the alpha and beta subunits of the gastric proton pump. *Gastroenterology 101*(2), 287–294.

Jorge, A.D. and Sanchez, D. (1973). The effect of azathioprine on gastric mucosal histology and acid secretion in chronic gastritis. *Gut 14*(2), 104–106.

Kaltenbach, G., Noblet-Dick, M., Barnier-Figue, G., Berthel, M., Kuntzmann, F. and Andres, E. (2002). Early normalization of low

vitamin B12 levels by oral cobalamin therapy in three older patients with pernicious anemia. *J. Am. Geriatr. Soc. 50*(11), 1914–1915.

Kapadia, C.R., Serfilippi, D., Voloshin, K. and Donaldson, R.M. Jr. (1983). Intrinsic factor-mediated absorption of cobalamin by guinea pig ileal cells. *J. Clin. Invest. 71*(3), 440–448.

Kaplan, H.S. and Riglar, L.G. (1945). Pernicious anemia and carcinoma of the stomach—autopsy studies concerning their interrelationships. *Am. J. Med. Sci. 290*, 339–348.

Karlsson, F.A., Burman, P., Loof, L. and Mardh, S. (1988). Major parietal cell antigen in autoimmune gastritis with pernicious anemia is the acid-producing H+,K+-adenosine triphosphatase of the stomach. *J. Clin. Invest. 81*(2), 475–479.

Katakai, T., Mori, K.J., Masuda, T. and Shimuzu, A. (1998 Sep). Differential localization of Th1 and Th2 cells in autoimmune gastritis. *Int. Immunol. 10*(9), 1325–1334.

Katakai, T., Mori, K.J., Masuda, T. and Shimuzu, A. (2002). Selective accumulation of type 1 effector cells expressing P-selectin ligand and/or alpha(4)beta(7)-integrin at the lesions of autoimmune gastritis. *Int. Immunol. 14*(2), 167–175.

Katakai, T., Hara, T., Sugai, M., Gonda, H. and Shimuzu, A. (2003). Th1-biased tertiary lymphoid tissue supported by CXC chemokine ligand 13-producing stromal network in chronic lesions of autoimmune gastritis. *J. Immunol. 171*(8), 4359–4368.

Kaye, M.D., Whorwell, P.J. and Wright, R. (1983). Gastric mucosal lymphocyte subpopulations in pernicious anemia and in normal stomach. *Clin. Immunol. Immunopathol. 28*(3), 431–440.

Kekki, M., Varis, K., Pohjanpalo, H., Isokoski, M., Ihamaki, T. and Siurala, M. (1983). Course of antrum and body gastritis in pernicious anemia families. *Dig. Dis. Sci. 28*(8), 698–704.

Khoury, E.L., Bottazzo, G.F., Pontes de Carvalho, L.C., Wick, G. and Roitt, I.M. (1982). Predisposition to organ-specific autoimmunity in Obese strain (OS) chickens: reactivity to thyroid, gastric, adrenal and pancreatic cytoplasmic antigens. *Clin. Exp. Immunol. 49*(2), 273–282.

King, C., Ilic, A., Koelsch, K. and Sarvetnick, N. (2004). Homeostatic expansion of T-cells during immune insufficiency generates autoimmunity. *Cell 117*(2), 265–277.

Kojima, A. and Prehn, R.T. (1981). Genetic susceptibility to post-thymectomy autoimmune diseases in mice. *Immunogenetics 14*(1–2), 15–27.

Kokkola, A., Sjoblom, S.M., Haapiainen, R., Sipponen, P., Puolakkainen, P. and Jarvinen, H. (1998). The risk of gastric carcinoma and carcinoid tumours in patients with pernicious anaemia. A prospective follow-up study. *Scand. J. Gastroenterol. 33*(1), 88–92.

Kontani, K., Taguchi, O. and Takahashi, T. (1992). Involvement of the H+/K(+)-ATPase alpha subunit as a major antigenic protein in autoimmune gastritis induced by neonatal thymectomy in mice. *Clin. Exp. Immunol. 89*(1), 63–67.

Krohn, K.J. and Finlayson, N.D. (1973). Interrelations of humoral and cellular immune responses in experimental canine gastritis. *Clin. Exp. Immunol. 14*(2), 237–245.

Kulke, M.H. and Mayer, R.J. (1999). Carcinoid tumors. *N. Engl. J. Med. 340*(11), 858–868.

Lane, L.A. and Rojas-Fernandez, C. (2002). Treatment of vitamin B (12)-deficiency anemia: oral versus parenteral therapy. *Ann. Pharmacother. 36*(7–8), 1268–1272.

Lennon, V.A., Lambert, E.H., Whittingham, S. and Fairbanks, V. (1982). Autoimmunity in the Lambert-Eaton myasthenic syndrome. *Muscle Nerve 5*(9S), S21–25.

Lester-Smith, E. (1948). Purification of anti-pernicious anaemia factors from liver. *Nature London* 161, 638–639.

Lindblom, A., Quadt, N., Marsh, T., Aeschlimann, D., Morgelin, M., Mann, K., Maurer, P. and Paulsson, M. (1999).The intrinsic factor-vitamin B12 receptor, cubilin, is assembled into trimers via a coiled-coil alpha-helix. *J. Biol. Chem. 274*(10), 6374–6380.

Lindenbaum, J., Healton, E.B., Savage, D.G., Brust, J.C., Garrett, T.J., Podell, E.R., Marcell, P.D., Stabler, S.P. and Allen, R.H. (1995). Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *Nutrition 11*(2), 181; discussion 180, 182.

Ma, J.Y., Borch, K. and Mardh, S. (1994). Human gastric H,K-adenosine triphosphatase beta-subunit is a major autoantigen in atrophic corpus gastritis. Expression of the recombinant human glycoprotein in insect cells. *Scand. J. Gastroenterol. 29*(9), 790–794.

Mackay, I.R. (1971). In M. Samter (ed.), *Immunological Diseases*, vol. 2, 2nd ed. Boston: Little, Brown.

Mackay, I.R. and Burnet, E.M. (1963). *Autoimmune Disease: pathogenesis, chemistry and therapy.* Springfield, IL: C.C. Thomas.

Markson, J.L. and Moore, J.M. (1962). Autoimmunity in pernicious anaemia and iron-deficiency anaemia. A complement-fixation test using human gastric mucosa. *Lancet 280*, 1240–1243.

Marshall, A.C., Alderuccio, F. and Toh, B.H. (2002). Fas/CD95 is required for gastric mucosal damage in autoimmune gastritis. *Gastroenterology 123*(3), 780–789.

Marshall, A.C., Toh, B.H. and Alderuccio, F. (2004).Tumor necrosis factor alpha is not implicated in the genesis of experimental autoimmune gastritis. *J. Autoimmun. 22*(1), 1–11.

Martinelli, T.M., van Driel, I.R., Alderuccio, F., Gleeson, P.A. and Toh, B.H. (1996). Analysis of mononuclear cell infiltrate and cytokine production in murine autoimmune gastritis. *Gastroenterology 110*(6), 1791–1802.

Masala, C., Smurra, G., Di Prima, M.A., Amendolea, M.A, Celestino, D. and Salsano, F. (1980). Gastric parietal cell antibodies: demonstration by immunofluorescence of their reactivity with surface of the gastric parietal cells. *Clin. Exp. Immunol. 41*(2), 271–280.

McHugh, R.S. and Shevach, E.M. (2002). Cutting edge: depletion of CD4+CD25+ regulatory T-cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J. Immunol. 168*(12), 5979–5983.

McHugh, R.S., Shevach, E.M., Margulies, D.H. and Natarajan, K. (2001). A T-cell receptor transgenic model of severe, spontaneous organ-specific autoimmunity. *Eur. J. Immunol. 31*(7), 2094–2103.

McIntyre, P., Hahn, R., Conley, C.L. and Glass, B. (1959). Genetic factors in predisposition to pernicious anemia. *Bull. Johns Hopkins Hosp. 104*(6), 309–342.

Minot, G.R. and Murphy, W.P. (2001). Treatment of pernicious anemia by a special diet: 1926. *Yale J. Biol. Med. 74*(5), 341–353.

Mosbech, J. and Videbaek, A. (1950). Mortality from and risk of gastric carcinoma among patients with pernicious anaemia. *Br. Med. J. 2*(4675), 390–394.

Muller, H.K., McGiven, A.R. and Nairn, R.C. (1971). Immunofluorescent staining of rat gastric parietal cells by human antibody unrelated to pernicious anaemia. *J. Clin. Pathol. 24*(1), 13–14.

Murphy, K., Biondo, M., Toh, B.H. and Alderuccio, F. (2003). Tolerance established in autoimmune disease by mating or bone marrow transplantation that target autoantigen to thymus. *Int. Immunol. 15*(2), 269–277.

Nishio, A., Katakai, T., Oshima, C., Kasakura, S., Sakai, M., Yonehara, S., Suda, T., Nagata, S. and Masuda, T. (1996). A possible involvement of Fas-Fas ligand signaling in the pathogenesis of murine autoimmune gastritis. *Gastroenterology 111*(4), 959–967.

Nishizuka, Y., Tanaka, Y., Sakakura, T. and Kojima, A. (1973). Murine thyroiditis induced by neonatal thymectomy. *Experientia 29*(11), 1396–1398.

Nyholm, E., Turpin, P., Swain, D., Cunningham, B., Daly, S., Nightingale, P. and Fegan, C. (2003). Oral vitamin B12 can change our practice. *Postgrad. Med. J. 79*(930), 218–220.

Okazaki, K., Ohana, M., Oshima, C., Uchida, K., Nishi, T., Iwano, M., Fukui, T., Kawasaki, K., Matsuura, M., Asada, M., Tamaki, H., Hiai, H. and Chiba, T. (2003). Interaction of *Helicobacter pylori*-induced fol-

licular gastritis and autoimmune gastritis in BALB/c mice with post-thymectomy autoimmune gastritis. *J. Gastroenterol. 38*(12), 1131–1137.

Pedersen, P.L. and Carofoli, E. (1987). Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. *Trends Biochem. Sci. 12*, 146–150.

Penhale, W.J., Farmer, A. and Irvine, W.J. (1975). Thyroiditis in T-cell-depleted rats. Influence of strain, radiation dose, adjuvants and anti-lymphocyte serum. *Clin. Exp. Immunol. 21*(3), 362–375.

Pettitt, J.M., Humphris, D.C., Barrett, S.P., Toh B.H, van Driel, I.R. and Gleeson, P.A. (1995). Fast freeze-fixation/freeze-substitution reveals the secretory membranes of the gastric parietal cell as a network of helically coiled tubule. A new model for parietal cell transformation. *J. Cell. Sci. 108* (Pt 3), 1127–1141.

Pettitt, J.M., van Driel, I.R., Toh, B.H. and Gleeson, P.A. (1996). From coiled tubules to a secretory canaliculus: a new model for membrane transformation and acid secretion by gastric parietal cells. *Trends Cell. Biol. 6*(2), 49–53.

Rabon, E.C. and Reuben, M.A. (1990).The mechanism and structure of the gastric H,K ATPase. *Annu. Rev. Physiol. 52*, 321–344.

Rickes, E.L., Brink, N.G., Koniuszy, F.R., Wood, T.R. and Folkers, K. (1948). Crystalline Vitamin B12. *Science 107*(2781), 396–397.

Rodbro, P., Dige-Petersen, H., Schwartz, M. and Dalgaard, O.Z. (1967). Effect of steroids on gastric mucosal structure and function in pernicious anemia. *Acta Med. Scand. 181*(4), 445–452.

Rode, J., Dhillon, A.P., Papadaki, L., Stockbrugger, R., Thompson, R.J., Moss, E. and Cotton, P.B. (1986). Pernicious anaemia and mucosal endocrine cell proliferation of the non-antral stomach. *Gut 27*(7), 789–798.

Rose, M.S., Doniach, D., Chanarin, I., Brostoff, J. and Ardeman, S. (1970). Intrinsic-factor antibodies in absence of pernicious anaemia: 3–7 year follow-up. *Lancet 2*(7662), 9–12.

Rothenberg, S.P., Kantha, K.R. and Ficarra, A. (1971). Autoantibodies to intrinsic factor: their determination and clinical usefulness. *J. Lab. Clin. Med. 77*(3), 476–484.

Russell, J.S.R., Batten, F.E. and Collier, J. (1900). Subacute combined degeneration of the spinal cord. *Brain 23*, 39–110.

Sakaguchi, S. (2004). Naturally arising $CD4^+$ regulatory T-cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. and Toda, M. (1995). Immunologic self-tolerance maintained by activated T-cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol. 155*(3), 1151–1164.

Samloff, I.M., Kleinman, M.S., Turner, M.D., Sobel, M.V. and Jeffries, G.H. (1968). Blocking and binding antibodies to intrinsic factor and parietal cell antibody in pernicious anemia. *Gastroenterology 55*(5), 575–583.

Samloff, I.M., Varis, K., Ihamaki, T., Siurala, M. and Rotter, J.I. (1982). Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. *Gastroenterology 83*(1 Pt 2), 204–209.

Savage, D.G. and Lindenbaum, J. (1995). Neurological complications of acquired cobalamin deficiency: clinical aspects. *Baillière's Clin. Haematol. 8*(3), 657–678.

Scarff, K.J., Pettitt, J.M., Van Driel, I.R., Gleeson, P.A. and Toh, B.H. (1997). Immunization with gastric H+/K(+)-ATPase induces a reversible autoimmune gastritis. *Immunology 92*(1), 91–98.

Schade, S.G., Feick, P., Muckerheide, M. and Schilling, R.F. (1966). Occurrence in gastric juice of antibody to a complex of intrinsic factor and vitamin B12. *N. Engl. J. Med. 275*(10), 528–531.

Scheinecker, C., McHugh, R., Shevach, E.M. and Germain, R.N. (2002). Constitutive presentation of a natural tissue autoantigen exclusively by dendritic cells in the draining lymph node. *J. Exp. Med. 196*(8), 1079–1090.

Schwartz, M. (1958). Intrinsic-factor-inhibiting substance in serum of orally treated patients with pernicious anaemia. *Lancet 2*(7037), 61–62.

Serafini, U., Masala, C. and Pala, A.M. (1970). Studies on gastric autoimmunity. *Folia Allergol. (Roma) 17*(4), 433–434.

Sercarz, E.E. (2000). Driver clones and determinant spreading. *J. Autoimmun. 14*(4), 275–277.

Sharp, E.A. (1929). An antianemic factor in desicated stomach. *JAMA 93*(10), 749–751.

Silveira, P.A., Baxter, A.G., Cain, W.E. and van Driel, I.R. (1999). A major linkage region on distal chromosome 4 confers susceptibility to mouse autoimmune gastritis. *J. Immunol. 162*(9), 5106–5111.

Silveira, P.A., Wilson, W.E., Esteban, L.M., Jordan, M.A., Hawke, C.G., van Driel, I.R. and Baxter, A.G. (2001). Identification of the Gasa3 and Gasa4 autoimmune gastritis susceptibility genes using congenic mice and partitioned, segregative and interaction analyses. *Immunogenetics 53*(9), 741–750.

Sjoblom, S.M., Sipponen, P. and Jarvinen, H. (1993). Gastroscopic follow up of pernicious anaemia patients. *Gut 34*(1), 28–32.

Song, Y.H., Ma, J.Y., Mardh, S., Liu, T., Sjostrand, S.E., Rask, L., Borch, K., Huang, G.C., Barnett, P. and McGregor, A.M. (1994). Localization of a pernicious anaemia autoantibody epitope on the alpha-subunit of human H,K-adenosine triphosphatase. *Scand. J. Gastroenterol. 29*(2), 122–127.

Steptoe, R.J., Ritchie, J.M. and Harrison, L.C. (2003). Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes. *J. Clin. Invest. 111*(9), 1357–1363.

Strickland, R.G. and Mackay, I.R. (1973). A reappraisal of the nature and significance of chronic atrophic gastritis. *Am. J. Dig. Dis. 18*(5), 426–440.

Strickland, R.G., Fisher, J.M. and Taylor, K.B. (1969). Effect of prednisolone on gastric function and structure in man. *Gastroenterology 56*(4), 675–686.

Sturgis, C.C. and Isaacs, R. (1929). Desiccated stomach in the treatment of pernicious anemia. *JAMA 93*(10), 747–749.

Suerbaum, S. and Michetti, P. (2002). *Helicobacter pylori* infection. *N. Engl. J. Med. 347*(15), 1175–1186.

Tai, C. and McGuigan, J.E. (1969). Immunologic studies in pernicious anemia. *Blood 34*(1), 63–71.

Tanaka, N. and Glass, G.B.J. (1970). Effect of prolonged aministration of parietal cell antibodies from patients with atrophic gastritis and pernicious anemia on the parietal cell mass and hydrochloric acid output in rats. *Gastroenterology 58*(4), 482–494.

Taylor, K.B. (1959). Inhibition of intrinsic factor by pernicious anaemia sera. *Lancet 2*, 106–108.

Taylor, K.B. (1961). Chronic atrophic gastritis and pernicious anemia. *Gastroenterology 41*, 147–151.

Taylor, K.B. and Morton, J.A. (1959). An antibody to Castle's intrinsic factor. *J. Pathol. Bacteriol. 77*(1), 117–122.

Taylor, K.B., Roitt, I.M., Doniach, D., Couchman, K.G. and Shapland, C. (1962). Autoimmune phenomena in pernicious anaemia: gastric antibodies. *Br. Med. J. 5316*, 1347–1352.

Telford, J.L., Ghiara, P., Dell'Orco, M., Comanducci, M., Burroni, D., Bugnoli, M., Tecce, M.F., Censini, S., Covacci, A. and Xiang, Z. (1994). Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. *J. Exp. Med. 179*(5), 1653–1658.

Toh, B.H., Gleeson, P.A., Simpson, R.J., Moritz, R.L., Callaghan, J.M., Goldkorn, I., Jones, C.M., Martinelli, T.M., Mu. F.T. and Humphris, D.C. (1990). The 60- to 90-kDa parietal cell autoantigen associated with autoimmune gastritis is a beta subunit of the gastric H+/K(+)-ATPase (proton pump). *Proc. Natl Acad. Sci. USA 87*(16), 6418–6422.

Toh, B.H., van Driel, I.R. and Gleeson, P.A. (1992). Autoimmune gastritis: tolerance and autoimmunity to the gastric H+/K+ ATPase (proton pump). *Autoimmunity 13*(2), 165–172.

Toh, B.H., Gleeson, P.A. and van Driel, I.R. (1993). Autoimmunity: the paradigm of autoimmune gastritis. *Today's Life Sci. 5*, 18–27.

Toh, B.H., van Driel, I.R. and Gleeson, P.A. (1997). Pernicious anemia. *N. Engl. J. Med. 337*(20), 1441–1448.

Toh, B.H., Sentry, J.W. and Alderuccio, F. (2000). The causative H+/K+ ATPase antigen in the pathogenesis of autoimmune gastritis. *Immunol. Today 21*(7), 348–354.

Tudhope, G.R. and Wilson, G.M. (1960). Anaemia in hypothyroidism. Incidence, pathogenesis, and response to treatment. *Q. J. Med. 29*, 513–537.

Tung, K.S., Smith, S., Matzner, P., Kasai, K., Oliver, J., Feuchter, F. and Anderson, R.E. (1987). Murine autoimmune oophoritis, epididymoorchitis, and gastritis induced by day 3 thymectomy. Autoantibodies. *Am. J. Pathol. 126*(2), 303–314.

Twomey, J.J., Jordan, P.H., Jarrold, T., Trubowitz, S., Ritz, N.D. and Conn, H.O. (1969). The syndrome of immunoglobulin deficiency and pernicious anemia. A study of ten cases. *Am. J. Med. 47*(3), 340–350.

Twomey, J.J., Jordan, P.H., Laughter, A.H., Meuwissen, H.J. and Good, R.A. (1970). The gastric disorder in immunoglobulin-deficient patients. *Ann. Intern. Med. 72*(4), 499–504.

Uibo, R. (2005). Contribution of epidemiological studies to gastritis immunology. *Int. Rev. Immunol. 24*(1–2), 31–54.

Uibo, R., Krohn, K., Villako, K., Tammur, R. and Tamm, A. (1984). The relationship of parietal cell, gastrin cell, and thyroid autoantibodies to the state of the gastric mucosa in a population sample. *Scand. J. Gastroenterol. 19*(8), 1075–1080.

Ungar, B., Whittingham, S. and Francis, C.M. (1967). Pernicious anaemia: incidence and significance of circulating antibodies to intrinsic factor and to parietal cells. *Australas. Ann. Med. 16*(3), 226–229.

Ungar, B., Stocks, A.E., Martin, F.I., Whittingham, S. and Mackay, I.R. (1968). Intrinsic-factor antibody, parietal-cell antibody, and latent pernicious anaemia in diabetes mellitus. *Lancet 2*(7565), 415–417.

Ungar, B., Francis, C.M. and Cowling, D.C. (1976). Antibody to parietal cells in patients with duodenal ulcer, and relationship to pernicious anaemia. *Med. J. Aust. 2*(24), 900–902.

Ungar, B., Mathews, J.D., Tait, B.D. and Cowling, D.C. (1977). HLA patterns in pernicious anaemia. *Br. Med. J. 1*(6064), 798–800.

Ungar, B., Mathews, J.D., Tait, B.D. and Cowling, D.C. (1981). HLA-DR patterns in pernicious anaemia. *Br. Med. J. (Clin. Res. Ed.) 282*(6266), 768–770.

van Driel, I.R. and Callaghan, J.M. (1995). Proton and potassium transport by H+/K(+)-ATPases. *Clin. Exp. Pharmacol. Physiol. 22*(12), 952–960.

Vargas, J.A., Alvarez-Mon, M., Manzano, L., Albillos, A., Fernandez-Corugedo, A., Albarran, F. and Durantez, A. (1995). Functional defect of T-cells in autoimmune gastritis. *Gut 36*(2), 171–175.

Varis, K. (1981). Family of behaviour of chronic gastritis. *Ann. Clin. Res. 13*(3), 123–129.

Varis, K., Samloff, I.M., Ihamaki, T. and Siurala, M. (1979). An appraisal of tests for severe atrophic gastritis in relatives of patients with pernicious anemia. *Dig. Dis. Sci. 24*(3), 187–191.

Vernanzi, E.S., Benoist, C. and Mathis, D. (2004). Good riddance: thymocyte clonal deletion prevents autoimmunity. *Curr. Opin. Immunol. 16*(2), 197–202.

Walker, I.R., Strickland, R.G., Ungar, B. and Mackay, I.R. (1971). Simple atrophic gastritis and gastric carcinoma. *Gut 12*(11), 906–911.

Wall, A.J., Whittingham, S., Mackay, I.R. and Ungar, B. (1968). Prednisolone and gastric atrophy. *Clin. Exp. Immunol. 3*(4), 359–366.

Wangel, A.G., Callender, S.T., Spray, G.H. and Wright, R. (1968). A family study of pernicious anaemia. I. Autoantibodies, achlorhydria, serum pepsinogen and vitamin B12. *Br. J. Haematol. 14*(2), 161–181.

Whittingham, S., Ungar, B., Mackay, I.R. and Mathews, J.D. (1969). The genetic factor in pernicious anaemia. A family study in patients with gastritis. *Lancet 1*(7602), 951–954.

Whittingham, S., Youngchaiyud, U., Mackay, I.R., Buckley, J.D. and Morris, P.J. (1975). Thyrogastric autoimmune disease. Studies on the cell-mediated immune system and histocompatibility antigens. *Clin. Exp. Immunol. 19*(2), 289–299.

Whittingham, S., Mackay, I.R. and Tait, B.D. *Immunogenetics of Autoimmune Diseases*. Boca Raton, FL: CRC Press.

Wilkinson, J.F. (1949). Megalocytic anaemia's (Oliver-Sharpey Lecture). *Lancet 1*, 249–255.

Wood, I.J., Doig, R.K., Motteram, R. and Hughes, A. (1949). Gastric biopsy; report on 55 biopsies using new flexible gastric biopsy tubes. *Lancet 1*, 18–21.

CHAPTER

40

Hypophysitis

PATRIZIO CATUREGLI AND SHEY-CHERNG TZOU
The Johns Hopkins University, Department of Pathology, Baltimore, MD

DEFINITION AND CLASSIFICATION

Hypophysitis is an inflammation of the pituitary gland that originates directly within the pituitary (primary forms) or from neighboring or systemic diseases (secondary forms). Primary hypophysitis comprises three main histologic types: granulomatous, xanthomatous, and lymphocytic.

Granulomatous hypophysitis was first described in 1917 by Simmonds (Simmonds, 1917). He reviewed 2000 pituitary glands at autopsy and found four cases not related to tuberculosis or syphilis, characterized by diffuse collections of multinucleated giant cells and histiocytes, with surrounding lymphocytes and plasma cells. The first antemortem patient was reported in 1980 (Taylon and Duff, 1980). The disease is rare, affects males and females equally, and can occur together with lymphocytic hypophysitis (McKeel, 1983; Miyamoto et al., 1988; Madsen and Karluk, 2000). For this reason, McKeel suggested that the two diseases are part of the same autoimmune spectrum, with a purely lymphocytic form constituting the predominant early lesion and a granulomatous component appearing later (McKeel, 1984). Different histologic appearance from other classic organ-specific autoimmune diseases and different epidemiologic data (lack of female bias and association with other autoimmune diseases) suggest, however, that lymphocytic and granulomatous hypophysitis are two distinct diseases.

Xanthomatous hypophysitis was originally described in three women in 1998 (Folkerth et al., 1998) and in six other patients thereafter (Burt et al., 2003). The pituitary is infiltrated by foamy histiocytes, small lymphocytes, and cyst-like areas of liquification. The rarity of this disease has hampered the understanding of its pathogenesis, natural history, and prognosis. Similar concepts apply to necrotizing hypophysitis, which has been demonstrated histologically only once in two patients (Ahmed et al., 1993) and suspected radiologically in another (Ogawa, 1995). Histologically, the pituitary appears destroyed by diffuse necrosis with surrounding lymphocytes, plasma cells, and a few eosinophils. It is unclear whether this represents a fourth histologic type or a variant of the other three.

Lymphocytic (autoimmune) hypophysitis is the most frequent among the primary hypophysitides (Caturegli et al., 2005). It is known in the literature by several names. Originally it was called "lymphocytic adenohypophysitis" (or just lymphocytic hypophysitis) because the infiltration, which was mainly lymphocytic, and the symptoms were thought to be limited to the anterior hypophysis. It was then realized that the autoimmune attack could also and exclusively involve the infundibular stem and the posterior lobe, and so the term "lymphocytic infundibulo-neurohypophysitis" was created. Finally, it was realized that both the adenohypophysis and the infundibulo-neurohypophysis could be affected; hence the term "lymphocytic panhy-

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

pophysitis" arose. We will refer to the disease as autoimmune hypophysitis, followed by the specific anatomic location when known.

HISTORICAL BACKGROUND

Autoimmune hypophysitis of the anterior lobe (lymphocytic adenohypophysitis, LAH) was first described in 1962 by Goudie and Pinkerton in Glasgow, UK (Goudie and Pinkerton, 1962). The authors reported a 22-year-old woman who died 14 months after her second delivery, probably because of adrenal insufficiency. Two months before admission, the patient felt increasingly tired and noticed a neck enlargement; she then developed severe lower abdominal pain, radiating to the right iliac fossa, associated with vomiting and diarrhea. She was brought to the operating room for suspected appendicitis. Surgery revealed an acutely inflamed, gangrenous appendix that, however, had not ruptured. The appendix was removed but 8 hours later the patient went into peripheral circulatory shock and died. The autopsy revealed a firm, enlarged thyroid gland infiltrated by lymphocytes, atrophic adrenal glands, and a small pituitary. Surprisingly for that time, the adenohypophysis was extensively infiltrated by lymphocytes and few plasma cells, aggregating in some areas to form lymphoid follicles. The neurohypophysis was normal. Noting the presence of Hashimoto's thyroiditis, a more extensively characterized autoimmune disease, the authors concluded their discussion by writing: "It seems reasonable to assume that the coexistence of Hashimoto's disease and the mononuclear cell infiltration of the anterior pituitary is not fortuitous. Both may be explained by the onset of autoimmune reaction to thyroid and pituitary antigens released during the puerperal involution of these glands." There is no doubt that Goudie and Pinkerton were the first to postulate the autoimmune nature of this condition, at a time (1962) when the field of autoimmunity had just begun. Earlier cases, however, are probably hidden in hospital archives or published without recognition of the disease (Duff and Bernstein, 1933; Rupp and Paschkis, 1953).

Autoimmune hypophysitis of the posterior lobe and infundibular stem (lymphocytic infundibulo-neurohypophysitis, LINH) was first described in 1970 by Saito et al. in Tokyo. They reported a 66-year-old asthmatic woman with a 1-month history of severe dehydration that responded strikingly to the administration of pitressin. Two months after discharge, however, she developed a severe attack of bronchial asthma and died. Autopsy revealed a marked infiltration of the neurohypophysis and the infundibular stem with lymphocytes and plasma cells, aggregating in some areas in lymphoid follicles. The adenohypophysis was normal, except for vacuolar degeneration of the basophilic cells, likely due to the prolonged use of steroids for asthma.

Autoimmune hypophysitis involving both the anterior and posterior lobe (lymphocytic panhypophysitis, LPH) was first described histologically in 1991 by Nussbaum et al. in New York. Their first case was a 40-year-old male with a 3-month history of headache, impotence, polyuria, and polydipsia. Transsphenoidal surgery showed that the sella turcica was filled with whitish, fibrous tissue that was almost completely removed. Histology revealed extensive infiltration of the adenohypophysis and the neurohypophysis by lymphocytes, plasma cells, and histiocytes.

CLINICAL AND EPIDEMIOLOGIC FEATURES

Very limited data exist on which to estimate the incidence of autoimmune hypophysitis. Sautner and Fehn analyzed 2500 surgical pituitary specimens collected at Hamburg, Germany, from 1970 to 1996 and found six cases (0.24%) (Sautner et al., 1995; Fehn et al., 1998). Honegger et al. analyzed 2362 specimens collected from 1982 to 1995 in Erlagen, Germany, and found seven cases (0.3%) (Honegger et al., 1997). Leung et al reported in Charlottesville, Virginia, 13 cases of autoimmune hypophysitis among 2000 patients who underwent transsphenoidal surgery for pituitary mass lesions from 1992 to 2003 (0.65%) (Leung et al., 2004). Buxton and Robertson analyzed 619 consecutive pituitary surgeries performed over 15 years at Nottingham, UK, and found five cases (0.8%) (Buxton and Robertson, 2001). Considering that their hospital served a population of approximately 3 million and that all surgery for pituitary masses was dealt with there, the yearly incidence in Nottingham can be estimated to be one case in every 10 million people. Thus, using the United States as an example, 28 new cases of autoimmune hypophysitis can be expected every year. This incidence may well be an underestimate of today's incidence, also considering that some autoimmune hypophysitis cases may go undiagnosed because of their indolent, subclinical course.

A total of 379 cases of autoimmune hypophysitis were reported in the literature from 1962 to 2004 (Caturegli et al., 2005). The incidence appears to have increased over time. In the first 20 years (1962–1981), in fact, only 16 cases were reported (11 at autopsy and five from surgical pathology specimens). In the next 20 years (1982–2001), 290 appeared in the literature, and 73 cases have been reported just in 2002–04. This exponential increase likely reflects an ascertainment bias, due to the widespread introduction of non-invasive imaging techniques of the sella turcica (mainly MRI), transsphenoidal surgery and also increased awareness in the medical community.

LAH is more common in women (F : M ratio of 6), who present at a younger age (35 ± 13) than men (43 ± 12). In a significant percentage of women (57%), LAH manifests

during pregnancy or post partum. Figure 40.1 shows the distribution of symptom appearance centered at delivery: most women present in the third trimester or early post partum. A history of previous pregnancies does not increase the risk of developing LAH in subsequent pregnancies. This striking temporal association is one of the most interesting features of autoimmune hypophysitis and, at the moment, remains unexplainable.

LINH affects males and females equally and is not associated with pregnancy. LPH is slightly more common in women (F : M ratio of 1.9), but does not show association with pregnancy.

The clinical presentation of autoimmune hypophysitis includes four categories of symptoms. Symptoms of local compression are the most common and usually the first complaint. They include headache and visual disturbances (either defects in the visual field or decreased acuity; more rarely, diplopia). Compression symptoms originate because a pituitary infiltrated by immune cells initially enlarges, expanding usually upward to stretch the dura mater and the optic chiasm. Next are symptoms due to a partial or complete deficit of the anterior pituitary function, more commonly ACTH deficiencies. These defects are considered the direct result of the autoimmune attack on the pituitary acinar cells. Next are symptoms due to deficit of the posterior pituitary (diabetes insipidus), which can be attributed either to direct immune destruction or to compression of the posterior lobe and infundibular stem. We favor the first mechanism considering that diabetes insipidus is very rare in pituitary adenomas. Last are the signs due to hyperprolactinemia (mainly amenorrhea/oligomenorrhea and galactorrhea), which occur in about a quarter of the patients. These clinical features of autoimmune hypophysitis are indistinguishable from those of any other expanding mass located in the sella turcica.

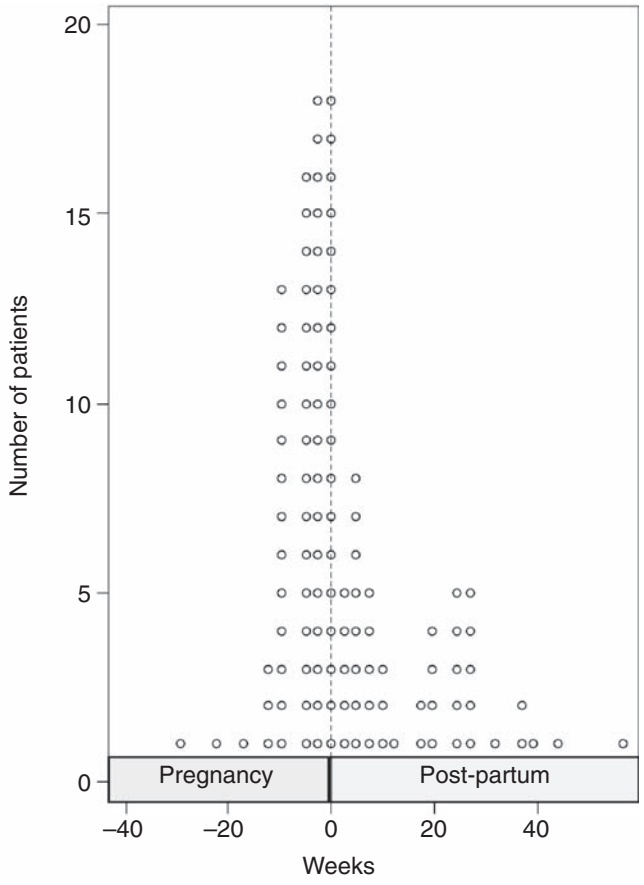


FIGURE 40.1 Distribution of symptom appearance in autoimmune hypophysitis in relation to delivery (indicated as week 0).

PATHOLOGIC FEATURES

The defining histologic feature of autoimmune hypophysitis is the diffuse infiltration of the pituitary gland with lymphocytes that sometimes aggregate to form true lymphoid follicles, often with germinal centers. Immunohistochemistry reveals a mixture of T and B lymphocytes (Gutenberg et al., 2005), without a dominant subset, as frequently seen in other autoimmune diseases. Other cell types are also present in the infiltrate and may play a dominant role in immunopathology. Plasma cells are frequently seen (53% of the cases) but also eosinophils (12%), macrophages, histiocytes and neutrophils (6%) and, more recently, mast cells (Vidal et al., 2002). Fibrosis is common (46%) and often severe, explaining the toughness and adherence the surgeon finds upon entering the sella turcica. Necrosis is rare (3%) and usually of modest and focal nature. Little is known of the mechanisms by which the infiltrate causes loss of function/destruction of the endocrine cells or impairment of vasopressin release. Little is also known about the natural history of autoimmune hypophysitis. It is believed that the initial phases are characterized by swelling and edema of the pituitary, which then later becomes small and fibrotic.

AUTOIMMUNE FEATURES

That autoimmune hypophysitis is truly an autoimmune disease is indicated by animal models (indirect evidence) and by circumstantial evidence (Rose and Bona, 1993). No case of autoimmune hypophysitis transmission from mother to newborn (direct evidence) has been reported, suggesting that pituitary antibodies described thus far in this disease are not pathogenic. Circumstantial evidence of autoimmunity derives from the association between autoimmune hypophysitis and other diseases of known autoimmune nature, such as Hashimoto's thyroiditis, Addison disease and systemic lupus erythematosus, and by the improvement of

symptoms upon usage of immunosuppressive drugs such as glucocorticoids, methotrexate, and azathioprine.

The autoantigen recognized by the autoimmune attack awaits identification. Kobayashi's laboratory first described sera from patients with pituitary disorders that, when reacted with pituitary cytosolic extracts, recognized a 22 kDa protein (Yabe et al., 1995; Kikuchi et al., 2000), later identified by Takao et al. (2001) as growth hormone. Crock's laboratory later reported that seven of 10 patients with biopsy-proven lymphocytic hypophysitis and 12 of 22 patients with suspected hypophysitis had a low titer antibody that recognized a 49 kDa cytosolic pituitary protein (Crock, 1998), subsequently identified as α-enolase (O'Dwyer et al., 2002b). The authors concluded that α-enolase is the autoantigen targeted by the immune system in autoimmune hypophysitis which, considering its coexpression in the placenta, explains the strong association between autoimmune hypophysitis and pregnancy (O'Dwyer et al., 2002a). Antibodies recognizing α-enolase, however, have been reported in many other diseases, ranging from endometriosis to discoid lupus and Wegener's granulomatosis (Crock, 1998). In addition, Tanaka et al. (2003) showed that the antibody was present in seven of 17 patients (41%) with autoimmune hypophysitis, but similarly in six of 13 (46%) patients with non-functioning pituitary adenoma, and in four of 17 (24%) patients with other pituitary diseases, making the α-enolase antibody inadequate as a diagnostic marker of autoimmune hypophysitis. Finally, Nishiki et al. (2001) reported that five of 13 patients with LAH and one of 12 patients with LINH had antibodies that recognized 68, 49, and 43 kDa proteins in pituitary membrane extracts.

GENETIC AND ENVIRONMENTAL INFLUENCES

Insufficient data are available to establish associations between the genes that are classically thought to influence autoimmunity, such as the MHC locus and CTLA-4, and autoimmune hypophysitis. Also no environmental agent has been involved, with the exception of four cases presenting after a viral infection of the meningi (Vanneste and Kamphorst, 1987; Honegger et al., 1997; Sandler et al., 1998; Matta et al., 2002).

ANIMAL MODELS

Only a few papers have been dedicated to the establishment of animal models of autoimmune hypophysitis. In 1964 Beutner and Witebsky immunized 16 rabbits up to five times with rabbit anterior pituitary extracts, emulsified in complete Freund's adjuvant. They were able to induce specific antibody responses but no pituitary pathology (Beutner et al., 1964). In 1967 Levine reported the first successful model of experimental autoimmune hypophysitis by immunizing rats with a single intracutaneous injection of rat pituitary tissue, emulsified in complete Freund's adjuvant. Two to three weeks after the injection, in six of the 14 rats (43%) the adenohypophysis showed focal and diffuse infiltration with mononuclear cells, mainly lymphocytes, monocytes, and occasional epithelioid cells. A few posterior and intermediate lobes had minimal inflammation. Disease incidence could be increased to 75% (15/20 rats) by addition of a second immunologic adjuvant, pertussis toxin. Levine subsequently showed that pituitary extracts from guinea pig were the most potent inducer of experimental autoimmune hypophysitis (6/6 rat recipients), whereas human and cow extracts were poorly effective, and dog and rabbit extracts not effective at all (Levine, 1969). In 1970 Beck and Melvin induced experimental autoimmune hypophysitis in one rhesus monkey by injecting her multiple times, over the course of 3 years, with human placental extracts and human chorionic gonadotropin, both emulsified with Freund's adjuvant. Histology showed infiltration of the adenohypophysis with lymphocytes and scattered plasma cells; the neurohypophysis was normal.

In 1982 Klein induced lymphoplasmacytic infiltration of the anterior pituitary by injecting 12 rabbits (seven cases and five controls) three times, at 2-week intervals, with rabbit pituitary tissue, emulsified in complete Freund's adjuvant. Eight weeks (10 rabbits) or 16 weeks (two rabbits) after the first injection, five of the seven experimental rabbits showed focal infiltration of the adenohypophysis with lymphocytes, some plasma cells, and a few eosinophils and fibrosis. None of the five controls showed histologic abnormalities. In 1992, Yoon et al. immunized over 100 hamsters by injecting intradermally three times, at 1-week intervals, recombinant rubella virus E1 and E2 glycoproteins. Three weeks after the first injection, specific antibodies against the adenohypophysis were found in 95% of the hamsters. Eleven weeks after the first injection, a diffuse lymphocytic infiltration occurred throughout the adenohypophysis. None of the hamsters that had received the control protein (nonglycosylated rubella nucleoprotein C) developed such lesions. The disease could be prevented by neonatal thymectomy and could not be produced by passive transfer of the autoantibodies, thus indicating that T cells are critical for disease induction and that antibodies are more important as markers of disease, rather than as a pathogenic player (Yoon et al., 1992). Finally, Watanabe et al. in 2001 immunized 12 Lewis rats twice, at 1-week intervals, with rat pituitary extract emulsified in complete Freund's adjuvant. Three (N = 6) or 6 weeks (N = 6) after the first immunization, rats showed minimal lymphocytic infiltration in the adenohypophysis and developed antibodies directed against growth hormone, thyroid-stimulating hormone and luteinizing hormone (Watanabe et al., 2001). It is unclear, however, whether these hormones represent the initiating autoantigens or are rather

the natural response of the immune system to the injection of hormone-rich pituitary extracts.

A somewhat different experimental approach has been recently published by Stockinger's laboratory (De Jersey et al., 2002; 2004). The authors have designed a transgenic mouse that expresses specifically in the anterior pituitary (because under transcriptional control of the growth hormone promoter) a nucleoprotein from the influenza virus, thus a foreign antigen. This influenza nucleoprotein contains a peptide that binds to the D^b allele of the mouse MHC locus and is recognized by specific $CD8^+$ T cells. When the authors crossed the nucleoprotein transgenic mice to TCR transgenic mice that have $CD8^+$ T cells specific for that nucleoprotein, they observed growth hormone defect due to destruction of the growth hormone cells expressing the viral antigen. Although informative about accessibility and homing of $CD8^+$ T lymphocytes to the pituitary, this transgenic model is likely remote from the human disease where $CD8^+$ T lymphocytes are rarely seen in the infiltrate and somatotrophs are usually spared by the autoimmune attack.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

The main diagnostic issue is the differentiation between the rare autoimmune hypophysitis and the overwhelmingly more common pituitary tumors (mainly adenomas). Of all pituitary expanding lesions, 92% are tumors; the remaining 8% are nontumorous and include cysts, hyperplasia, vascular lesions, bony lesions, primary and secondary hypophysitides (Saeger, 2003). All expanding lesions located in the sella turcica have similar clinical findings, so the distinction among them can be achieved with certainty only by examining under the microscope the pituitary tissue obtained from surgical procedures. Surgery, however, is invasive and costly and can injure the patient. For this reason, authors have struggled to define criteria more suggestive of autoimmune hypophysitis. These include clinical hints, endocrinologic testing, imaging studies, and immunologic markers.

Clinical suspicion of autoimmune hypophysitis should be raised if symptoms appear in striking temporal relationship with pregnancy and post partum, if the degree of hypopituitarism (partial or complete) appears disproportionate to the size of the radiologically evidenced pituitary mass (Guay et al., 1987), and if the hypopituitarism involves mainly the adrenal and thyroidal axis, rather than growth hormone and gonadotropins. Clinical criteria, however, have extremely low predictive values.

Endocrine evaluation of anterior pituitary hormones shows that corticotrophs are the most frequently impaired cells (59% of patients), followed by gonadotrophs, somatotrophs, and thyrotrophs. Based on these data and on the notion that the deficiency of ACTH can be observed in isolation (Jensen et al., 1986; Escobar-Morreale et al., 1994), some authors consider it the earliest functional alteration in autoimmune hypophysitis. It is important to note, however, that isolated ACTH deficiency can be observed in the absence of autoimmune hypophysitis (Sauter et al., 1990; Nagai et al., 1997) and that isolated deficiencies of other pituitary hormones have been described in autoimmune hypophysitis. The greater frequency of ACTH defects may simply represent a selection bias, considering that these patients may come to medical attention more promptly than patients with other tropin deficiencies.

Imaging studies are currently the most powerful tool to differentiate autoimmune hypophysitis from pituitary tumors, although this distinction is not always possible (Caturegli et al., 2005). Computed tomography was used in the first patient diagnosed ante mortem (Mayfield et al., 1980), and exclusively until 1988. Since then, MRI has become the most commonly used imaging study. In patients with LAH, MRI shows a symmetrical enlargement of the pituitary, with symmetrical suprasellar extension; the stalk may be thickened but not deviated. In pituitary adenomas, in contrast, the mass and the suprasellar extension are asymmetric and the stalk is deviated contralaterally. After injection of the contrast medium (gadolinium), the enhancement in LAH is intense and homogeneous and creates strips of abnormal enhancing tissue adjacent to the enlarged pituitary (the so-called "dura tail"), whereas in adenomas the enhancement is slight, delayed, inhomogeneous, and without a dura tail. In patients with LINH, MRI shows a thickened stalk and a swollen posterior lobe that has lost its normal hyperintense signal, observed after contrast enhancement.

Antibodies against pituitary antigens were measured in 83 of 379 patients, mainly by indirect immunofluorescence (Bottazzo et al., 1975) or Western blotting (Crock et al., 1993). They were found positive in a minority of patients, yielding an extremely poor sensitivity (37%) (Caturegli et al., 2005). They are also not specific for autoimmune hypophysitis, considering that they have been described in type 1 diabetes (Mirakian et al., 1982), Hashimoto's thyroiditis (Kobayashi et al., 1988), Graves' disease (Hansen et al., 1989), and normal women post partum (Engelberth and Jezkova, 1965). This lack of sensitivity and specificity may have several explanations. Patients with autoimmune diseases come to medical attention long after the onset of disease and it is known that antibodies against endocrine glands disappear over the years. In addition, the autoantigen/s targeted by autoimmune attack in autoimmune hypophysitis is/are still unknown.

TREATMENT AND OUTCOME

The treatment of autoimmune hypophysitis is, at the moment, only symptomatic. It includes reduction of the pituitary mass and replacement of the defective hormones. Mass

reduction treatment comprises surgery, lympholytic drugs, and radiotherapy. Surgery has been the most commonly used form of treatment thus far and in some cases is the only means to control rapidly increasing serious defects of the visual pathway. Surgery is usually performed using the transsphenoidal approach, more rarely by craniotomy. If a diagnosis of autoimmune hypophysitis is suspected and there are no urgent compressive symptoms, the best management is to reduce the pituitary mass with glucocorticoids, monitoring the patient clinically and radiologically (Caturegli et al., 2005).

The majority of patients (72%) require long-term replacement with one or more pituitary hormones. More rarely, autoimmune hypophysitis resolves after mass reduction treatment without need of hormone replacement (16%) or spontaneously (4%). In the remaining 8% of patients, autoimmune hypophysitis has resulted in death, considered secondary to irreversible adrenal insufficiency. Deaths have occurred almost constantly through the years (11 deaths from 1962 to 1982 and 14 deaths from 1983 to 2003), to remind us that autoimmune hypophysitis can be fatal if unrecognized.

CONCLUDING REMARKS— FUTURE PERSPECTIVES

Autoimmune hypophysitis is rare but increasingly recognized. It offers a fascinating model to study the effect of pregnancy on autoimmune diseases and the interactions between nervous, endocrine, and immune systems. Patient management will be improved when a reliable serologic test that identifies the autoimmune nature of the pituitary disease becomes available. Much remains to be learned about the natural history of autoimmune hypophysitis. Establishment of experimental models in mice and of a disease registry in humans will greatly advance the field.

References

Ahmed, S.R., Aiello, D.P., Page, R., Hopper, K., Towfighi, J. and Santen, R.J. (1993). Necrotizing infundibulo-hypophysitis: a unique syndrome of diabetes insipidus and hypopituitarism. *J. Clin. Endocrinol. Metab. 76*, 1499–1504.

Beutner, E.H., Djanian, A. and Witebsky, E. (1964). Serological studies on rabbit antibodies to the rabbit anterior pituitary. *Immunology 7*, 172–181.

Bottazzo, G.F., Pouplard, A., Florin-Christensen, A. and Doniach, D. (1975). Autoantibodies to prolactin-secreting cells of human pituitary. *Lancet 2*, 97–101.

Burt, M.G., Morey, A.L., Turner, J.J., Pell, M., Sheehy, J.P. and Ho, K.K. (2003). Xanthomatous pituitary lesions: a report of two cases and review of the literature. *Pituitary 6*, 161–168.

Buxton, N. and Robertson, I. (2001). Lymphocytic and granulocytic hypophysitis: a single centre experience. *Br. J. Neurosurg. 15*, 242–245, discussion 245–246.

Caturegli, P., Newschaffer, C., Olivi, A., Pomper, M.G., Burger, P.C. and Rose, N.R. (2005). Autoimmune hypophysitis. *Endocr. Rev. 26*, 599–614.

Crock, P.A. (1998). Cytosolic autoantigens in lymphocytic hypophysitis. *J. Clin. Endocrinol. Metab. 83*, 609–618.

Crock, P.A., Salvi, M., Miller, A., Wall, J. and Guyda, H. (1993). Detection of anti-pituitary antibodies by immunoblotting. *J. Immunol. Methods 162*, 31–40.

De Jersey, J., Carmignac, D., Barthlott, T., Robinson, I. and Stockinger, B. (2002). Activation of CD8 T cells by antigen expressed in the pituitary gland. *J. Immunol. 169*, 6753–6759.

De Jersey, J., Carmignac, D., Le Tissier, P., Barthlott, T., Robinson, I. and Stockinger, B. (2004). Factors affecting the susceptibility of the mouse pituitary gland to CD8 T-cell-mediated autoimmunity. *Immunology 111*, 254–261.

Duff, G. L. and Bernstein, C. (1933). Five cases of Addison's disease with so-called atrophy of the adrenal gland. *Bull. Johns Hopkins Hosp. 52*, 67–83.

Engelberth, O. and Jezkova, Z. (1965). Autoantibodies in Sheehan's syndrome. *Lancet 1*, 1075.

Escobar-Morreale, H., Serrano-Gotarredona, J. and Varela, C. (1994). Isolated adrenocorticotropic hormone deficiency due to probable lymphocytic hypophysitis in a man. *J. Endocrinol. Invest. 17*, 127–131.

Fehn, M., Sommer, C., Ludecke, D.K. and Saeger, W. (1998). Lymphocytic hypophysitis: light and electron microscopic findings and correlation to clinical appearance. *Endocr. Pathol. 9*, 71–78.

Folkerth, R.D., Price, D.L. Jr., Schwartz, M., Black, P.M. and De Girolami, U. (1998). Xanthomatous hypophysitis. *Am. J. Surg. Pathol. 22*, 736–741.

Goudie, R.B. and Pinkerton, P.H. (1962). Anterior hypophysitis and Hashimoto's disease in a woman. *J. Pathol. Bacteriol. 83*, 584–585.

Guay, A.T., Agnello, V., Tronic, B.C., Gresham, D.G. and Freidberg, S.R. (1987). Lymphocytic hypophysitis in a man. *J. Clin. Endocrinol. Metab. 64*, 631–634.

Gutenberg, A., Buslei, R., Fahlbusch, R., Buchfelder, M. and Bruck, W. (2005). Immunopathology of primary hypophysitis: implications for pathogenesis. *Am. J. Surg. Pathol. 29*, 329–338.

Hansen, B.L., Hegedus, L., Hansen, G.N., Hagen, C., Hansen, J.M. and Hoier-Madsen, M. (1989). Pituitary-cell autoantibody diversity in sera from patients with untreated Graves' disease. *Autoimmunity 5*, 49–57.

Honegger, J., Fahlbusch, R., Bornemann, A., Hensen, J., Buchfelder, M., Muller, M. and Nomikos, P. (1997). Lymphocytic and granulomatous hypophysitis: experience with nine cases. *Neurosurgery 40*, 713–722.

Jensen, M.D., Handwerger, B.S., Scheithauer, B.W., Carpenter, P.C., Mirakian, R. and Banks, P.M. (1986). Lymphocytic hypophysitis with isolated corticotropin deficiency. *Ann. Intern. Med. 105*, 200–203.

Kikuchi, T., Yabe, S., Kanda, T. and Kobayashi, I. (2000). Antipituitary antibodies as pathogenetic factors in patients with pituitary disorders. *Endocr. J. 47*, 407–416.

Kobayashi, I., Inukai, T., Takahashi, M., Ishii, A., Ohshima, K., Mori, M., Shimomura, Y., Kobayashi, S., Hashimoto, A. and Sugiura, M. (1988). Anterior pituitary cell antibodies detected in Hashimoto's thyroiditis and Graves' disease. *Endocrinol. Jpn 35*, 705–708.

Leung, G.K., Lopes, M.B., Thorner, M.O., Vance, M.L. and Laws, E.R. Jr. (2004). Primary hypophysitis: a single-center experience in 16 cases. *J. Neurosurg. 101*, 262–271.

Levine, S.(1967). Allergic adenophypophysitis: new experimental disease of the pituitary gland. *Science 158*, 1190–1191.

Levine, S. (1969). Allergic adrenalitis and adenohypophysitis: further observations on production and passive transfer. *Endocrinology 84*, 469–475.

Madsen, J.R. and Karluk, D. (2000). Case records of the Massachusetts General Hospital, case 34–2000: a 71-year-old woman with an enlarging pituitary mass. *N. Engl. J. Med. 343*, 1399–1406.

Matta, M.P., Kany, M., Delisle, M.B., Lagarrigue, J. and Caron, P.H. (2002). A relapsing remitting lymphocytic hypophysitis. *Pituitary 5*, 37–44.

Mayfield, R.K., Levine, J.H., Gordon, L., Powers, J., Galbraith, R.M. and Rawe, S.E. (1980). Lymphoid adenohypophysitis presenting as a pituitary tumor. *Am. J. Med. 69*, 129–133.

McKeel, D.W. (1983). Common histopathological and ultrastructural features in granulomatous and lymphoid adenohypophysitis. *Endocrinology 112* (Suppl), 190.

McKeel, D.W. (1984). Primary hypothyroidism and hypopituitarism in a young woman: pathological discussion. *Am. J. Med. 77*, 326–329.

Mirakian, R., Cudworth, A.G., Bottazzo, G.F., Richardson, C.A. and Doniach, D. (1982). Autoimmunity to anterior pituitary cells and the pathogenesis of insulin-dependent diabetes mellitus. *Lancet 1*, 755–759.

Miyamoto, M., Sugawa, H., Mori, T., Hashimoto, N. and Imura, H. (1988). A case of hypopituitarism due to granulomatous and lymphocytic adenohypophysitis with minimal pituitary enlargement: a possible variant of lymphocytic adenohypophysitis. *Endocrinol. Jpn 35*, 607–616.

Nagai, Y., Ieki, Y., Ohsawa, K. and Kobayashi, K. (1997). Simultaneously found transient hypothyroidism due to Hashimoto's thyroiditis, autoimmune hepatitis and isolated ACTH deficiency after cessation of glucocorticoid administration. *Endocr. J. 44*, 453–458.

Nishiki, M., Murakami, Y., Ozawa, Y. and Kato, Y. (2001). Serum antibodies to human pituitary membrane antigens in patients with autoimmune lymphocytic hypophysitis and infundibuloneurohypophysitis. *Clin. Endocrinol. (Oxf.) 54*, 327–333.

O'Dwyer, D.T., Clifton, V., Hall, A., Smith, R., Robinson, P.J. and Crock, P.A. (2002a). Pituitary autoantibodies in lymphocytic hypophysitis target both gamma- and alpha-enolase: a link with pregnancy? *Arch. Physiol. Biochem. 110*, 94–98.

O'Dwyer, D.T., Smith, A.I., Matthew, M.L., Andronicos, N.M., Ranson, M., Robinson, P.J. and Crock, P.A. (2002b). Identification of the 49-kDa autoantigen associated with lymphocytic hypophysitis as alpha-enolase. *J. Clin. Endocrinol. Metab. 87*, 752–757.

Ogawa, R. (1995). A child with necrotizing infundibulo-neurohypophysitis. *Hormone to Rinsho 43*, 33–36.

Rose, N.R. and Bona, C. (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today 14*, 426–430.

Rupp, J.J. and Paschkis, K.E. (1953). Panhypopituitarism and hypocalcemic tetany in a male: case presentation. *Ann. Intern. Med. 39*, 1103–1106.

Saeger, W. (2003). [Space occupying processes of the sellar region with emphasis on tumor-like lesions.] *Pathologe 24*, 247–254.

Sandler, R., Danks, K.R., Hennigan, S.H., Hui, A.N. and McElroy, K.C. (1998). The widening spectrum of lymphocytic hypophysitis. *J. Ark. Med. Soc. 95*, 197–200.

Sauter, N.P., Toni, R., McLaughlin, C.D., Dyess, E.M., Kritzman, J. and Lechan, R.M. (1990). Isolated adrenocorticotropin deficiency associated with an autoantibody to a corticotroph antigen that is not adrenocorticotropin or other proopiomelanocortin-derived peptides. *J. Clin. Endocrinol. Metab. 70*, 1391–1397.

Sautner, D., Saeger, W., Ludecke, D.K., Jansen, V. and Puchner, M.J. (1995). Hypophysitis in surgical and autoptical specimens. *Acta Neuropathol. 90*, 637–644.

Simmonds, M. (1917). Über das Vorkommen von Riesenzelle in der Hypophyse. *Virchows Arch. 223*, 281–290.

Takao, T., Nanamiya, W., Matsumoto, R., Asaba, K., Okabayashi, T. and Hashimoto, K. (2001). Antipituitary antibodies in patients with lymphocytic hypophysitis. *Horm. Res. 55*, 288–292.

Tanaka, S., Tatsumi, K I., Takano, T., Murakami, Y., Takao, T., Yamakita, N., Tahara, S., Teramoto, A., Hashimoto, K., Kato, Y. and Amino, N. (2003). Anti-alpha-enolase antibodies in pituitary disease. *Endocr. J. 50*, 697–702.

Taylon, C. and Duff, T.A. (1980). Giant cell granuloma involving the pituitary gland. *J. Neurosurg. 52*, 584–857.

Vanneste, J.A. and Kamphorst, W. (1987). Lymphocytic hypophysitis. *Surg. Neurol. 28*, 145–149.

Vidal, S., Rotondo, F., Horvath, E., Kovacs, K. and Scheithauer, B.W. (2002). Immunocytochemical localization of mast cells in lymphocytic hypophysitis. *Am. J. Clin. Pathol. 117*, 478–483.

Watanabe, K., Tada, H., Shimaoka, Y., Hidaka, Y., Tatsumi, K., Izumi, Y. and Amino, N. (2001). Characteristics of experimental autoimmune hypophysitis in rats: major antigens are growth hormone, thyrotropin, and luteinizing hormone in this model. *Autoimmunity 33*, 265–274.

Yabe, S., Murakami, M., Maruyama, K., Miwa, H., Fukumura, Y., Ishii, S., Sugiura, M. and Kobayashi, I. (1995). Western blot analysis of rat pituitary antigens recognized by human antipituitary antibodies. *Endocr. J. 42*, 115–119.

Yoon, J.W., Choi, D.S., Liang, H.C., Baek, H.S., Ko, I.Y., Jun, H.S. and Gillam, S. (1992). Induction of an organ-specific autoimmune disease, lymphocytic hypophysitis, in hamsters by recombinant rubella virus glycoprotein and prevention of disease by neonatal thymectomy. *J. Virol. 66*, 1210–1214.

CHAPTER

41

Hemolytic Anemia

BRUCE C. GILLILAND

Department of Medicine, Division of Rheumatology, and Department of Laboratory Medicine, University of Washington School of Medicine, Washington, DC, USA

HISTORIC BACKGOUND

Autoimmune hemolytic anemia (AIHA) is characterized by the presence of autoantibodies directed to antigens on the individuals' own red cell membrane, and by evidence of decreased red cell survival mediated by the red cell autoantibody. AIHA was the first autoimmune disorder in which an autoantibody was clearly shown to be involved in its pathogenesis. Historically, paroxysmal cold hemoglobinuria, described by Donath and Landsteiner (1904), was the first example of an autoimmune disease. Chauffard and Colleagues (1908, 1909) reported a case of acute hemolytic anemia in which an active hemolysin was demonstrated. Over the next three decades, the failure to identify a hemolytic serologic factor led many investigators to the concept that acquired hemolytic anemia was caused by a latent cell defect similar to that observed in familial hemolytic anemia. During these years, the main focus of interest was familial forms of hemolytic anemia. Interest in an extrinsic hemolytic factor was renewed when Dameshek and Schwartz (1938) demonstrated a hemolysin in the sera of three patients that caused destruction of transfused normal red cells. Dacie and Mollison (1943) distinguished familial hemolytic anemia from acquired hemolytic anemia. They demonstrated that normal red cells transfused into patients with familial hemolytic anemia had a normal lifespan, in contrast to a drastically shortened lifespan when transfused into patients with acquired hemolytic anemia. These observations indicated that patients with acquired hemolytic anemia had an extrinsic factor, presumably antibody, which led to accelerated destruction of normal transfused red dells. Young et al. (1951) applied the term "autoimmune" to those cases of acquired hemolytic anemia in which patients had developed antibodies reacting with their own red cells.

Coombs et al. (1945) developed an antiglobulin test for the detection of weak and incomplete Rhesus (Rh) agglutinins, thus providing a method for detection of antibody on the red cell surface. Boorman et al. (1946) and Loutit and Mollison (1946) applied the antiglobulin (Coombs) technique to detect autoantibodies on the red cells of patients with acquired hemolytic anemia, thus establishing a role for these antibodies in the pathogenesis of AIHA. Dacie (1951) reported that the red cells in acquired hemolytic anemia were either sensitized with gamma-globulin or non-gamma-globulin. Subsequently, Harboe et al. (1963) demonstrated that complement components were responsible for the non-gamma-globulin reactions. Today, specific antisera allow for the detection of immunoglobulins and their subclasses, as well as complement components on the red cell membrane.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

CLASSIFICATION

Autoimmune hemolytic anemias are usually classified into warm reactive type and cold reactive type based on the optimal temperature at which autoantibodies react with red cells *in vitro* (Box 41.1). Drug-induced autoantibodies comprise a third group and are usually of the warm reactive type. Warm reactive type autoantibodies react optimally at 37°C but also react at lower temperatures. Cold reactive type autoantibodies react optimally at 0–5°C and show decreasing red cell affinity as the temperature is raised to 37°C. They do not usually bind to the red cell at 37°C. A small number of patients have both cold and warm reactive autoantibodies, each having its own red cell antigen specificity.

Both warm and cold reactive types of AIHA are referred to as idiopathic or primary when no underlying or associated disorder can be recognized. When AIHA occurs in associations with another disorder, it is referred to as secondary. Secondary AIHA is most often associated with lymphoproliferative disorders, including lymphomas. A smaller number of cases occur in patients with systemic lupus erythematosus (SLE) and other multisystem autoimmune disorders, and also in inflammatory bowel disease (see Section II-A and Chapter 52).

Box 41.1
Classification of Autoimmune Hemolytic Anemia (Gilliland, 1996)

Warm autoantibody type
1. Idiopathic
2. Secondary: lymphoma, chronic lymphocytic leukemia, systemic lupus erythematosus, other autoimmune disorders, ovarian tumors, chronic inflammatory disorders

Cold autoantibody type
- Cold hemagglutinin disease
  1. Idiopathic
  2. Secondary: transient (acute)—*M. pneumoniae* infection, infectious mononucleosis, other infections; chronic—lymphoreticular malignancies
- Paroxysmal cold hemoglobinuria
  1. Idiopathic
  2. Secondary: viral and other infections, syphilis

Drug-induced immune hemolytic anemia
- Drug adsorption (hapten) type (e.g., penicillin)
- Drug-dependent antibody type, also referred to as immune complex (e.g., second- or third-generation cephalosporins)
- Autoimmune induction type (e.g., α-methyldopa)
- Nonimmunologic adsorption of protein (e.g., cephalothin)

WARM AUTOANTIBODY TYPE

Warm autoantibodies are most frequently encountered in AIHA. The onset of hemolytic disease is usually after the age of 40, and the incidence increases with age, reflecting the association of AIHA with lymphoproliferative disorders. Idiopathic warm type AIHA is more common in women, as is secondary AIHA associated with SLE. Idiopathic AIHA occurs sporadically and usually without a precipitating event such as an infection. Warm autoantibodies occasionally are accompanied by autoantibodies to platelets, which is referred to as Evans syndrome (Evans and Duane, 1949). Patients with warm type AIHA usually present with symptoms attributable to their anemia, such as fatigue, weakness, and pallor. In older patients, the anemia may lead to angina, myocardial infarction or heart failure. In patients with brisk hemolysis, urine is dark secondary to hemoglobulinuria. Jaundice may also be present. Patients usually have only a moderate degree of splenomegaly; however, with severe hemolytic anemia there may be significant hepatosplenomegaly. In patients with mild hemolytic anemia, a large spleen is usually indicative of an underlying lymphoproliferative disorder.

Pathophysiology

The coating of the red cell with autoantibody leads to the destruction of red cells by intravascular, extravascular, and/or cell-mediated mechanisms. The spleen plays a key role in the removal of IgG autoantibody-coated red cells (Jandl et al., 1957; Mollison et al., 1965; Schreiber and Frank, 1972; Chang et al., 1993). When blood enters the spleen, red cells become highly concentrated and pass through fine fenestrations between macrophages, enhancing their interaction with these cells. Macrophages have receptors for the Fc portion of IgG (subclasses IgG1 and IgG3), which bind IgG-coated red cells (LoBuglio and Jandl, 1967; Huber et al., 1968; Abramson et al., 1970). This interaction of red cell-bound autoantibody with macrophages results in either phagocytosis of the entire red cell or partial internalization of a portion of the red cell membrane. In the latter situation, the remaining red cell reseals and re-enters the circulation as a microspherocyte. Microspherocytes, because of their rigidity and increased osmotic fragility, have a greatly shortened lifespan (Fleer et al., 1978). They are fragmented or destroyed in the circulation and in subsequent passages through the spleen. Microspherocytes in the peripheral circulation signify ongoing hemolysis, usually through the process of IgG-mediated erythrophagocytosis.

The degree of microspherocytosis correlates with the severity of the hemolytic process. Some warm type autoantibodies fix complement on the red cell; however, complement-mediated hemolysis is unusual in warm type AIHA. Human red cells are relatively resistant to complement lysis because of several membrane regulating proteins that block complement activation at specific steps. Decay-accelerating factor (DAF, CD55) acts by binding to C3 convertases of both the classical and alternative complement pathways, leading to increased spontaneous decay of C3 convertase, and thereby limiting the amount of C3 membrane deposition (Fujita et al., 1986; Kinoshita et al., 1986). CD59 is a regulatory protein which is able to bind to C8 in the C5b-8 complex and interfere with the incorporation of C9 (Rollins et al., 1991). CD59 also can bind to C9 in the membrane attack complex, preventing polymerization of C9 and the full development of the transmembrane pore (Rollins and Sims, 1990). Another membrane protein proposed to regulate the formation of C5b-9 complex is homologous restriction factory/C8-binding protein (HRF/C8bp) (Zalman, 1992). This protein has been reported to block C8 and C9 deposition into membranes, thereby preventing red cell lysis.

The rate of hemolysis is dependent on several factors, including the quantity of cell-bound IgG subclass, other cell-bound immunoglobulin classes, the affinity of the autoantibody, the presence of cell-bound complement, and activated receptors on macrophages for IgG and/or complement (Chaplin, 1990). In addition, the chemical and physical characteristics of the antigen on the red cell also are determinants for the effectiveness of autoantibodies to cause hemolytic anemia. Although there are exceptions, the rate of hemolysis correlates with the amount of IgG autoantibody on the red cell membrane, particularly in the individual patient (Garratty and Nance, 1990; Garratty, 1991; Dubarry et al., 1993). The quantity, however, of autoantibody required to produce hemolytic anemia varies among patients. The presence of IgA and/or IgM in association with IgG was reported by Sokol et al. (1990) to be more likely associated with hemolytic anemia than those cells coated with IgG alone. IgG1 and IgG3 are most often found in patients with active hemolysis. IgG3 was reported by Engelfriet et al. (1981) to be commonly associated with active hemolytic anemia. The quantity of IgG1 and/or IgG3 is an important determinant for red cell destruction (Abramson et al., 1970; Huber et al., 1971). When red cells were coated with IgG1 alone or in combination with IgG2 or IgG4, 2000 or more molecules of IgG1 per red cell were necessary to stimulate phagocytosis and rosette formation *in vitro*, while an average of only 230 molecules of IgG3 per red cell were required to produce monocyte binding (Zupa'nska et al., 1986). When complement accompanies IgG on the red cell, binding to monocytes *in vitro* was enhanced and fewer molecules of IgG were required.

Autoantibody Characteristics

Warm type autoantibodies are usually IgG and polyclonal. Their restriction to the Gm allotype and/or light chain type has been reported (Leddy and Bakemeier, 1965; Litwin et al., 1973). In patients with AIHA, the subclass of IgG is most often IgG1 and frequently is the only subclass identified on the red cell. IgG3 alone on the red cell was reported by Engelfriet et al. (1982) in 2% of patients and in combination with other subclasses in approximately 5%. IgA or monomeric IgM was found in only 1% of patients and was frequently associated with complement on the red cell.

The specificity of many of these autoantibodies has been thought to be directed to the Rh antigen complex. This was based on the observation that these antibodies reacted weakly or not at all with Rh_{null} red cells that lacked determinants of the Rh complex. Furthermore, some autoantibodies reacted with Rh antigens such as E, e, or C (Weiner and Vos, 1963; Leddy et al., 1970; Dacie, 1975; Garratty, 1991). Rh_{null} cells have now been recognized to be deficient in the membrane antigens LW and Fy, and to have decreased amounts of U and Ss, and of glycophorin B (Dahr et al., 1987; Avent et al., 1988). It is now appreciated that many of these autoantibodies that did not react in the past with Rh_{null} cells had specificities outside the Rh system (Celano and Levine, 1967; Marsh et al., 1972). Red cell autoantibodies reacting equally well with Rh_{null} and normal red cells have specificities outside of the Rh system, including Wr^b, En^a, Ge, A, B, and antigens within the Kidd and Kell blood group systems (Issitt et al., 1976; Bell and Zwicker, 1978; Marsh et al., 1979; Mollison et al., 1993a; Garratty, 1994).

Red cell autoantibodies have been shown to be reactive with specific membrane proteins by immunochemical techniques. Some autoantibodies have been shown to recognize red cell protein Band 4.1 or red cell membrane Band 3 glycoprotein (Wakui et al., 1988; Victoria et al., 1990). Red cell protein Band 4.1 binds the spectrin–actin matrix to transmembrane proteins of phospholipids (Sato and Ohnishi, 1983; Anderson and Lovrien, 1984). Protein Band 3 traverses the lipid membrane and functions as an anion transport channel (Steck, 1978; Jennings, 1984). Victoria et al. (1990) demonstrated the reactivity of 12 warm IgG autoantibodies with Band 3. Barker et al. (1992), however, were unable to show reactivity with Band 3 with 11 eluates obtained from patients with AIHA. Eluates did react with a polypeptide band of 32 kDa and a band of 38–51 or 40–51 kDa, which corresponded with a pattern produced by two Rh-specific monoclonal antibodies. Leddy et al. (1993) studied the autoantibodies of 20 patients and found that these antibodies reacted with four distinct membrane proteins alone or in combinations. Nine of the eluates recognized Rh-containing polypeptides, five reacted strongly with only Band 3, and six reacted with both Band 3 and

glycophorins A (GPA). In a subsequent study, Leddy et al. (1994) examined these six eluates for their reactivity with $Wr(b^+)$ red cells because the Wr^b antigen is formed by the interaction of Band 3 and GPA (Telen and Chasis, 1990). One eluate was found to have a specificity to Wr^b, while two had evidence of Wr^b reactivity as well as other specificities, and three did not show any reactivity to Wr^b antigens. The latter three eluates apparently reacted with an antigen(s) dependent on the Band 3-GPA interaction, which was not $Wr(b^+)$ related.

A naturally occurring red cell autoantibody has been identified that recognizes an antigen on aging red cells (Kay, 1986 and 1994; Branch et al., 1984; Galili et al., 1986). The antigen (referred to as senescent red cell antigen) has been identified as a degradation product of Band 3 (Kay, 1975; 1986; 1994). It is speculated that these autoantibodies serve to remove old red cells from the circulation.

Autoimmune hemolytic anemia has been associated with antiphospholipid autoantibodies (Cabral et al., 1990; Arvieux et al., 1991; Sthoeger et al., 1993). Red cell eluates were shown to have IgG anticardiolipin reactivity. These autoantibodies may account for the antiglobulin (Coombs)-positive hemolytic anemia found in some patients with primary antiphospholipid syndrome or with antiphospholipid syndrome secondary to SLE. It also may explain the finding of IgG and complement on the red cells of some patients with SLE who do not have immune hemolytic anemia.

Antiglobulin Test

Antiglobulin tests are usually performed with a broad-spectrum antiglobulin antiserum directed primarily against IgG and C3. Antiserum should recognize C3d/C3dg, which remain on the red cell membrane after degradation of C3. Under special circumstances, IgA and IgM antisera are used. Three basic patterns are observed: IgG only, IgG plus C3, and C3 alone (Table 41.1). In various reports of warm-reacting type AIHA (Dacie and Worlledge, 1969; Petz and Garratty, 1980a; Engelfriet et al., 1982), IgG alone ranged from 18 to 64%, IgG plus C3 from 34 to 65%, and complement alone from 10 to 33%. A positive indirect antiglobulin test signifying free serum autoantibodies is not common in the absence of a positive direct antiglobulin test (DAT). Sensitivity of the indirect antiglobulin test can be increased by using enzyme-treated donor red cells, but care must be taken to exclude alloantibodies when interpreting the test.

Hemolytic anemia occurs in a small number of patients with warm autoantibodies who have a negative antiglobulin test as performed by conventional methods. The DAT requires the presence of 300–500 molecules of IgG per red cell using quantitative methods (Gilliland et al., 1971). AIHA, therefore, can occur in some instances with levels of IgG autoantibody below this threshold. The frequency of antiglobulin-negative disease has been reported as 2–4% of patients, but with more sensitive techniques, small amounts of red cell-bound autoantibody can be identified in many of these patients (Chaplin, 1973; Worlledge, 1978).

Treatment

Glucocorticoids remain the mainstay of treatment. The initial dose is usually 40–60 mg/day given in divided doses for the first 14 days. Patients will respond with an increase in hematocrit and decreased hemolysis. Prednisone is gradually reduced to the lowest level to maintain an adequate hematocrit. Prednisone is thought to decrease red cell destruction by interfering with the monocyte–red cell interac-

TABLE 41.1 Autoimmune hemolytic anemia (Gilliland, 1996)

Type	DAT	Serum antibody	Antibody specificity
Warm autoantibody	IgG	Serum antibody in ~50% with untreated red cells	Rh complex antigens in the majority; others include LW, U, Wr^b, En^a, Kidd, Kell, and Ge
	IgG†		
	C3d		
	C3d*		
Cold autoagglutinin	C3d	Pathologic cold agglutinins 1. 1:1000 at 4°C 2. Positive at 30°C in albumin	Mainly I, also I and Pr
Paroxysmal cold hemoglobinuria	C3d	Biphasic hemolysin antibody reacts with red cell in cold; hemolysis occurs at 37°C in presence of complement (fresh serum)	P

DAT, direct antiglobulin test.

*IgG can be detected on red cells apparently coated with C3d alone by more sensitive techniques, warm-reacting IgM antibodies also may fix complement to red cells.

tion that occurs predominantly in the spleen and liver (Schreiber et al., 1975; 1977; Kay and Douglas, 1977). It also leads to a reduction of autoantibody production and decreased binding affinity of autoantibody to red cell antigens (Rosse, 1971).

In patients who have not had a satisfactory response to prednisone, splenectomy should be considered. Approximately two-thirds of patients will have a complete or partial response to splenectomy, allowing the dose of prednisone to be reduced or even discontinued (Christensen, 1973; Bowdler, 1976; Petz and Garratty, 1980b; Zupa'nska et al., 1981). Splenectomy increases the risk of pneumococcal sepsis, especially in children; therefore, pneumococcal vaccine should be given prior to the surgery and in subsequent years (Allgood and Chaplin, 1967).

Immunosuppressive therapy is reserved for those patients who have not responded well to glucocorticoids and/or splenectomy (Mueller-Eckhardt and Kretschmer, 1972; Skinner and Schwartz, 1972; Habibi et al., 1974; Worlledge, 1974; Murphy and LoBulgio, 1976; Panceri et al., 1992). In patients who cannot tolerate a splenectomy, immunosuppressive therapy is a reasonable next step. When an immunosuppressive agent does not lead to a reduction in the prednisone dose after 4–6 months, other immunosuppressive agents may be tried, including azathioprine (1–2 mg/kg/day), cyclophosphamide (1–2 mg/kg/day), and chlorambucil (0.1–0.2 mg/kg/day). Cyclophosphamide can also be given intravenously once a month as in the treatment of lupus nephritis (Austin et al., 1986). The intervals and duration of pulsed cyclophosphamide therapy depend on the clinical response. The dose is 0.5–1.0 g/m^2 of body surface area. Cyclosporine A (4 mg/kg/day) has been reported to be effective in some patients (Hershko et al. (1990). Intravenous gamma-globulin (IVIG) may also be effective (Macintyre et al., 1985; Bussel et al., 1986; Leickly and Buckley, 1987; Blanchette et al., 1992; Flores et al., 1993). In resistant disease, treatment with rituximab, a monoclonal anti-CD20 antibody, has been successful (Ahrens et al., 2001; Zecca et al., 2001; Gupta et al., 2002). Another monoclonal antibody, anti-CD52 Campath-1H (alemtuzumab), has also been beneficial in some patients (Willis et al., 2001). Other forms of treatment include danazol (Ahn, 1990; Chan and Sack, 1991; Pignon et al., 1993), antilymphocyte or antithymocyte globulin, vincristine-loaded platelets (Ahn et al., 1983), and plasmapheresis. Patients with chronic hemolytic anemia should receive folic acid (1 mg/day).

COLD AUTOANTIBODY TYPE

Cold-reacting red cell autoantibodies comprise 15–25% of patients with AIHA (Dausset and Colombani, 1959; Van Loghem et al., 1963; Dacie and Worlledge, 1969; Petz and Garratty, 1980c; Engelfriet et al., 1982). Cold autoantibodies react optimally with red cells at 0–5°C, but their thermal amplitude must extend to 25–31°C to be reactive *in vivo* to cause hemolytic anemia. These temperatures are normally encountered in the microvasculature of the skin in the distal extremities, ears, and tip of the nose. The two types of cold autoantibody disorders are cold hemagglutinin disease and paroxysmal cold hemoglobinuria. Both types depend on autoantibodies fixing and activating complement on the red cell membrane, which leads to varying degrees of intra- and extra-vascular hemolysis.

Cold Hemagglutinin Disease

Landsteiner (1903) described that the serum of an animal could agglutinate its own red cells at temperatures near 0°C. Subsequently, Landsteiner and Levine (1926) reported that sera from most normal persons agglutinated their own red cells at 0°C. The term "cold agglutinin disease" was credited to Schubothe (1952) who used this designation to distinguish it from paroxysmal cold hemoglobinuria. Cold hemagglutinin disease can manifest as an acute/transient disease, which is most often associated with infectious mononucleosis or mycoplasma pneumonia. Young persons are most commonly affected. The cold agglutinin in acute/transient disease is usually a polyclonal IgM. Cold hemagglutinin disease also occurs as a chronic disease, found most often in persons over the age of 50. The associated cold agglutinin is usually a monoclonal IgM. Chronic cold hemagglutinin disease occurs as a primary or idiopathic disorder in which no underlying disease can be identified. Cold agglutinin disease can also be associated with chronic lymphocytic leukemia or Waldenström macroglobulinemia, but a malignant lymphocytic disorder occurs in only a few patients.

Patients with the chronic monoclonal cold agglutinin disease usually present with manifestations of chronic anemia, the most common being shortness of breath, fatigue, and in older patients congestive heart failure. Patients may have episodes of hemoglobinemia and hemoglobinuria manifested by passage of dark urine after cold exposure. Patients who live in cooler climates may experience acrocyanosis of the fingers and blueness of the nose and ears when outdoors. Raynaud phenomenon may also be a manifestation. Prolonged cold exposure of an extremity may lead to vascular occlusion and gangrene. In patients with monoclonal IgA cold agglutinins, these cutaneous manifestations occur in the absence of hemolytic anemia because IgA cold agglutinins do not activate the classical complement pathway. Mild splenomegaly is usually present. The spleen may become enlarged during an episode of acute hemolysis. A persistently enlarged spleen suggests an underlying malignant lymphoma. Patients with secondary chronic cold agglutinin disease may present with features of lymphoma (e.g., lymphadenopathy, hepatosplenomegaly,

anemia), and the finding of a cold agglutinin may be incidental.

The hemolytic anemia that develops in patients with infectious mononucleosis or *Mycoplasma pneumoniae* infection usually has an abrupt onset in the second or third week of the illness. It is transient, usually disappearing in 1–2 weeks. Patients may have marked hemoglobinemia and hemoglobinuria, which can lead to transient renal failure. Cold agglutinins are found in approximately 45% of patients with *M. pneumoniae* infections, but hemolytic anemia is unusual. Hemolytic anemia in infectious mononucleosis is also uncommon. Cold agglutinins uncommonly develop following chicken pox or other viral infectious diseases (Friedman and Dracker, 1992).

Pathophysiology

Cold hemagglutinins produce red cell destruction through activation of the complement system, resulting in red cell destruction. The hemolytic potential of cold agglutinins depends on their titer and thermal amplitude, which is the temperature *in vivo* at which cold agglutinins bind to red cells and fix complement (Dacie, 1962; Evans et al., 1968; Brown et al., 1970; Atkinson and Frank, 1974; Jaffe et al., 1976; Rosse and Adams, 1980). The temperature in the microvasculature of the skin in distal extremities usually ranges from 28 to 31°C; therefore, cold agglutinins must be able to react with red cells at these temperatures in order to cause hemolytic anemia. Cold agglutinins with a lower thermal amplitude produce significant hemolytic anemia only when the patient is exposed to cold temperatures. In patients with high titers of cold agglutinins, the thermal amplitude is usually in the temperature range to produce hemolytic anemia at ordinary room temperatures. Acute episodes of hemolysis are exacerbated by cold exposure. Hemolytic anemia has been reported in patients with low titers of IgM cold agglutinins with a higher thermal range (Schreiber et al., 1977; Rousey and Smith, 1990).

When IgM cold agglutinins react with red cells at sites of cooler temperatures in the extremities, the classical complement pathway is activated (Sokol et al., 1982; Frank, 1988). The binding and activation of C1 requires only one IgM molecule to react with the red cell membrane. C1 then activates C4 and C2 to form a C3 convertase, which in turn activates several hundred molecules of C3 that bind to the red cell membrane on return of the red cells to warmer body temperatures. The subsequent binding of C5-9 leads to the lysis of red cells. Red cell destruction to a lesser extent occurs by the mechanism of erythrophagocytosis. Circulating red cells coated with C3b or iC3b bind, respectively, to CR1 and CR3 receptors on macrophages, predominantly in the liver (Berger et al., 1981–1982; Fearon and Wong, 1983). This interaction, as described previously for IgG-coated red cells, results in sphering and eventual destruction of red cells (Huber et al., 1968).

The interaction of C3b- and iC3b-coated red cells with CR1 and CR3 receptors, respectively, on hepatic macrophages can produce degradation of C3 without damaging red cells. C3b is degraded in iC3b by factor 1, with factor H and CR1 acting as cofactors. Further degradation results in the formation of C3dg and C3d. A substantial number of C3d- and C3dg-coated red cells are then released from the liver back into the circulation and are no longer recognized by complement receptors (Jandl et al., 1957; Schreiber and Frank, 1972; Lachmann et al, 1982). Red cells with a sufficient coating of C3d and C3dg become resistant to further interactions with cold agglutinins and to further activation of complement, and have a normal survival (Evans et al., 1967).

Autoantibody Characteristics

The specificity of the majority of cold agglutinins is directed to the i/I blood group system. These antigens are oligosaccharides and are closely related to the ABH and Lewis blood groups (Feizi, 1977). They are also expressed on human neutrophils, lymphocytes, and monocytes (Pruzanski and Shumak, 1977). The adult red cells of normal individuals express predominantly I antigen. At birth, however, cord (neonatal) red cells express i antigen, which is gradually replaced by I antigen in the first 18 months of life (Jenkins et al., 1960; Marsh and Jenkins, 1960; Marsh, 1961). The transformation of i to I is through the action of a branching enzyme on this glycoprotein (Watanabe and Hakomori, 1976). This process occurs as red cells leave the marrow and mature. Reticulocytes express more i antigen than adult red cells. Stimulation of the bone marrow by repeated phlebotomy results in the emergence of red cells into the peripheral circulation that have more i reactivity (Hillman and Giblett, 1965). The amount of i is inversely proportional to the length of the transit time of red cells from the bone marrow. A rare normal individual expresses only i on adult red cells, indicating a lack of branching enzyme (Race and Sanger, 1975). When the glycoprotein antigen is extracted from the red cell membrane, cold agglutinins react with their respective I or i antigen at 37°C, indicating that cold-induced conformational changes of the antigen are required for *in vivo* reactivity (Rosse and Lauf, 1970; Lau and Rosse, 1975).

Cold agglutinins may infrequently be specific for Pr antigen, which is so designated because of its sensitivity to protease treatment. Cold agglutinins directed against Pr antigens and other antigens outside the I/i system are recognized by the finding that the patient's serum reacts equally well with adult and cord (neonatal) red cells (Roelcke, 1974; Roelcke and Kreft, 1984). Cold agglutinins with anti-Pr specificity will not bind to papain-treated red cells. Other

specificities of cold agglutinins are rarely observed (Mollison et al., 1993b).

Molecular studies have shown that the highly restrictive V_H4–21 germline gene segment is essential for the generation of cold agglutinins with either anti-I or anti-i specificity (Silverman and Carson, 1990; Silberstein et al., 1991; Pascual et al., 1992). Cold agglutinins share a common idiotype as first shown by Williams et al. (1968). Later, in studies using a rat monoclonal anti-idiotype antibody (9G4), a conformation-dependent idiotype encoded by the V_H4–21 gene segment was found in cold agglutinins from different individuals (Pascual et al., 1992; Thompson et al., 1991). The anti-idiotype antibody also has been shown to inhibit cold agglutinin activity, suggesting that it recognizes an epitope close to the antigen-binding site (Thompson et al., 1991). The idiotype generated by the V_H4–21 gene segment has also been identified in a high proportion of circulating B-cells and marrow lymphoplasmacytoid cells in patients with chronic cold agglutinin disease, and in a small proportion of B-cells in the circulation and lymphoid tissue of normal individuals (Stevenson et al., 1989). Cold agglutinins also have restricted light chain variable gene segment expression. Anti-I cold agglutinins preferentially use κ light chains (Harboe et al., 1965; Capra et al, 1972) restricted to the V_κ III family (Silverman and Carson, 1990), whereas cold agglutinins with anti-i specificity use several V_L gene segment families also involving λ light chains (Feizi, 1967; Pruzanski et al., 1974; Roelcke et al., 1974; Silberstein et al., 1991). The restriction of V_L gene segment usage may be a factor in determining anti-I or anti-i specificity.

Thompson et al. (1991) reported the usage of the V_H4–21 gene segment by human alloantibodies against a diverse group of red cell antigens. This would indicate that the V_H4–21 gene segment can encode IgM anti-red cell antibodies having specificities to structurally diverse red cell antigens. In addition, antibodies to exogenous carbohydrate antigens have been shown to be encoded by a restricted set of V_H and V_L genes (Adderson et al., 1992, 1993).

The characteristics and specificity of the cold agglutinins have clinical associations. Monoclonal IgM cold agglutinins with i specificity have been reported to be associated with malignant lymphoma (Crisp and Pruzanski, 1982). In patients with infectious mononucleosis, the IgM cold agglutinins are polyclonal and frequently have i specificity, whereas in *M. pneumoniae* infection the cold agglutinins are directed to the I antigen (Jenkins et al., 1965; Rosenfield et al., 1965; Costea et al., 1966; Feizi and Schumacher, 1968; Dacie and Worlledge, 1969). In some patients with infectious mononucleosis or angioimmunoblastic lymphadenopathy, a polyclonal nonagglutinating IgG reactive with i antigen was shown to bind to red cells at reduced temperatures. Hemagglutination observed *in vitro* was produced by IgM anti-IgG autoantibodies that react with red cells coated with IgG anti-i in the cold (Goldberg and Barnett, 1967; Capra et al., 1969).

Antiglobulin Test

The DAT shows only C3d or C3dg on the red cell membrane (see Table 41.1). The titer of cold agglutinins in patients with chronic cold hemagglutinin disease is usually 1:1000 or greater at 4°C (Petz and Garratty, 1980d). When titers are below 1:1000, the thermal range of cold agglutinins reactivity should be determined. Cold agglutinins that are not able to agglutinate cells in saline at 20°C are usually not pathologic. When agglutination is seen at 20°C, the cold agglutinin titer is determined at 30°C with the red cells suspended in albumin; positive results indicate that these cold agglutinins are capable of producing hemolytic anemia *in vivo* and are of clinical significance (Petz and Garratty, 1980d).

Treatment

Treatment of acute/transient cold agglutinin disease is mainly supportive. Patients should be protected from the cold and kept well hydrated to minimize the effects of hemoglobin on the kidney. Corticosteroids and/or cytotoxic drugs are usually not indicated. Transfusion should be avoided except in life-threatening situations.

In chronic cold hemagglutinin disease treatment depends on the degree of hemolysis and anemia. In patients with well-compensated hemolytic anemia, no specific treatment is required. These patients should, however, be given approximately 1 mg of folic acid/day and advised to avoid cold exposure, including exposure through drinking large quantities of cold beverages. Cytotoxic drugs are reserved for patients with more severe hemolytic anemia. The two most commonly used drugs are cyclophosphamide and chlorambucil (Dacie and Worlledge, 1969; Schubothe, 1966; Hippe et al., 1970; Evans et al, 1973). These drugs lead to a decrease in the amount of cold agglutinins and therefore decreased hemolysis. Corticosteroids and splenectomy usually are not beneficial except in those patients who have low titers of IgM cold agglutinins with high thermal range and in patients with IgG cold agglutinins (Silberstein et al., 1987). They also may be beneficial in patients who have evidence of red cell destruction occurring by erythrophagocytosis, a process that involves the spleen and is responsive to glucocorticoids. Plasmapheresis may provide transient improvement (Andrzejewski et al., 1988). There are conflicting reports of the effectiveness of interferon-α, being beneficial in some patients (O'Connor et al., 1989) and ineffective in others (Hillen and Bakker, 1994). An overall response rate of 54% was reported in patients treated with rituximab, a monoclonal anti-CD20 antibody (Berentsen et al., 2004). Transfusion should be reserved for those patients

with life-threatening degrees of anemia. The patient should be kept warm during the transfusion and the donor red cells warmed to body temperature before administration (Rosenfeld and Jagathambal, 1976).

Paroxysmal Cold Hemoglobinuria

Paroxysmal cold hemoglobinuria (PCH) is a rare form of AIHA which is most commonly observed in children following a viral infection. Historically, Donath and Landsteiner (1904) were the first to describe the biphasic hemolysis test necessary to demonstrate the presence of the responsible autoantibody. A positive test was attributable to a cold-reacting autohemolysin and a warm-reacting lytic factor. Acute episodes of hemolysis following cold exposure led to this disorder being termed "paroxysmal cold hemoglobinuria" (Donath and Lansteiner, 1925). PCH was initially observed to be often associated with syphilis, but today is most commonly associated with a variety of viral infections (Wolach et al., 1981; Sokol et al., 1982; Lau et al., 1983; Göttsche et al., 1990).

Hemolytic anemia usually develops at the time when patients are recovering from a recent upper respiratory infection. Episodes are characterized by the sudden onset of shaking chills, back and leg pain, and abdominal cramps followed by fever which may be as high as 40°C. Hemoglobin is present in urine. Attacks are not always associated with cold exposure. The constitutional symptoms usually abate after several hours. In most cases of acute/transient PCH the patient recovers in a few days to several weeks without further occurrences. Severe attacks may be life-threatening. Cold urticaria has also been associated with episodes. Renal failure, secondary to hemolysis, may occur and anemia can be quite severe.

Pathophysiology and Autoantibody Characteristics

The autohemolysin in PCH is termed the Donath–Landsteiner (D–L) antibody. The autoantibody is IgG and reacts with red cells at reduced temperatures found in the microvasculature of extremities, resulting in fixation of the early components of complement. Complement activation continues as the blood returns to the warmer core temperature with eventual lysis of the red cells. In patients with the acute transient form of disease, the D–L antibody does not bind to the red cells *in vitro* at temperatures higher than 20°C, yet hemolysis occurs even in the absence of significant cold exposure, indicating that this antibody may react with red cells at higher temperatures *in vivo*. The reason for this is unknown. On the other hand, patients with chronic PCH usually experience hemolysis with cold exposure, similar to patients with cold agglutinin disease. The D–L antibody is usually IgG and has anti-P specificity (Levine et al., 1965; Worlledge and Rousso, 1965). The P antigen is a globoside. Reactivity of the D–L autoantibody with P antigen can be inhibited by globoside and Forssman glycolipids, which are found in a variety of microorganisms (Schwarting et al., 1979). This suggests that the autoantibody response may be to a cross-reacting antigen present in some microorganisms. Infections associated with PCH include measles (also measles vaccination), mumps, infectious mononucleosis, chicken pox, mycoplasmal pneumonia, cytomegalovirus virus, *Haemophilus influenzae, Klebsiella pneumoniae*, and *Escherichia coli*. Even though an infectious etiology is suspected, the microorganism is usually not identified (Vogel et al., 1972; Bird et al., 1976).

Antiglobulin Test

The DAT shows C3d (see Table 41.1). The D–L antibody is detected by mixing serum from the patient with donor red cells in the presence of normal serum as a source of complement (Petz and Branch, 1983). The mixture is incubated at 4°C and then warmed to 37°C, resulting in hemolysis when D–L antibody is present. The D–L antibody may persist for several weeks to months following an acute episode.

Treatment

PCH is usually a self-limited disease and treatment is largely symptomatic. When transfused blood is necessary, it should be warmed. Corticosteroids are sometimes given but are usually of little value in this disorder.

DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

Drug-induced immune hemolytic anemia (IHA) has been reported in as many as 18% of patients with AIHA (Petz and Garratty, 1980e). The frequency of drug-induced disease may be even higher because the association between anemia and the drug may not be immediately evident. In addition, patients are often on several drugs and there may be other explanations for anemia. Another problem is that most clinical laboratories are not prepared to perform tests for drug-related antibodies. The concept of the pathogenesis of drug-induced IHA has evolved over the past several decades. Three mechanisms have been defined (Petz and Garratty, 1980e): 1) drug adsorption (hapten); 2) production of drug-dependent antibody (formerly called immune complex/innocent bystander); and 3) induction of autoantibodies (Table 41.2). In the case of some drugs, more than one of these mechanisms may be operative. In addition, certain drugs can produce a coating of red cells with immunoglobulins and other serum proteins (nonimmuno-

TABLE 41.2 Drug-induced immune hemolytic anemia (IHA) (Gilliland, 1996)

Mechanism	DAT	Detection of antibody	Clinical features
Drug absorption, e.g., penicillin	IgG C3d*	Antibody reacts with drug-coated red cells; eluate reacts only with drug-coated red cells	Moderate degree of hemolysis, usually extravascular mechanism
Drug-dependent antibody type, e.g., cefotetan	C3d	Antibody + drug + red cell → sensitization, agglutination, or hemolysis of red cells. Antibody is IgG or IgM; eluate is negative	Abrupt onset of severe intravascular hemolysis; renal failure
Autoimmune induction, e.g., α-methyldopa	IgG	Autoantibodies against red cell; eluate reacts with red cells	Mild-to-moderate degree of extravascular type hemolysis

DAT, direct antiglobulin test.
*Present in approximately 40% of patients with penicillin-induced IHA.

logic adsorption), resulting in a positive DAT but not hemolytic anemia.

Drug Adsorption Mechanism

In the drug adsorption mechanism, drug-induced antibodies react with a drug or one of its metabolic products that is firmly bound to the red cell membrane. Penicillin is the prototype for this mechanism of drug-induced IHA (Van Arsdel and Gilliland, 1965; Swanson et al., 1966; Levine and Redmond, 1967; White et al., 1968). Penicillin is one of a few drugs that have been shown to strongly bind to the red cell membrane and to not be removed with washing of the red cells. Approximately 90% of patients who have been given penicillin in the past will have IgM antipenicillin antibodies, but these antibodies are not involved in the positive antiglobulin test or hemolytic anemia (Garratty and Petz, 1975). Those patients who do develop hemolytic anemia have an IgG antibody directed against the benzylpenicilloyl determinant or other metabolites of penicillin (White et al., 1968). The onset of hemolytic anemia usually occurs after 7 days of treatment but can appear shortly after the beginning of treatment in patients previously sensitized to penicillin. Hemolysis is usually moderate and usually subsides once the drug is discontinued. Prednisone is usually not required. The DAT is positive for IgG accompanied in some instances by complement. The indirect antiglobulin test is negative unless donor red cells coated *in vitro* with penicillin are used.

Drug-Dependent Antibody Mechanism

The drug-dependent antibody mechanism (formerly termed the immune complex/innocent bystander mechanism) was first described by Ackroyd (1949) who observed drug-induced thrombocytopenia following Sedormid administration. He demonstrated that serum antibody against Sedormid was only able to produce agglutination of platelets in the presence of this drug. The drug could be easily removed from the cells by conventional washing or by dialysis against saline. He proposed that the drug transiently bound with the cell membrane protein to form an immunogen which stimulated antibodies that recognize the drug as part of a drug–cell membrane protein antigen. More recent studies have indicated that in many cases, antibody and drug do not bind to the red cells in a nonspecific (innocent bystander) manner. Instead, it appears that a red cell glycoprotein in combination with drug is the immunogen for antibody stimulation and the target for antibody reactivity (Mueller-Eckhardt and Salama, 1990; Salama and Mueller-Eckhardt, 1992). Demonstration of antidrug antibody reactivity with red cells requires the presence of free drug or its metabolite. Furthermore, the antidrug antibodies plus drug may react only with red cells of a specific blood group.

The onset of hemolysis is usually abrupt. Hemolytic anemia can be quite severe, manifested by hemoglobinemia and hemoglobinuria, and complicated by real failure, shock, and/or disseminated intravascular coagulation. Renal failure is a common complication. Hemolysis usually subsides once the putative drug is discontinued; however, drug-dependent antibodies may persist for years, placing the patient at risk for subsequent hemolysis when re-exposed to the drug. The responsible antibodies are either IgG or IgM, which are capable of activating complement, which binds to the red cell. The DAT is positive only for C3d as the drug and antidrug antibody dissociate from the red cell by conventional washing.

Autoimmune Induction Mechanism

Drugs may stimulate the production of autoantibodies, leading to hemolytic anemia. Drug-induced autoantibodies react with antigens intrinsic to the red cell membrane and do not require the presence of drug for their detection. The prototype drug is α-methyldopa. Autoantibody usually appears after 3–6 months of α-methyldopa therapy. The reason for the infrequency of hemolysis, even though approximately 20% of patients develop a positive DAT, was addressed by Kelton (1985), who demonstrated that α-methyldopa impaired Fc-dependent reticuloendothelial

function. A patient who had a positive DAT and hemolysis was shown to have normal reticuloendothelial function, whereas patients who were DAT positive without hemolysis or were DAT negative were found to have impaired reticuloendothelial function, indicating that the drug was responsible.

The DAT may remain positive several months to years after the drug is stopped (Worlledge, 1969b). When the patient is re-exposed to α-methyldopa after autoantibodies have disappeared, it again takes 3–6 months for the autoantibody to appear. The mechanism by which α-methyldopa induces autoantibody is not known. Initially, Worlledge et al. (1966) and Green et al. (1980) suggested that the red cell membrane was altered by α-methyldopa, which resulted in autoantibodies to this neoantigen. Subsequent studies made this unlikely. α-Methyldopa has been shown to increase lymphocyte cyclic adenosine monophosphate, leading to the inhibition of suppressor T-cells (Kirtland et al., 1980) and therefore overproduction of autoantibody-producing B-cells. Garratty et al. (1993), however, were unable to demonstrate depression of suppressor T-cell function.

The DAT shows IgG, occasionally accompanied by complement. Serum autoantibody and eluates react with normal red cells in the absence of free drug. The specificity of autoantibodies has been shown to be directed to the Rh complex (Worlledge, 1969a; LoBuglio and Jandl, 1967; Bakemeier and Leddy, 1968). Studies by Leddy et al. (1993) showed that autoantibodies induced by α-methyldopa recognized a 34-kDa polypeptide and a 37–55-kDa glycoprotein, which are both related to the Rh complex.

The degree of hemolytic anemia is usually mild to moderate. Once the drug is discontinued, hemolysis usually subsides within a few days, but in some cases may continue for several weeks to months (Ewing et al., 1968). Corticosteroids may be required in patients with more severe hemolytic anemia, shortening the period of recovery. The DAT may remain positive for several weeks or even years after discontinuation of the drug (Worlledge, 1969b). Induction of red cell autoantibodies has also been associated with levodopa (Henry et al., 1971; Lindstrom et al., 1977), mefenamic acid (Scott et al., 1968), and procainamide (Kleinman et al., 1984). α-Methyldopa occasionally induces other immunopathic reactions affecting the liver, e.g., hepatitis, as described in Chapter 53.

AUTOIMMUNITY

Most cases of primary warm type AIHA arise spontaneously and appear to be unrelated to a specific HLA haplotype or other genetic factor. In primary or idiopathic disease, the red cell autoantibody is usually not accompanied by polyclonal gammopathy. A small number of cases of AIHA occur in patients with disorders that have other features of autoimmunity, e.g., SLE, lymphoproliferative disorders, and inflammatory bowel disease.

Evidence indicates that warm autoantibodies arise from a selective process that is most likely antigen driven. Warm type autoantibodies also have a well-defined specificity to red cell antigen and a high binding affinity. Clonal selection is also supported by the restriction of IgG subclasses and light chain types of these autoantibodies (Leddy and Bakemeier, 1965; Litwin et al., 1973) and electrophoretic dispersion (Andrzejewski et al., 1991). The specificity and high binding affinity of these autoantibodies indicate somatic mutations of immunoglobulin variable region genes. Many of the findings in warm type AIHA are similar to observations in New Zealand Black mice AIHA (see below).

The emergence of anti-red cell autoantibodies is related to the breakdown of immunologic tolerance (see Chapters 8 and 13). Autoreactive T and B cells are clonally removed in the thymus and bone marrow (central tolerance); however, there remain in normal individuals self-reactive T- and B-cells. These self-reactive cells are controlled by mechanisms of peripheral tolerance, including T-cell anergy and ignorance, and by a unique population of $CD4^+CD25^+$ lymphocytes (see Chapter 9). The anti-self reactivity of these cells is well controlled in view of the low incidence of positive DATs in the normal population and the infrequency of AIHA (Pirofsky, 1969).

T and B cells specific for self-antigens can be activated when exposed to antigens that are normally not presented *in vivo* by antigen-presenting cells (APCs). Helper T (Th) cells from normal individuals respond *in vitro* to synthetic peptides from the red blood cell (RBC) Rh complex (Barker and Elson, 1995; Barker et al., 1997). The proliferation of these T cells was blocked by anti HLA-DR antibodies. The presence of self-reactive T cells in normal individuals indicates that these T cells escaped removal by the thymus. These T cells respond *in vitro* to Rh synthetic peptides, which are cryptic antigens not normally processed *in vivo* by APCs (see Chapter 14). The T cells from normal individuals that recognize these cryptic antigens remain inactive unless stimulated by cross-reactive environmental agents and/or change in antigen processing with presentation of these cryptic antigens (Elson et al., 1995).

Natural self-reactive autoantibodies found in normal individuals include those directed against nuclear antigens, plasma proteins, and cell membrane components (Guilbert, et al., 1982; Coutinho et al., 1995). In normal individuals, IgG and IgM anti-idiotype autoantibodies directed against natural self-reactive autoantibodies act to control the expression of these potentially pathologic autoantibodies (Hurez et al., 1993). Self-reactive antibodies in normal individuals have been identified that react with ABO blood groups (Spalter et al., 1999), with the red cell anion transporter, Band 3 (Lutz et al., 1984), and with the red cell cytoskele-

tal protein, spectrin (Lutz and Wipf, 1982). In addition, T-cell reactivity with the Rh polypeptide has been observed in normal individuals (Barker and Elson, 1994). Spalter et al. (1999) showed that purified IgG and IgM from normal individuals reacted with autologous ABO blood group antigens, but when nonfractionated serum was used, the expression of these autoantibodies was restricted, suggesting an idiotype complementary interaction between the IgG and IgM. Similar findings were reported in a study of patients with warm type AIHA (Stahl et al., 2000). These investigators demonstrated that autologous IgM in patients with AIHA was defective in restricting the reactivity of anti-red cell autoantibodies. This study suggests that within the IgM population of normal individuals there are anti-idiotype antibodies controlling the expression of anti-red cell autoantibodies and their absence in patients with AIHA may be important in the development of clinical AIHA.

The type of T-cell response and resultant cytokine profile play a role in the pathogenesis of AIHA. Barcellini et al. (2000), using whole blood cultures from patients with warm type hemolytic anemia, examined the cytokine profile after mitogen stimulation, and in parallel experiments, measured anti-red cell antibody production after mitogen and cytokine stimulation. In the AIHA patients, mitogen stimulation of the whole blood culture increased the Th2 cytokines, interleukin (IL)-4, IL-6, and IL-13, and reduced production of interferon (IFN)-γ, a Th1 cytokine. IFN-γ along with IL-2 were further reduced in those patients with active hemolytic anemia. In addition, the secretion of transforming growth factor (TGF)-β was increased in these patients. The addition of Th2 cytokines (IL4, IL-6, IL10 and IL-13) to whole red cell cultures from patients stimulated increased anti-red cell antibody production and increased binding to red cells. The production of anti-red cell antibodies also increased after stimulation with TGF-β. These results suggest that AIHA is mediated by a Th2 response. TGF-β may facilitate B-cell hyperactivity and anti-red cell antibody production by downregulating the Th1 response. Subsequent studies by Fagiolo and Torrani-Terenzi (2002) examined the *in vitro* secretion of cytokines from peripheral blood mononuclear cells of AIHA patients, basally and after mitogen stimulation. Basal secretion of IL-4 was increased, while IFN-γ synthesis was decreased compared to healthy controls. Also, basal levels of IL-10 were elevated in AIHA patients and further increased with mitogen stimulation. IL-12 levels did not increase with mitogen stimulation. This cytokine has been shown to promote development of Th1 cells, leading to inhibition of antibody production. The increased levels of Th2 cytokines and decreased levels of Th1 cytokines suggest an imbalance of IL-10/IL-12 that leads to heightened Th2 cell differentiation and antibody production in AIHA patients.

The role of $CD4^+CD25^+$ regulatory T cells (Tregs) in AIHA has been described in a mouse model (see below). These regulatory cells, derived from the thymus, require activation via their T-cell receptor (TCR) to become suppressive and to inhibit proliferation of $CD4^+$ or $CD8^+$ T cells (see Chapter 9).

Molecular mimicry may be involved in the development of transient postinfectious warm type AIHA in children following a brief viral illness or of cold type AIHA in adults with acute cold hemagglutinin disease associated with mycoplasmal pneumonia or infectious mononucleosis. Approximately 45% of patients with mycoplasmal pneumonia develop cold agglutinins with anti-I specificity (Chanock et al., 1961). This close association between an infectious microorganism and the development of a red cell autoantibody suggests the possibility of cross-antigenicity between red cell antigens and membrane antigens on microorganisms. Rabbits injected with *M. pneumoniae* were demonstrated to develop cold agglutinins with anti-I specificity that cross-reacted with antigenic determinants on the membrane of the microorganism (Janney et al., 1978). Another concept proposed is that *M. pneumoniae* binds to the I antigen, rendering this self-antigen immunogenic. Studies have shown that the I antigen, which is a branched oligosaccharide, has sialic acid-containing receptors for *M. pneumoniae* (Loomis et al., 1983). Injection of human I-positive red cells treated initially with live *M. pneumoniae* into rabbits resulted in enhanced elevated titers of anti-I antibodies in about 30% of rabbits (Feizi et al, 1969). Another study, however, demonstrated that modification of the I red cell antigen was not required for induction of cold agglutinins with anti-I specificity (Costea et al., 1971). Injection of *M. pneumoniae* into mice, which do not possess I antigen on red cells, still resulted in the production of cold agglutinins with anti-I specificity. Furthermore, prior incubation of I-positive human red cells with *M. pneumoniae* did not enhance the cold agglutinin response in rabbits following immunization with these red cells. In infectious mononucleosis, cold agglutinins may develop as a result of polyclonal stimulation by Epstein–Barr virus. Why the specificity of these cold agglutinins is frequently anti-i is not understood. Perhaps here also molecular mimicry is involved.

ANIMAL MODELS

Several murine models have furthered our understanding of AIHA. New Zealand Black (NZB) mice spontaneously develop AIHA (Bielschowsky et al., 1959). Red cell autoantibodies and AIHA are not seen before the age of 3–6 months and by 15 months all mice have AIHA. These mice also develop anti-DNA autoantibodies and renal lesions resembling human SLE. Polyclonal B-cell activation occurs spontaneously early in the life of the NZB mice and is characterized by increased secretion of IgM and production of IgM anti-DNA, but no anti-mouse RBC (MRBC)

autoantibodies (Moutsopoulos et al., 1977; Jyonouchi and Kincade, 1984). The development of AIHA in these mice therefore is not a result of polyclonal B-cell activation but requires T cells. Young Coombs-negative NZB mice (12–14 weeks old) given nondepleting monoclonal anti-CD4 over several weeks did not develop MRBC autoantibodies (Oliveira et al., 1994). Older female Coombs-positive NZB mice were also given anti-CD4 for several weeks and eventually became Coombs negative. On withdrawal of the anti-CD4 antibodies, anti-MRBC autoantibodies appeared in the young mice and reappeared in the older mice.

Antigen specificity of the CD4 cells in Coombs-positive NZB mice was shown by Perry et al. (1996) to be directed to the membrane glycoprotein Band 3, a specificity also found in human AIHA. In subsequent studies, Elson and Barker (2000) and Shen et al. (1999) demonstrated that the dominant epitope eliciting a CD4 T-cell response was the 861–874 peptide sequence of Band 3.

The transgenic mice (anti-RBC Tg) model developed by Okamoto et al. (1992) has furthered our understanding of B-cell autoreactivity, tolerance and the role of cytokines in AIHA. This mouse model carries the immunoglobulin genes that encode an anti-MRBC autoantibody isolated from the anti-red cell autoantibody-producing hybridoma derived from NZB mice (Ozaki et al., 1984). Approximately half of these mice develop AIHA, with individual mice showing variable phenotypes ranging from severe disease to no disease. The source of anti-RBC autoantibody is B-1 cells found primarily in the peritoneum and lamina propria of these mice. These mice do not have conventional B-cells (B-2 cells), which reside primarily in the bone marrow, spleen, and lymph nodes, as these cells are clonally deleted on exposure to red cells. The B1 cells in the peritoneum, however, do not come into contact with red cells and thus persist. On injection of red cells into the peritoneum of these mice, the B-1 cells are removed through clonal deletion by apoptosis. Administration of lipopolysaccharide (LPS) into asymptomatic anti-RBC Tg mice led to activation of B-1 cells and autoantibody production, followed by AIHA (Murakami et al., 1994). When these mice are raised under germ-free and specific pathogen-free conditions, few peritoneal B-1 cells are found. When these mice are placed in a conventional environment or given LPS, there is activation and differentiation of these B-1 cells into autoantibody-producing cells and subsequent AIHA (Murakami et al., 1997). These studies suggest that the intestinal bacteria play a role in the stimulation of anti-red cell antibody production in these mice, possibly by acting as an adjuvant.

Activation and differentiation of B-1 cells into autoantibody-producing cells was also induced by administration of Th2-cell-derived cytokines (Nisitani et al., 1995). To further define the role of IL-10, Nisitani et al. (1998) crossed $RAG2^{-/-}$ mice with anti-RBC Tg mice, the former lacking mature T and B cells. These recombinant mice have few B-1 antibody-producing cells. The transfer of fetal thymus from C57BL/6 mice into these recombinant mice resulted in proliferation and differentiation of B-1 cells into antibody-producing cells, indicating a requirement for T cells. The requirement for T cells, however, could be bypassed by the administration of LPS, IL-5 or IL-10. LPS was shown to increase serum levels of IL-10. Activation of B-1 cells by LPS, IL-5 or IL10 was blocked by administration of an anti-IL-10 antibody, indicating a pivotal role for this cytokine.

Further studies by Sakiyama et al. (1999) defined the role of IL-5 in the induction of AIHA in anti-RBC Tg mice. IL-5 receptor α chain-deficient mice were crossed with anti-RBC Tg mice. The number of B-1 antibody-producing cells in the recombinant mice was negligible, although the total number of peritoneal B-1 cells was approximately 30% that found in anti-RBC Tg mice. When the recombinant mice were given LPS or IL-10, the differentiation of B-1 cells into antibody-producing cells was greatly reduced; however, these cells still showed spontaneous proliferation. IL-5 therefore was essential for the terminal differentiation of B-1 cell into antibody-producing cells.

Recent studies by Mqadmi et al. (2005) demonstrated a critical role of $CD4^+CD25^+$ regulatory cells in a murine model of AIHA. The investigators utilized the Marshall/Clarke and Playfair murine model in which AIHA is induced by repeatedly immunizing C57/B16 mice with rat RBCs (Playfair and Marshall-Clarke, 1973). These mice develop autoantibodies to MRBCs, as well as alloantibodies to rat RBCs. Approximately 30% of these mice develop AIHA. When these mice are depleted of $CD4^+CD25^+$ T cells by injecting anti-CD25 prior to immunization with rat RBCs, 90% develop AIHA. Transfusion of $CD4^+CD25^+$ T cells but not $CD4^+CD25^-$ T cells, which were purified from the spleens of mice immunized with rat RBCs, completely suppressed autoantibody production. When these mice were subsequently immunized with rat RBCs, they did not make anti-MRBC autoantibodies. They continued to produce anti-rat alloantibodies, indicating that suppression by these cells was autoantigen specific.

CONCLUDING REMARKS

AIHA was the first autoimmune disorder in which autoantibodies were shown to be involved in the pathogenesis of the disease state. Early studies were directed to the characterization of the putative autoantibodies and the mechanisms of how these antibodies produced red cell damage. Later, the specificities of anti-red cell autoantibodies were defined by determining their reactivity with antigens isolated from the red cell membrane. T cells from AIHA patients have been shown to recognize specific blood group antigens. The type of T-cell response and accompa-

nying cytokine profile in AIHA appears to be predominantly Th2, a pattern seen in other autoimmune diseases. Studies have shown abnormal anti-idiotype responses in patients with AIHA, which might lead to a loss of peripheral tolerance. The NZB mouse, transgenic mouse, and other animal models have furthered our understanding of the role of immunoregulatory T cells and the effects of cytokines in AIHA. Selected peptides from red cell membranes have been shown to alter the immune response to one that ameliorates the disease. It is possible that the administration of such peptides might be beneficial in the treatment of AIHA. Recent studies in a mouse model demonstrated the importance of $CD4^+CD25^+$ T reg cells for the control and induction of AIHA. These Tregs offer the possibility of novel treatment intervention. In the treatment of warm type AIHA, the chimeric, human, IgGκ monoclonal antibody (rituximab) specific for the CD20 antigen expressed on the surface of mature B cells has been found to be effective in those patients refractory to conventional treatment. It has also been beneficial to a lesser extent in cold agglutinin disease. As in other autoimmune disorders, the future research in AIHA should be directed to the further understanding of the intricacies of immunoregulation and the influence of genetics and environment on the immune system.

References

Abramson, N., LoBuglio, A.F., Jandl, J.H., and Cotran, R.S. (1970). The interaction between human monocytes and red cells. Binding characteristics. *J. Exp. Med. 132*, 1191–1206.

Ackroyd, J.F. (1949). The pathogenesis of thrombocytopenic purpura due to hypersensitivity to Sedormid (allyisopropyl-acetylcarbamide). *Clin. Sci. 7*, 249–283.

Adderson, E.E., Shackelford, P.G., Insel, R.A., Quinn, A., Wilson, P.M., and Carroll, W.L. (1992). Immunoglobulin light chain variable region gene sequences for human antibodies to *Haemophilus influenzae* type b capsular polysaccharide are dominated by a limited number of V_κ and V_λ segments and VJ combinations. *J. Clin. Invest. 89*, 729–738.

Adderson, E. ., Shackelford, P.G., Quinn, A., Wilson, P.M., Cunningham, M.W., Insel, R.A., and Carroll, W.L. (1993). Restricted immunoglobulin V_H usage and VDJ combinations in the human response to *Haemophilus influenzae* type b capsular polysaccharide. Nucleotide sequences of monospecific anti-*Haemophilus* antibodies and polyspecific antibodies cross-reacting with self antigens. *J. Clin. Invest. 91*, 2734–2743.

Ahn, Y.S. (1990). Efficacy of danazol in hematologic disorders. *Acta Haematol. 84*, 122–129.

Ahn, Y.S., Harrington, W.J., Byrnes, J.J., Pall, L., and McCrainie, J. (1983). Treatment of autoimmune hemolytic anemia with Vinca-loaded platelets. *JAMA 249*, 2189–2194.

Ahrens, N., Kingreen, D., Seltsam, A., and Salama, A. (2001). Treatment of refractory autoimmune haemolytic anaemia with anti-CD20 (rituximab) [letter]. *Br. J. Haematol. 114*, 244–245.

Allgood, J.W. and Chaplin, H., Jr. (1967). Idiopathic acquired autoimmune hemolytic anemia. A review of forty-seven cases treated from 1955 through 1965. *Am. J. Med. 43*, 254–273.

Anderson, R.A. and Lovrien, R.E. (1984). Glycophorin is linked by band 4.1 protein to the human erythrocyte membrane skeleton. *Nature (Lond.) 307*, 655–658.

Andrzejewski, C., Jr., Gault, E., Briggs, M., and Silberstein, L. (1988). Benefit of a 37°C extra-corporeal circuit in plasma exchange therapy for selected cases with cold agglutinin disease. *J. Clin. Apheresis 4*, 13–17.

Andrzejewski, C., Jr., Young, P.J., Cines, D.B., and Silberstein, L.E. (1991). Heterogeneity of human red cell autoantibodies assessed by isoelectric focusing. *Transfusion 31*, 236–244.

Arvieux, J., Schweizer, B., Roussel, B., and Colomb, M.G. (1991). Autoimmune haemolytic anaemia due to anti-phospholipid antibodies. *Vox Sang. 61*, 190–195.

Atkinson, J. . and Frank, M.M. (1974). Studies on the *in vivo* effects of antibody. Interactions of IgM antibody and complement in the immune clearance and destruction of erythrocytes in man. *J. Clin. Invest. 54*, 339–348.

Austin III, H.A., 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.

Avent, N., Judson, P.A., Parsons, S.F., Mallinson, G., Anstee, D.J., Tanner, M.J., Evans, P.R., Hodges, E., Maciver, A.G., and Holmes, C. (1988). Monoclonal antibodies that recognize different membrane proteins that are deficient in Rh_{null} human erythrocytes. *Biochem. J. 251*, 499–505.

Bakemeier, R.F. and Leddy, J.P. (1968). Erythrocyte autoantibody association with alpha-methyldopa: Heterogeneity of structure and specificity. *Blood 31*, 1–14.

Barcellini, W., Clerici, G., Montesano, R., Taioli, E., Morelati, F., Rebulla, P., and Zanella, A. (2000). *In vitro* quantification of anti-red blood cell antibody production in idiopathic autoimmune haemolytic anaemia: effect of mitogen and cytokine stimulation. *Br. J. Haematol. 111*, 452–460.

Barker R.N. and Elson, C.J. (1994). Multiple self-epitopes on the Rhesus polypeptides stimulate immunologically ignorant human T-cells *in vitro*. *Eur. J. Immunol. 24*, 1578–1582.

Barker, R.N. and Elson, C.J. (1995). Red blood cell glycophorins as B and T-cell antigens in canine autoimmune haemolytic anaemia. *Vet. Immunol. Immunopathol. 47*, 225–238.

Barker, R.N., Casswell, K.M., Reid, M.E., Sokol, R.J., and Elson, C.J. (1992). Identification of autoantigens in autoimmune haemolytic anaemia by a nonradioisotope immunoprecipitation method. *Br. J. Haematol. 82*, 126–132.

Barker, R.N., Hall, A.M., Standen, G.R., Jones, J., and Elson, C.J. (1997). Identification of T-cell epitopes on the rhesus polypeptides in autoimmune hemolytic anemia. *Blood 90*, 2701–2715.

Bell, C.A. and Zwicker, H. (1978). Further studies on the relationship of $antiEn^a$ and $antiWr^b$ in warm autoimmune hemolytic anemia. *Transfusion 18*, 572–575.

Berentsen, S., Ulvestad, E., Gjertsen, B.T., Hjorth-Hansen, H., Landholm, R., Knutsen, H, Ghanima, W., Shammas, F.V., and Tjonnfjord, G.E. (2004). Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. *Blood 103*, 925–2928.

Berger, M., Gaither, I.A., and Frank, M.M. (1981–1982). Complement receptors. *Clin. Immunol. Rev. 1*, 471–545.

Bielschowsky, M., Helyer, B.J., and Howie, J.B. (1959). Spontaneous hemolytic anaemia in mice of the NZB/BL strain. *Proc. Univ. Otago Med. Sch. 37*, 9–11.

Bird, G.W., Wingham, J., Martin, A.J., Richardson, S.G., Cole, A.P., Payne, R.W., and Savage, B.F. (1976). Idiopathic non-syphilitic paroxysmal cold haemaglobinuria in children. *J. Clin. Pathol. 29*, 215–218.

Blanchette, V.S., Kirby, M.A., and Turner, C. (1992). Role of intravenous immunoglobulin G in autoimmune hemolytic disorders. *Semin. Hematol. 29*(Suppl. 2), 72–82.

Boorman, K.E., Dodd, B.B., and Loutit, J.F. (1946). Haemolytic icterus (acholuric jaundice), congenital and acquired. *Lancet 2*, 812–814.

Bowdler, A.J. (1976). The role of the spleen and splenectomy in autoimmune hemolytic disease. *Semin. Hematol. 13*, 335–348.

Branch, D.R., Shulman, I.A., Sy Siok Hian, A. ., and Petz, L.D. (1984). Two distinct categories of warm autoantibody reactivity with age-fractionated red cells. *Blood 63*, 177–180.

Brown, D.L., Lachmann, P.J., and Dacie, J.V. (1970). The vivo behaviour of complement-coated red cells: Studies in C6-deficient, C3-depleted and normal rabbits. *Clin. Exp. Immunol. 7*, 401–421.

Bussel, J.B., Cunningham-Rundles, C., and Abraham, C. (1986). Intravenous treatment of autoimmune hemolytic anemia with very high dose gammaglobulin. *Vox Sang. 51*, 264–269.

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. Autoimmunity 3*, 773–787.

Capra, J.D., Dowling, P., Cook, S., and Kunkel, H.G. (1969). An incomplete cold-reactive gamma G antibody with i specificity in infectious mononucleosis. *Vox Sang. 16*, 10–17.

Capra, J.D., Kehoe, J.M., Williams, R.C., Feizi, T., and Kunkel, H.G. (1972). Light chain sequences of human IgM cold agglutinins. *Proc. Natl. Acad. Sci. USA 69*, 40–43.

Celano, M.J. and Levine, P. (1967). Anti-LW specificity in autoimmune acquired hemolytic anemia. *Transfusion 7*, 265–268.

Chan, A.C. and Sack, K. (1991). Danazol therapy in autoimmune hemolytic anemia associated with systemic lupus erythematosus. *J. Rheumatol. 18*, 280–282.

Chang, C.S., Li, C.Y., Liang, Y.H., and Cha, S.S. (1993). Clinical features and splenic pathologic changes in patients with autoimmune hemolytic anemia and congenital hemolytic anemia. *Mayo Clin. Proc. 68*, 757–762.

Chanock, R.M., Mufson, M.A., Bloom, H.H., James, W.D., Fox, H.H., and Kingston, J.R. (1961). Eaton agent pneumonia. *JAMA 175*, 213–220.

Chaplin, H. (1973). Clinical usefulness of specific antiglobulin reagents in autoimmune hemolytic anemias. In E. B. Brown (ed.), *Progress in Hematology*, Vol. 8 (pp.25–49). New York: Grune & Stratton.

Chaplin, H. (1990). Red cell-bound immunoglobulin as a predictor of severity of hemolysis in patients with autoimmune hemolytic anemia (editorial). *Transfusion 30*, 576–577.

Chauffard, M.A. and Troisier, J.(1908). Anémie grave avec hemolysine dans le serum; ictère hémolysinique. *Sem. Med. 28,* 345.

Chauffard, M.A. and Vincent, C. (1909). Hémoglobinurie hémolysinique a ictère polycholique aligu. *Sem. Med. 29,* 601.

Christensen, B.E. (1973). The pattern of erythrocyte sequestration in immunohaemolysis: Effects of prednisone treatment and splenectomy. *Scand. J. Haematol. 10*, 120–129.

Coombs, R.R.A., Mourant, A.E., and Race, R.R. (1945). A new test for the detection of weak and "incomplete" Rh agglutinins. *Lancet 2*, 15–16.

Costea, N., Yakulis, V.J., and Heller, P. (1966). Light-chain heterogeneity of cold agglutinins. *Science 152*, 1520–1521.

Costea, N., Yakulis, V.J., and Heller, P. (1971). The mechanism of induction of cold agglutinins by *Mycoplasma pneumoniae*. *J. Immunol. 106*, 598–604.

Coutinho, A., Kazatchkine, M.D., and Avrameas. S. (1995). Natural autoantibodies. *Curr. Opin. Immunol. 7*, 812–818.

Crisp, D. and Pruzanski, W. (1982). B-cell neoplasms with homogeneous cold-reacting antibodies (cold agglutinins). *Am. J. Med. 72*, 915–922.

Dacie, J.V. (1951). Differences in the behavior of sensitized red cells to agglutination by antiglobulin sera. *Lancet 261*, 954–955.

Dacie, J.V. (1962). The haemolytic anemias, congenital and acquired: Part II. In *The Autoimmune Haemolytic Anaemias*, 2nd ed. (pp. 666–671). New York: Grune & Stratton.

Dacie, J.V. (1975). Autoimmune hemolytic anemia. *Arch. Intern. Med. 135*, 1293–1300.

Dacie, J.V. and Mollison, P.L. (1943). Survival of normal erythrocytes after transfusion to patients with familial haemolytic anemia (acholuric jaundice). *Lancet 1*, 550–552.

Dacie, J.V. and Worlledge, S.M. (1969). Auto-immune hemolytic anemias. In E. B. Brown and C. V. Moore (eds), *Progress in Hematology*, Vol. 6 (p. 82). New York: Grune & Stratton.

Dahr, W., Kordowicz, M., Moulds, J., Gielen, W., Lebeck, L., and Kruger, J. (1987). Characterization of the Ss sialoglycoprotein and its antigens in Rh_{null} erythrocytes. *Blut 54*, 13–24.

Dameshek, W. and Schwartz, S.O. (1938). The presence of hemolysins in acquired hemolytic anemia. *N. Engl. J. Med. 218*, 75–80.

Dausset, J. and Colombani, J. (1959). The serology and the prognosis of 128 cases of autoimmune hemolytic anemia. *Blood 14*, 1280–1301.

Donath, J. and Landsteiner, K. (1904). Über paroxysmale Hämoglobinurie. *München Med. Wochenschr. 51*, 1590–1593.

Donath, J. and Landsteiner, K. (1925). Über Kältehämoglobinurie. *Ergeb. Hyg. Bakteriol. 7*, 184.

Dubarry, M., Charron, C., Habibi, B., Bretagne, Y., and Lambin, P. (1993). Quantitation of immunoglobulin classes and subclasses of autoantibodies bound to red cells in patients with and without hemolysis. *Transfusion 33*, 466–471.

Elson, C.J. and Barker, R.N. (2000). Helper T cells in antibody-mediated, organ-specific autoimmunity. *Curr. Opin. Immunol. 12*, 664–669.

Elson, C.J., Barker, R.N., Thompson, S.J., and Williams, N.A. (1995). Immunologically ignorant T-cells, epitope spreading and repertoire limitation. *Immunol. Today 16*, 71–76.

Engelfriet, C.P., von dem Borne, A.E., Beckers, D., van der Meulen, F.W., Fleer, A., Roos, D., and Ouwehand, W.H. (1981). Immune destruction of red cell. In C. A. Bell (ed.) *A Seminar on Immune-Mediated Cell Destruction*. Washington, DC: American Association of Blood Banks.

Engelfriet, C. ., Beckers, D., van't Veer, M.B., von dem Borne, A.E., and Ouwehand, W.H. (1982). Recent advances in immune haemolytic anemia. In S.R. Hollan (ed.), *Recent Advances in Haematology* (pp. 235–251). Budapest: Akademia Kiado.

Evans, R.S. and Duane, R.T. (1949). Acquired hemolytic anemia: Relation of erythrocyte antibody to activity of the disease; significance of thrombocytopenia and leukopenia. *Blood 4*, 1196–1213.

Evans, R.S., Turner, E., and Bingham, M. (1967). Chronic hemolytic anemia due to cold agglutinins: The mechanism of resistance of red cells to C' hemolysis by cold agglutinins. *J. Clin. Invest. 46*, 1461–1474.

Evans, R.S., Turner, E., Bingham, M., and Woods, R. (1968). Chronic hemolytic anemia due to cold agglutinins. II. The role of C1 in red cell destruction. J. *Clin. Invest. 47*, 691–701.

Evans, R.S., Baxter, E., and Gilliland, B.C. (1973). Chronic hemolytic anemia due to cold agglutinins: A 20-year history of benign gammopathy with response to chlorambucil. *Blood 42*, 463–470.

Ewing, D. ., Hughes, C. ., and Wardle, D.F. (1968). Methyldopa-induced autoimmune haemolytic anaemia—a report of two further cases. *Guy's Hosp. Rep. 117*, 111–118.

Fagiolo, E. and Toriani-Terenzi, C. (2002). Th1 and Th2 cytokine modulation by IL-10/IL-12 imbalance in autoimmune haemolytic anaemia (AIHA). *Autoimmunity 35*, 39–44.

Fearon, D.T. and Wong, W.W. (1983). Complement ligand receptor interactions that mediate biological responses. *Annu. Rev. Immunol. 1*, 243–271.

Feizi, T. (1967). Lambda chains in cold agglutinins. *Science 156*, 1111–1112.

Feizi, T. (1977). Immunochemistry of the Ii blood group antigens. In J. F. Mohn, R. W. Plunkett, R. K. Cunningham, and R. M. Lambert (eds), Human Blood Groups (pp. 164–171). Basel: Karger.

Feizi, T. and Schumacher, M. (1968). Light chain homogeneity of post-infective cold agglutinins. *Clin. Exp. Immunol. 3*, 923–929.

Feizi, T., Taylor-Robinson, D., Shields, M.D., and Carter, R.A. (1969). Production of cold agglutinins in rabbits immunized with human erythrocytes treated with *Mycoplasma pneumoniae*. *Nature (Lond.) 222*, 1253–1256.

Fleer, A., Koopman, M.G., von dem Borne, A.E., and Engelfriet, C.P. (1978). Monocyte-induced increase in osmotic fragility of human red cells sensitized with anti-D alloantibodies. *Br. J. Haematol. 40*, 439–446.

Flores, G., Cunningham-Rundles, C., Newland, A.C., and Bussel, J.B. (1993). Efficacy of intravenous immunoglobulin in the treatment of autoimmune hemolytic anemia: Results in 73 patients. *Am. J. Hematol. 44*, 237–242.

Frank, M.M. (1988). The complement system. In M. Samter, D.W. Talmage, M.M. Frank, K.F. Austen, and H.N. Claman (eds), *Immunologic Diseases*, 4th edn (pp. 203–232). Boston: Little, Brown.

Friedman, H.D. and Dracker, R.A. (1992). Cold agglutinin disease after chicken pox. An uncommon complication of a common disease. *Am. J. Clin. Pathol. 97*, 92–96.

Fujita, T., Inoue, T., Ogawa, K., Iida, K., and Tamura, N. (1986). The mechanism of action of decay-accelerating factor (DAF). DAF inhibits the assembly of C3 convertases by dissociating C2a and Bb. *J. Exp. Med. 166*, 1221–1228.

Galili, U., Flechner, I., Knyszynski, A., Danon, D., and Rachmilewitz, E.A. (1986). The natural anti-α-galactosyl IgG on human normal senescent red blood cells. *Br. J. Haematol. 62*, 317–324.

Garratty, G. (1991). Effect of cell-bound proteins on the *in vivo* survival of circulating blood cells. *Gerontology 37*, 68–94.

Garratty, G. (1994). Autoimmune hemolytic anemia. In G. Garratty (ed.) *Immunobiology of Transfusion Medicine* (pp. 497–499). New York: Dekker.

Garratty, G. and Nance, S.J. (1990). Correlation between *in vivo* hemolysis and the amount of red cell-bound IgG measured by flow cytometry. *Transfusion 30*, 617–621.

Garratty, G. and Petz, L.D. (1975). Drug-induced hemolytic anemia. *Am. J. Med. 58*, 398–407.

Garratty, G., Arndt, P., Prince, H.E., and Shulman, I.A. (1993). The effect of methyldopa and procainamide on suppressor cell activity in relation to red cell autoantibody production. *Br. J. Haematol. 81*, 579–584.

Gilliland, B.C., Baxter, E., and Evans, R.S. (1971). Red-cell antibodies in acquired hemolytic anemia with negative antiglobulin serum tests. *N. Engl. J. Med. 285*, 252–256.

Gilliland, B.C. (1996). Autoimmune hemolytic anemia. In E.C. Rossi, T.L. Simon, G.S. Moss, and S.A. Gould (eds) *Principles of Transfusion Medicine*. Balimore: Williams and Wilkins.

Goldberg, L.S. and Barnett, E.V. (1967). Mixed gamma G-gamma M cold agglutinin. *J. Immunol. 99*, 803–809.

Göttsche, B., Salama, A., and Mueller-Eckhardt, C. (1990). Donath–Landsteiner autoimmune hemolytic anemia in children. A study of 22 cases. *Vox Sang. 58*, 281–286.

Green, F.A., Jung, C.Y., Rampal, A., and Lorusso, D.J. (1980). Alpha-methyldopa and the erythrocyte membrane. *Clin. Exp. Immunol. 40*, 554–560.

Guilbert, B., Digheiro, G., and Avrameas, S. (1982). Naturally occurring antibodies against nin common antigens in normal human sera. 1. Detection, isolation and characterization. *J. Immunol. I28*, 2779–2787.

Gupta, N., Kavuru, S., Patel, D., Janson, D., Driscoll, N., Ahmed, S., and Rai, K.R. (2002). Rituximab-based chemotherapy for steroid-refractory autoimmune hemolytic anemia of chronic lymphocytic leukemia. *Leukemia 16*, 2092–2095.

Habibi, B., Homberg, J.C., Schaison, G., and Salmon, C. (1974). Autoimmune hemolytic anemia in children. A review of 80 cases. *Am. J. Med. 56*, 61–69.

Harboe, M., Muller-Eberhard, H.I., Fudenberg, H.H., Polley, M.J., and Mollison, P.L. (1963). Identification of the components of complement participating in the anti-globulin reaction. *Immunology 6*, 412–420.

Harboe, M., van Furth, R., Schubothe, H., Lind, K., and Evans, R.S. (1965). Exclusive occurrence of K chains in isolated cold haemagglutinins. *Scand. J. Hematol. 2*, 259.

Henry, R.E., Goldberg, L.S., Sturgeon, P., and Ansel, R.D. (1971). Serologic abnormalities associated with L-dopa therapy. *Vox Sang. 20*, 306–316.

Hershko, C., Sonnenblick, M., and Ashkenazi, J. (1990). Control of steroid-resistant autoimmune haemolytic anaemia by cyclosporine. *Br. J. Haematol. 76*, 436–437.

Hillen, H.F., and Bakker, S.J. (1994). Failure of interferon-α-2b therapy in chronic cold agglutinin disease [letter]. *Eur. J. Haematol. 53*, 242–243

Hillman, R.S. and Giblett, E.R. (1965). Red cell membrane alteration associated with "marrow stress." *J. Clin. Invest. 44*, 1730–1736.

Hippe, E., Jensen, K.B., Olesen, H., Lind, K., and Thomsen, P.E. (1970). Chlorambucil treatment of patients with cold agglutinin syndrome. *Blood 35*, 68–72.

Huber, H., Polley, M.J., Linscott, W.D., Fudenberg, H.H., and Muller Eberhard, H.J. (1968). Human monocytes: Distinct receptor sites for the third component of complement and for immunoglobulin G. *Science 162*, 1281–1283.

Huber, H., Douglas, S.D., Nusbacher, J., Kochwa, S., and Rosenfield, R. E. (1971). IgG subclass specificity of human monocyte receptor sites. *Nature (Lond.) 229*, 419–420.

Hurez, V., Kaveri, S-V., and Kazathchkine, M.D. (1993). Expression and control of the natural autoreactive IgG repertoire in normal human serum. *Eur. J. Immunol. 23*, 783–789.

Issitt, P.D., Pavone, B.G., Goldfinger, D., Zwikder, H., Issitt, C.H., Tessel, J.A., Kroovand, S.W., and Bell, C.A. (1976). AntiWr^b and other autoantibodies responsible for positive direct antiglobulin tests in 150 individuals. *Br. J. Haematol. 34*, 5–18.

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.

Jandl, J.H., Jones, A.R., and Castle, W.B. (1957). The destruction of red cells by antibodies in man. *J. Clin. Invest. 36*, 1428–1459.

Janney. F.A., Lee, L.T., and Howe. C. (1978). Cold hemaglutinin cross-reactivity with *Mycoplasma pneumoniae*. *Infect. Immun. 22*, 29–33.

Jenkins, W.J., Marsh, W.L., Noades, J., Tippett, P., Sanger, R., and Race, R.R. (1960). The I antigen and antibody. *Vox Sang. 5*, 97–106.

Jenkins, W.J., Koster, H.G., Marsh, W.L., and Carter, R.L. (1965). Infectious mononucleosis: an unsuspected source of anti-I. *Br. J. Haematol. 11*, 480–483.

Jennings, M.L. (1984). Oligomeric structure and the anion transport function of human erythrocyte Band 3 protein. *J. Membr. Biol. 80*, 105–117.

Jyonouchi, H. and Kincade, P.W. (1984). Precocious and enhanced functional maturation of B lineage cells in New Zealand Black mice during embryonic development. *J. Exp. Med. 159*, 1277–1282.

Kay, M.M. (1975). Mechanism of removal of senescent cells by human macrophages *in situ*. *Proc. Natl. Acad. Sci. USA 72*, 3521–3525.

Kay, N.E. and Douglas, S.D. (1977). Monocyte–erythrocyte interaction *in vitro* in immune hemolytic anemias. *Blood 50*, 889–897.

Kay, M.M.B. (1986). Senescent cell antigen: A red cell aging antigen. In 'Red Cell Antigens and Antibodies' (G. Garrity ed) pp 173–198. American Association of Blood Banks, Arlington, Virginia.

Kay, M.M.B. (1994). Cellular and monocular biology of senescent cell antigen. In 'Immunobiology of Transfusion Medicine' (G. Garrity, ed) pp 173–198. Dekker, New York.

Kelton, J.G. (1985). Impaired reticuloendothelial function in patients treated with methyldopa. *N. Engl. J. Med. 313*, 596–600.

Kinoshita, T., Medof, M.E., and Nussenzweig, V. (1986). Endogenous association of decay-accelerating factor (DAF) with C4b and C3b on cell membranes. *J. Immunol. 136*, 3390–3395.

Kirtland III, 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*, 825–832.

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.

Lachmann, P.J., Pangburn, M.K., and Oldroyd, R.G. (1982). Breakdown of C3 after complement activation. Identification of a new fragment, C3g, using monoclonal antibodies. *J. Exp. Med. 156*, 205–216.

Landsteiner, K. (1903). Über Beziehungen zwischen dem Blutserum und den Körperzellen. *München. Med. Wochenschr. 40*, 1812.

Landsteiner, K. and Levine, P. (1926). On the cold agglutinins in human serum. *J. Immunol. 12*, 441.

Lau, F.O. and Rosse, W.F. (1975). The reactivity of red blood cell membrane glycophorin with "cold-reacting" antibodies. *Clin. Immunol. Immunopathol. 4*, 1–8.

Lau, P., Sererat, S., Moore, V., McLeish, K., and Alousi, M. (1983). Paroxysmal cold hemoglobinuria in a patient with *Klebsiella* pneumonia. *Vox Sang. 44*, 167–172.

Leddy, J.P. and Bakemeier, R.F. (1965). Structural aspects of human erythrocyte autoantibodies. I. L chain types and electrophoretic dispersion. *J. Exp. Med. 121*, 1–17.

Leddy, J.P., Peterson, P., Yeaw, M.A., and Bakemeier, R.F. (1970). Patterns of serologic specificity of human G erythrocyte autoantibodies. *J. Immunol. 105*, 677–686.

Leddy, J.P., Falany, J.L., Kissel, G.E., Passador, S.T, and Rosenfeld, S.I. (1993). Erythrocyte membrane proteins reactive with human (warm-reacting) anti-red cell autoantibodies. *J. Clin. Invest. 91*, 1672–1680.

Leddy, J.P., Wilkinson, S.L., Kissel, G.E., Passador, S.T., Falany, J.L., and Rosenfeld, S.I. (1994). Erythrocyte membrane proteins reactive with IgG (warm-reacting) anti-red blood cell autoantibodies: II. Antibodies coprecipitating Band 3 and glycophorin A. *Blood 84*, 650–656.

Leickly, F.E. and Buckley, R.H. (1987). Successful treatment of autoimmune hemolytic anemia in common variable immunodeficiency with high-dose intravenous gamma globulin. *Am. J. Med. 82*, 159–162.

Levine, B. and Redmond, A. (1967). Immunochemical mechanisms of penicillin induced Coombs positivity and hemolytic anemia in man. *Int. Arch. Allergy Appl. Immunol. 31*, 594–606.

Levine, P., Celano, M.J., and Falkowski, F. (1965). The specificity of the antibody in paroxysmal cold hemaglobuinuria (P.C.H.) *Ann N.Y. Acad. Sci. 124,* 456–461.

Linstrom, F.D., Lieden, G., and Enstrom, M.S. (1977). Dose-related levodopa-induced haemolytic anaemia. *Ann. Intern. Med. 86*, 298–300.

Litwin, S.D., Balaban, S., and Eyster, M.E. (1973). Gm allotype preference in erythrocyte IgG antibodies of patients with autoimmune hemolytic anemia. *Blood 42*, 241–246.

LoBuglio, A.F. and Jandl, J.H. (1967). The nature of alpha-methyldopa red cell antibody. *N. EngI. J. Med. 276*, 658–665.

Loomis, L.M., Uemura, K., Childs, R.A., Paulson, J.C., Rogers, G.N., Scudder, P.R., Michalski, J.-C., Hounsell, E.F., Taylor-Robinson, D., and Feizi, T. (1983). Erythrocyte receptors for *Mycoplasma pneumoniae* are sialylated oligosaccharides of Ii antigen type. *Nature (Lond.) 307*, 560–563.

Loutit, J.F. and Mollison, P.L. (1946). Haemolytic icterus (acholuric jaundice), congenital and acquired. *J. Pathol. Bacteriol 58*, 711–728.

Lutz, H.U. and Wipf, G. (1982). Naturally occurring autoantibodies to skeletal proteins from human red blood cells. *J. Immunol. 128*, 1695–1699.

Lutz, H.U., Flepp, R., and Stringaro-Wipf, G. (1984). Naturally occurring autoantibodies to exoplasmic and cryptic regions of band 3 protein, the major integral membrane protein of human red blood cells. *J. Immunol. 133*, 2610–2618.

Macintyre, E.A., Linch, D.C., Macey, M.G., and Newland, A.C. (1985). Successful response to intravenous immunoglobulin in autoimmune haemolytic anaemia. *Br. J. Haematol. 60*, 387–388.

Marsh, W.L. (1961). Anti-Ii a cold antibody defining the I's relationship in human red cells. *Br. J. Haematol. 7*, 200–209.

Marsh, W.L. and Jenkins, W.J. (1960). Anti-li: A new cold antibody. *Nature (Lond.) 188*, 753.

Marsh, W.L., Reid, M.E., and Scott, E.P. (1972). Autoantibodies of U blood group specificity in autoimmune haemolytic anaemia. *Br. J. Haematol. 22*, 625–629.

Marsh, W.L., Oyea, R., Alicia, E., Linter, M., and Horton, S. (1979). Autoimmune hemolytic anemia and the Kell blood groups. *Am. J. Hematol. 7,* 155–162.

Mollison, P.L., Crome, P., Hughes-Jones, N.C., and Rochna, E. (1965). Rate of removal from the circulation of red cells sensitized with different amounts of antibody. *Br. J. Haematol. 11*, 461–470.

Mollison, P.L., Engelfriet, C.P., and Contreras, M. (1993a). *Blood Transfusion in Clinical Medicine*, 9th edn (pp. 304–305). Oxford: Blackwell.

Mollison, P.L., Engelfriet, C.P., and Contreras, M. (1993b). Blood Transfusion in Clinical Medicine, 9th edn (p. 289). Oxford: Blackwell.

Moutsopoulos, H.M., Boehm-Truitt, M., Kassan, S.S., and Chused, T.M. (1977). Demonstration of activation of B lymphocytes in New Zealand black mice at birth by an immunoradiometric assay for murine IgM. *J. Immunol. 119*, 1639–1644.

Mqadmi, A., Zheng, X., and Yazdanbakhsh, K. (2005). $CD4^+CD25^+$ regulatory T cells control induction of autoimmune hemolytic anemia. *Blood 105*, 3746–3748.

Mueller-Eckhardt, C. and Kretschmer, V. (1972). Immunosuppressive therapy in blood disease. In *Immunosuppressive Therapy: Proceedings of the International Wiesbaden Symposium* (p. 85). New York: Schwabe.

Mueller-Eckhardt, C. and Salama, A. (1990). Drug-induced immune cytopenias: A unifying pathogenetic concept with special emphasis on the role of drug metabolites. *Transfus. Med. Rev. 4*, 69–77.

Murakami, M., Tsubata, T., Shinkura, R., Nisitani, S., Okamoto, M., Yoshioka, H., Usui, T., Miyawaki, S., and Honjo, T. (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*, 111–121.

Murakami, M., Nakajima, K., Yamazaki, K., Muraguchi, T., Serikawa, T., and Honjo T. (1997). Effects 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.

Murphy, S. and LoBuglio, A.F. (1976). Drug therapy of autoimmune hemolytic anemia. *Semin. Hematol. 13*, 323–334.

Nisitani, S., Tsubata, T., Murakami, M., and Honjo, T. (1995). Administration of interleukin-5 or -10 activates peritoneal B-1 cells and induces autoimmune hemolytic anemia in anti-erythrocyte autoantibody-transgenic mice. *Eur. J. Immunol. 25*, 3047–3052.

Nisitani, S., Sakiyama, T., and Honjo, T. (1998). Involvement of IL–10 in induction of autoimmune hemolytic anemia in anti-erythrocyte Ig transgenic mice. *Int. Immunol. 10*, 1039–1047.

O'Connor, B.M., Clifford, J.S., Lawrence, W.D., and Logue, G.L. (1989). Alpha-interferon for severe cold agglutinin disease. *Ann. Intern. Med. 111*, 255–256.

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.

Oliveira, G.G.S., Hutchings, P.R., Roitt, I.M., and Lydyard, P.M. (1994). Production of erythrocyte autoantibodies in NZB mice is inhibited by CD4 antibodies. *Clin. Exp. Immunol. 96*, 297–302.

Ozaki, S., Nagasawa, R., Sato, H., and Shirai, T. (1984). Hybridoma autoantibodies to erythrocytes from NZB mice and the induction of hemolytic anemia. *Immunol. Lett. 8*, 115–119.

Panceri, R., Fraschini, D., Tornotti, G., Masera, G., Locasciulli, A., and Bacigalupo, A. (1992). Successful use of high-dose cyclophosphamide in a child with severe autoimmune hemolytic anemia. *Haematologica 77*, 76–78.

Pascual, V., Victor, K., Spellerberg, M., Hamblin, T.J., Stevenson, F.K., and Capra, J.D. (1992). V_H restriction among human cold agglutinins. The V_H4–2l gene segment is required to encode anti-I and anti-i specificities. *J. Immunol. 149*, 2337–2344.

Perry, F.E., Barker, R.N., Mazza, G., Day, M.J., Wells, A.D., Shen, C.R., Schofield, A.E., and Elson, C.J. (1996). Autoreactive T cell specificity in autoimmune hemolytic anemia of the NZB mouse. *Eur. J. Immunol. 26*, 136–141.

Petz, L.D., and Branch, D.R. (1983). Serological tests for the diagnosis of immune hemolytic anemias. In R. McMillan (ed.) *Methods in Haematology: Immune Cytopenias* (p. 24). New York: Churchill Livingstone.

Petz, L.D. and Garratty, G. (1980a). *Acquired Immune Hemolytic Anemias* (p. 177). New York: Churchill Livingstone.

Petz, L.D. and Garratty, G. (1980b). *Acquired Immune Hemolytic Anemias* (pp. 396–403). New York: Churchill Livingstone.

Petz, L.D. and Garratty, G. (1980c). *Acquired Immune Hemolytic Anemias* (p. 39). New York: Churchill Livingstone.

Petz, L.D. and Garratty, G. (1980d). *Acquired Immune Hemolytic Anemias* (pp. 213–223). New York: Churchill Livingstone.

Petz, L.D., and Garratty, G. (1980e). *Acquired Immune Hemolytic Anemias* (pp. 267–304). New York: Churchill Livingstone.

Pignon, J.-M., Poirson, E., and Rochant, H. (1993). Danazol in autoimmune haemolytic anaemia. *Br. J. Haematol. 83*, 343–345.

Pirofsky, B. (1969). *Autoimmunization and the Autoimmune Hemolytic Anemias*. Baltimore: Williams & Wilkins.

Playfair, J.H. and Marshall-Clarke, S. (1973). Induction of red cell autoantibodies in normal mice. *Nat. New Biol. 243*, 213–214.

Pruzanski, W. and Shumak, K.H. (1977). Biologic activity of cold-reacting autoantibodies (second of two parts). *N. Engl. J. Med. 297*, 583–589.

Pruzanski, W., Cowan, D.H., and Parr, D.M. (1974). Clinical and immunochemical studies of IgM cold agglutinins with lambda type light chains. *Clin. Immunol. Immunopathol. 2*, 234–245.

Race, R.R. and Sanger, R. (1975). *Blood Groups in Man*, 6th edn. Oxford: Blackwell.

Roelcke, D. (1974). Cold agglutination. Antibodies and antigens. *Clin. Immunol. Immunopathol. 2*, 266–280.

Roelcke, D. and Kreft, H. (1984). Characterization of various anti-Pr cold agglutinins. *Transfusion 24*, 210–213.

Roelcke, D., Ebert, W., and Feizi, T. (1974). Studies on the specificities of two IgM lambda cold agglutinins. *Immunology 27*, 879–886.

Rollins, S.A. and Sims, P.J. (1990). The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. *J. Immunol. 144*, 3478–3483.

Rollins, S.A., Zhao, J., Ninomiya, H., and Sims, P.J. (1991). Inhibition of homologous complement by CD59 is mediated by a species-selective recognition conferred through binding to C8 within C5b-8 or C9 within C5b-9. *J. Immunol. 146*, 2345–2351.

Rosenfield, R.E. and Jagathambal (1976). Transfusion therapy for autoimmune hemolytic anemia. *Semin. Hematol. 13*, 311–321.

Rosenfield, R.E., Schmidt, P.J., Calvo, R.C., and McGinnis, M.H. (1965). Anti-i, a frequent cold agglutinin in infectious mononucleosis. *Vox Sang. 10*, 631–634.

Rosse, W.F. (1971). Quantitative immunology of immune hemolytic anemia. II. The relationship of cell-bound antibody to hemolysis and the effect of treatment. *J. Clin. Invest. 50*, 734–743.

Rosse, W.F. and Adams, J.P. (1980). The variability of hemolysis in the cold agglutinin syndrome. *Blood 56*, 409–416.

Rosse, W.F. and Lauf, P.K. (1970). Reaction of cold agglutinins with I antigen solubilized from human red cells. *Blood 36*, 777–784.

Rousey, S.R. and Smith, R.E. (1990). A fatal case of low titer anti-PR cold agglutinin disease. *Am. J. Hematol. 35*, 286–287.

Sakiyama, T., Ikuta, K., Nisitani, S., Takatsu, K., and Honjo, T. (1999). Requirement of IL-5 for induction of autoimmune hemolytic anemia in anti-red blood cell autoantibody transgenic mice. *Int. Immunol. 11*, 995–1000.

Salama, A. and Mueller-Eckhardt, C. (1992). Immune-mediated blood cell dyscrasias related to drugs. *Semin. Hematol 29*, 54–63.

Sato, S.B. and Ohnishi, S. (1983). Interaction of a peripheral protein of the erythrocyte membrane, band 4.1, with phosphatidylserine-containing liposomes and erythrocyte inside-out vesicles. *Eur. J. Biochem. 130*, 19–25.

Schreiber, A.D. (1977). Clinical immunology of the corticosteroids. *Prog. Clin. Immunol. 3*, 103–114.

Schreiber, A.D. and Frank, M.M. (1972). Role of antibody and complement in the immune clearance and destruction of erythrocytes. II: Molecular nature of IgG and IgM complement fixing sites and effects of their interaction with serum. *J. Clin. Invest. 52*, 583–589.

Schreiber, A.D., Parsons, J., McDermott, P., and Cooper, R.A. (1975). Effect of corticosteroids on the human monocyte IgG and complement receptors. *J. Clin. Invest. 56*, 1189–1197.

Schreiber, A.D., Herskovitz, B.S., and Goldwein, M. (1977). Low-titer cold hemagglutinin disease: Mechanism of hemolysis and response to corticosteroids. *N. Engl. J. Med. 296*, 1490–1494.

Schubothe, H. (1952). Antikorperbedingte hamolytische Anamien. *Verh. Dtsch. Ges. Inn. Med. 58*, 679.

Schubothe, H. (1966). The cold hemagglutinin disease. *Semin. Hematol. 3*, 27–47.

Schwarting, G.A., Kundu, S.K., and Marcus, D.M. (1979). Reaction of antibodies that cause paroxysmal cold hemoglobinuria (P.C.H.) with globoside and Forrsman glycosphingolipids. *Blood 53*, 186–192.

Scott, G.L, Myles, A.B., and Bacon, P.A. (1968). Autoimmune haemolytic anaemia and mefenamic acid therapy. *BMJ 3*, 534–535.

Shen, C.R., Wraith, D.C., and Elson, C.J. (1999). Splenic but not thymic autoreactive T cells from New Zealand Black mice respond to a dominant erythrocyte Band 3 peptide. *Immunology 96*, 595–599.

Silberstein, L.E., Berkman, E.M., and Schreiber, A.D. (1987). Cold hemagglutinin disease associated with IgG cold-reactive antibody. *Ann. Intern. Med. 106*, 238–242.

Silberstein, L.E., Jefferies, L.C., Goldman, J., Friedman, D., Moore, J.S., Nowell, P.C., Roelcke, D., Pruzanski, W., Roudier, J., and Silverman, G.J. (1991). Variable region gene analysis of pathologic human autoantibodies to the related i and I red blood cell antigens. *Blood 78*, 2372–2386.

Silverman, G.J. and Carson, D.A. (1990). Structural characterization of human monoclonal cold agglutinins: Evidence for a distinct primary sequence-defined V_H4 idiotype. *Eur. J. Immunol. 20*, 351–356.

Skinner, M.D. and Schwartz, R.S. (1972). Immunosuppressive therapy. *N. Engl. J. Med. 287*, 221–227.

Sokol, R.J., Hewitt, S., and Stamps, B.K. (1982). Autoimmune haemolysis associated with Donath–Landsteiner antibodies. *Acta Haematol. 68*, 268–277.

Sokol, R.J., Hewitt, S., Booker, D.J., and Bailey, A. (1990). Red cell autoautibodies, multiple immunoglobulin classes, and autoimmune hemolysis. *Transfusion 30*, 714–717.

Spalter, S.H., Kaveri, S.V., Bonnin, E., Mani, J.C., Cartron, J.P., and Kazatchkine, M.D. (1999). Normal human serum contains natural anti-

bodies reactive with autologous ABO blood group antigens. *Blood 93*, 4418–4424.

Stahl, D., Lacroix-Desmazes, S., Heudes, D., Mouthon, L., Kaveri, S.V., and Kazatchkine, M.D. (2000). Altered control of self-reactive IgG by autologous IgM in patients with warm autoimmune hemolytic anemia. *Blood 95*, 328–335.

Steck, T.L. (1978). The band 3 protein of the human red cell membrane: A review. *J. Supramol. Struct. 8*, 311–324.

Stevenson, F.K., Smith, G.J., North, J., Hamblin, T.J., and Glennie, M.J. (1989). Identification of normal B-cell counterparts of neoplastic cells which secrete cold agglutinins of anti-I and anti-i specificity. *Br. J. Haematol. 72*, 9–15.

Sthoeger, Z., Sthoeger, D., Green, L., and Geltner, D. (1993). The role of anticardiolipin autoantibodies in the pathogenesis of autoimmune hemolytic anemia in systemic lupus erythematosus. *J. Rheumatol. 20*, 2058–2061.

Swanson, M.A., Chanmougan, D., and Schwartz, R.S. (1966). Immunohemolytic anemia due to anti-penicillin antibodies. Report of a case. *N. Engl. J. Med. 274*, 178–181.

Telen, M.J. and Chasis, J.M. (1990). Relationship of the human erythrocyte Wrb antigen to an interaction between glycophorin A and band 3. *Blood 76*, 842–848.

Thompson, K.M., Sutherland, J., Barden, G., Melamed, M.D., Randen, I., Natvig, J.B., Pascual, V., Capra, J.D., and Stevenson, F.K. (1991). Human monoclonal antibodies against blood group antigens preferentially express a V_H4–2l variable region gene-associated epitope. *Scand. J. Immunol. 34*, 509–518.

Van Arsdel, P.P., Jr. and Gilliland, B.C. (1965). Anemia secondary to penicillin treatment: Studies on two patients with non-allergic serum hemagglutinins. *J. Lab. Clin. Med. 65*, 277–285.

Van Loghem, J.J., Peetoom, F., van der Hart, M., van der Veer, M., van der Giessen, M., Prins, H.K., Zurcher, C., and Engelfriet, C.P. (1963). Serological and immunochemical studies in hemolytic anaemia with high-titer cold agglutinins. *Vox Sang. 8*, 33–46.

Victoria, E.J., Pierce, S.W., Branks, M.J., and Masouredis, S.P. (1990). IgG red blood cell autoantibodies in autoimmune hemolytic anemia bind to epitope on red blood cell membrane band 3 glycoprotein. *J. Lab. Clin. Med. 115*, 74–88.

Vogel, J.M., Hellman, M., and Moloshok, R.E. (1972). Paroxysmal cold hemoglobinuria of nonsyphilitic etiology in two children. *J. Pediatr. 81*, 974–977.

Wakui, H., Imai, H., Kobayashi, R., Itoh, H., Notoya, T., Yoshida, K., Nakamoto, Y., and Miura, A.B. (1988). Autoantibody against erythrocyte protein 4.1 in a patient with autoimmune hemolytic anemia. *Blood 72*, 408–412.

Watanabe, K. and Hakomori, S. (1976). Status of blood group carbohydrate chains in ontogenesis and in oncogenesis. *J. Exp. Med. 144*, 645–653.

Weiner, W. and Vos, G.H. (1963). Serology of acquired hemolytic anemias. *Blood 22*, 606–613.

White, J.M., Brown, D.L., Hepner, G.W., and Worlledge, S.M. (1968). Penicillin induced hemolytic anaemia. *BMJ 3*, 26–29.

Williams, R.C., Kunkel, H.G., and Capra, J.D. (1968). Antigenic specificities related to the cold agglutinin activity of Gamma M globulins. *Science, 161,* 379–381.

Willis, F., Marsh, J.C., Bevan, D.H., Killick, S.B., Lucas, G., Griffiths, R., Ouwehand, W., Hale, G., Waldmann, H., and Gordon-Smith, E.C. (2001). The effect of treatment with Campath-1H in patients with autoimmune cytopenias. *Br. J. Haematol. 114*, 891–898.

Wolach, B., Heddle, N., Barr, R.D., Zipursky, A., Mohan Pai, K.R., and Blajchman, M.A. (1981). Transient Donath–Landsteiner haemolytic anaemia. *Br. J. Haematol. 48*, 425–434.

Worlledge, S.M. (1969a). Immune drug-induced haemolytic anaemias. *Semin. Hematol. 6*, 181–200.

Worlledge, S.M. (1969b). Autoantibody formation associated with methyldopa (aldomet) therapy. *Br. J. Haematol. 16*, 5–8.

Worlledge, S. (1974). Immune haemolytic anemias. In R.M. Hardesty and D.S. Weatherall (eds), *Blood and Its Disorders* (p. 714). Oxford: Blackwell.

Worlledge, S.M. (1978). *Blood Transfusion in Clinical Medicine*, 6th edn (p. 397). Oxford: Blackwell.

Worlledge, S.M. and Dacie, J.V. (1969). Haemolytic and other anaemias in infectious mononucleosis. In "Infectious mononucleosis" (H.G. Garter and R.L. Penman, eds). Pp 82–98, Blackwell, Oxford.

Worlledge, S.M. and Rousso, C. (1965). Studies of the serology of paroxysmal cold hemoglobinuria (P.C.H.) with special reference to its relationship with P blood group system. *Vox Sang. 10*, 293–298.

Worlledge, S.M., Carstairs, D.C., and Dacie, J.V. (1966). Autoimmune haemolytic anaemia associated with alpha-methyldopa therapy. *Lancet 2*, 135–139.

Young, L.E., Miller, G., and Christian, R.M. (1951). Clinical and laboratory observations on autoimmune hemolytic disease. *Ann. Int. Med. 35*, 507–517.

Zalman, L.S. (1992). Homologous restriction factor. *Curr. Top. Microbiol. Immunol. 178*, 87–99.

Zecca, M., De Stefano, P., Nobili, B., and Locatelli, F. (2001). Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. *Blood 97*, 3995–3997.

Zupa'nska, B., Sylwestrowicz, T., and Pawelski, S. (1981). The results of prolonged treatment of autoimmune haemolytic anaemia. *Haematologia 14*, 425–433.

Zupa'nska, B., Thomson, E.E., and Merry, A.H. (1986). Fc receptors for IgG1 and IgG3 on human mononuclear cells—an evaluation with known levels of erythrocyte-bound IgG. *Vox Sang. 50*, 97–183.

CHAPTER

42

Thrombocytopenic Purpura

DAVID GREEN AND SIMON KARPATKIN
New York University Medical School, New York, New York, USA

HISTORIC BACKGROUND

The humoral nature of autoimmune thrombocytopenic purpura (ATP) has been recognized since the seminal and dramatic studies conducted by Harrington in the early 1950s. Prompted by the observation that pregnant women with ATP often give birth to infants with thrombocytopenia, he infused himself and other volunteer subjects with ATP plasma. This resulted in the rapid onset of thrombocytopenic purpura, followed by recovery within 6–8 days (Harrington et al., 1951). Interestingly, only 63% of ATP plasmas studied by Harrington were capable of inducing thrombocytopenia in transfused recipients, indicating that platelet destruction by autoantibody is probably not the sole effector of autoimmune pathogenesis. The antiplatelet factor was present in the IgG-containing serum fraction (Shulman et al., 1965). IgG autoantibody was first detected in the serum of ATP patients by a platelet immunoinjury assay (Karpatkin et al., 1972a; 1972b). A quantitative immunoassay of antiplatelet antibody was developed in 1975 (Dixon et al., 1975). In 1982, van Leeuwen et al. (1982) demonstrated that antibody eluates from ATP platelets bound to normal platelets, but not to thrombasthenic platelets lacking platelet glycoprotein (GP) IIb and GPIIIa; similar conclusions were drawn by Beardsley et al. (1984). Varon and Karpatkin (1983) demonstrated the absence of binding of a monoclonal antiplatelet GPIIb antibody to platelets of ATP patients with thrombocytopenia, but the presence of binding to platelets of patients in remission, suggesting that GPIIb is a major antigen on the platelet surface but which is not detectable in thrombocytopenic patients because it is blocked by patient antibody. This has recently been confirmed (McMillan et al., 2002). Direct binding of antiplatelet antibody to platelet membrane GPIIb, GPIIIa, and GPIb has been reported using antigen-specific assays (McMillan, 2000); however, the pathophysiology of these *in vitro* studies has not been studied *in vivo*, i.e., some of the antibodies were directed against intracellular components of GPIIIa. The spleen is the predominant site of destruction initially, as well as a major source of autoantibody production (McMillan et al., 1972; Karpatkin et al., 1972b).

GENERAL FEATURES

ATP, also referred to as idiopathic thrombocytopenic purpura (ITP) (Karpatkin, 1997), is an autoimmune condition causing platelet destruction, as documented by kinetic studies showing shortened platelet survival and/or platelet

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

production (Ballem et al., 1987). In ATP, autoantibody binds to platelets, resulting in clearance of the opsonized platelets by the phagocytic cells of the reticuloendothelial system (RES) (Figure 42.1). It also crosses the placenta and can result in neonatal ATP (Burrows and Kelton, 1993).

Specific criteria to establish the diagnosis are lacking since the etiology is unknown and other causes of thrombocytopenia must be ruled out. The diagnostic criteria for ATP include: 1) thrombocytopenia and/or shortened platelet survival, as shown by kinetic studies or increased large "stress" platelets or megathrombocytes (which represent increased platelet turnover) (Garg et al., 1971); 2) increased (or occasionally normal) numbers of megakaryocytes in the bone marrow; 3) bound antiplatelet antibody; 4) absence of palpable splenomegaly; 5) increase in platelet count in response to corticosteroids or intravenous gamma-globulin (IVIG); 6) history of a thrombocytopenic mother giving birth to a thrombocytopenic infant; and 7) exclusion of other causes of thrombocytopenia. All are usually present.

ATP is classified as either chronic (duration longer than 6 months) or acute, with the former more common in adults and the latter in children. Chronic ATP is more common in women than in men (3:1), particularly in younger adult patients. The overall incidence based on limited epidemiologic data is 16–66 cases in a million (George et al., 1996; Frederikson and Schmidt, 1999; Neylon et al., 2003). Acute ATP in children often follows an acute viral illness in the winter or spring. It is self-limiting, remitting within 6 months of onset, affects both sexes equally, and has an incidence of 1–4 cases in 100,000 (Cohn, 1976; Walker and Walker, 1984). Adult patients typically have a chronic and variable course lasting more than 6 months. Spontaneous complete remission occurs infrequently and usually in the setting of mild ATP with or without compensated thrombocytolysis (shortened platelet survival with normal platelet count). In contrast, remissions of purpura are commonly seen as the majority of patients will achieve a platelet count sufficient for normal hemostasis (>30,000/mm^3) spontaneously or with treatment.

Coexisting autoimmune cytopenias may be seen, including hemolytic anemia (Evans syndrome) or autoimmune neutropenia. ATP may also be seen in association with other autoimmune diseases such as SLE (Rabinowitz and Dameshek, 1960), rheumatoid arthritis, and autoimmune thyroid disease (Hymes et al., 1981). Presence of antiphospholipid antibody or lupus anticoagulant is a frequent finding in ATP but does not correlate with disease severity or progression (Stasi et al., 1994). ATP has been reported in connection with lymphoproliferative diseases (Lim and Ifthikharuddin, 1994) and has been reported after autologous (Jillella et al., 2000) and allogeneic bone marrow transplantation. A variety of infectious diseases are associated with ATP, particularly human immunodeficiency virus (HIV) infection (Morris et al., 1982), but also hepatitis B and C (Samuel et al., 1999). In 1998, Gasbarrini et al. reported that eradication of *Helicobacter pylori* was associated with a significant rise in platelet count in patients with ATP. This has been confirmed in studies in Italian and Japanese patient populations (Franchini and Veneri, 2004). However, lack of correlation and response to treatment has also been reported (Jarque et al., 2001; Michael et al., 2004). A recent review of the published experience noted a complete or partial response to successful eradication therapy in 101 of 191 (52.9%) ATP patients (Franchini and Veneri, 2004). The pathophysiologic mechanism wherein *H. pylori* infection contributes to the development or progression of ATP is unknown; however, molecular mimicry of the CagA protein may play a role (Takahashi et al., 2004).

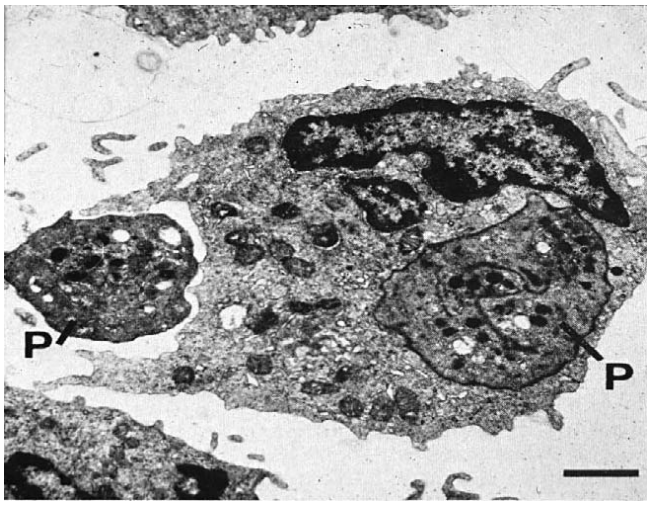


FIGURE 42.1 Monocyte (from the buffy coat of a patient with autoimmune thrombocytopenic purpura), containing one intact platelet and, apparently, in the process of phagocytosing another. The phagocytosed platelet is not degranulated. P, platelet.

Reproduced from Zucker-Franklin and Karpatkin (1977), with permission.

CLINICAL FEATURES

The clinical presentation of ATP is highly variable and unpredictable. Patients may initially present with severe mucocutaneous bleeding, including petechiae, ecchymoses, and hemorrhagic bullae, signs that may herald more serious bleeding complications. Intracranial hemorrhage is a feared complication, although fortunately quite rare. The overall disease-specific mortality in chronic ATP in a reviewed case series was 0.0162–0.0389 cases per patient-year at risk (platelet count <30,000) (Cohen et al., 2000). Significant treatment-related mortality resulting from infections is an important consideration (Portielje et al., 2001). Some patients tolerate exceedingly low platelet counts and remain

asymptomatic for years without treatment. Putative factors that may influence predilection to bleeding in addition to the severity of the thrombocytopenia may include platelet age and size (Karpatkin, 1978), capillary vascular integrity (Spaet, 1952), and functional inhibition of platelet membrane glycoproteins by bound platelet autoantibody (McMillan et al., 1996).

GENETICS

Major histocompatibility complex (MHC) susceptibility genes have been associated with chronic ATP, as well as with treatment response to steroids or splenectomy and autoantibody specificity. A study in Japanese patients showed increased prevalence of DRB1*0410 in ATP patients (Nomura et al., 1998) and associated poor response to steroids. Taken together, the results of numerous studies in white and ethnic populations are inconsistent, limited by small sample size, and at best support weak associations between ATP and HLA class I and class II alleles.

THROMBOKINETICS

Studies evaluating platelet turnover are conflicting. Platelet survival is often reduced to 10% of normal and has been reported to be inversely related to antiplatelet antibody in older studies (Najean et al., 1967; Harker, 1970; Branehog et al., 1975). Turnover is increased 2 to 5 times above normal in some studies (Harker, 1970; Branehog et al., 1975), whereas studies using autologous platelets demonstrated impaired production in the majority of untreated ATP patients (Ballem et al., 1987). In this respect, binding of antiplatelet antibody to shared antigens on megakaryocytes may inhibit platelet production (McMillan et al., 1978). *In vitro* megakaryocytopoiesis is inhibited by ATP plasma and by monoclonal antiplatelet antibody (Chang et al., 2003; McMillan et al., 2004). Impaired thrombopoiesis may be clinically significant in some patients with ATP and could explain favorable responses to thrombopoietin therapy (Nomura et al., 2002). However, increased platelet destruction is likely to be the predominant mechanism. This is supported by the classical experiments of Harrington et al. (1951), who, as described above, reported a precipitous drop in platelet count shortly after infusion of ITP plasma into 63% of volunteers with a nadir of 4 h (Figure 42.2); see also Figure 42.3. It is unclear whether the two mechanisms described for the induction of thrombocytopenia represent two different autoimmune diseases or heterogeneity within the same disease.

Megakaryocytes are usually increased in number and size (Garg et al., 1971). Platelets are decreased in number and increased in volume (Garg et al., 1971).

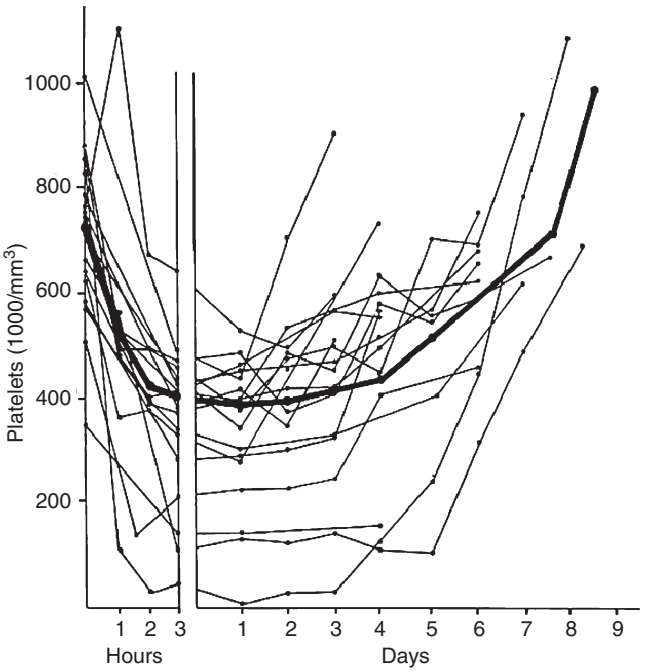


FIGURE 42.2 Thrombocytopenic effect produced by transfusing 500 ml of citrated blood or its plasma equivalent from 17 patients with idiopathic thrombocytopenic purpura. Recipients were healthy laboratory workers or patients with inoperable carcinoma; line shows mean. Platelet counts are older, indirect measurements, which are about twice current direct measurements.

Adapted from Harrington et al. (1951), with permission.

Platelets

Megathrombocytes are commonly seen in ATP. They are representative of a change in the platelet volume distribution curve and are indicative of stress platelets released upon increased turnover of megakaryocytes (Garg et al., 1971). Approximately 50% of ATP patients in remission have increased megathrombocytes and increased platelet IgG, which may reflect a compensated thrombocytolytic state (Garg et al., 1971). An inverse relationship exists between platelet diameter and lifespan (Branehog et al., 1975).

AUTOANTIGENS

Specific antiplatelet antibodies targeting platelet membrane glycoproteins have been described in ATP (Karpatkin and Siskind, 1969; McMillan et al., 1972; Dixon et al., 1975; van Leeuwen et al., 1982; Varon and Karpatkin, 1983; Beardsley et al., 1984; Fujisawa et al., 1991; 1993; Kekomaki et al., 1991; McMillan, 1990; 2000; McMillan et al., 2002). Antigen-specific antibody is detected in up to 70% of ATP patients and in most cases localizes to platelet glycoprotein IIb–IIIa or Ib–IX complexes (McMillan, 1990). Epitope mapping to both linear and conformational

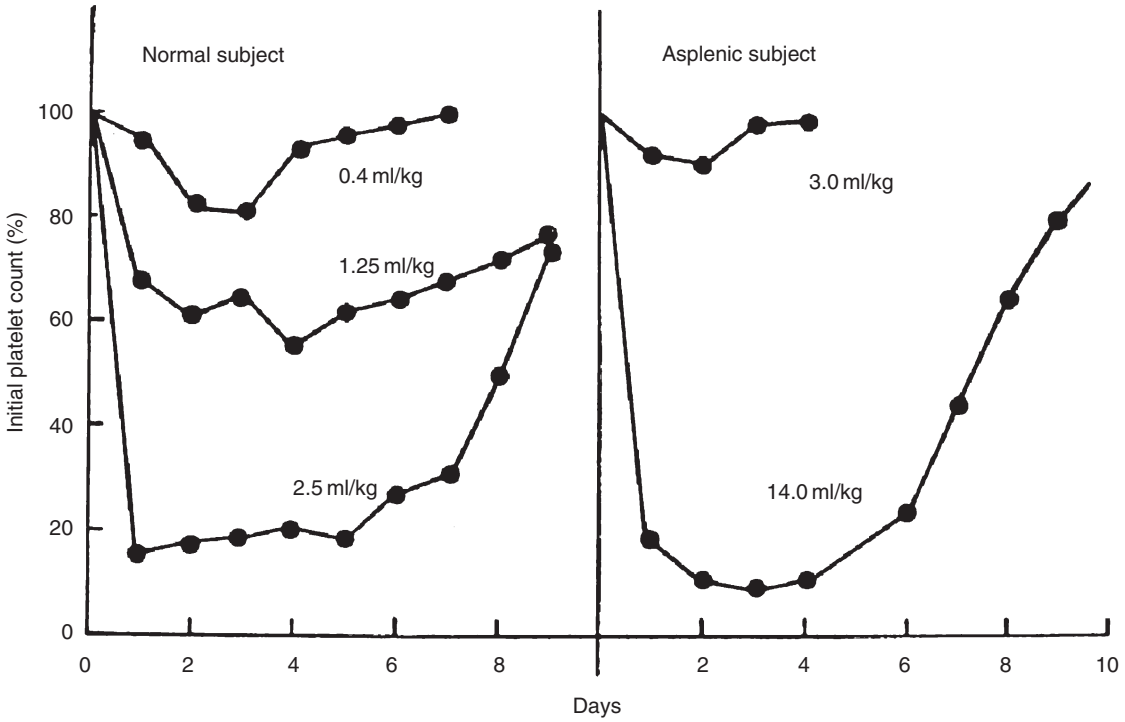


FIGURE 42.3 Effect of plasma from a patient with autoimmune idiopathic thrombocytopenic purpura (ATP) on platelet counts in a normal and asplenic volunteer subject.
Reproduced from Shulman et al. (1965), with permission.

sites on the IIb–IIIa and Ib–IX receptors has been achieved using synthetic or recombinant peptides and phage display libraries of random peptides (McMillan, 2000). Autoepitopes are frequently conformational and calcium-dependent (Fujisawa et al., 1993). Cross-reactivity of ATP autoantibody (sharing of epitope) has been reported, suggesting that epitopes are often shared or clustered, but these observations need to be confirmed by more extensive epitope scanning (McMillan, 2000). The cysteine-rich and intracytoplasmic regions of GPIIIa are targets of autoantibody (Fujisawa et al., 1991; Kekomaki et al., 1991). Many of the IIb–IIIa autoepitopes localize to the N-terminus of IIb (McMillan et al., 2002). The pathophysiologic significance of these antigen-specific antibodies is not clear. In most cases, the receptor function appears to be spared by bound autoantibody; although platelet dysfunction similar to Glanzmann thrombasthenia (Balduini et al., 1992) and Bernard–Soulier (Varon et al., 1992) has been reported, as has platelet activation and thrombosis (Pfueller et al., 1990).

ANTIPLATELET ANTIBODY

Numerous immunologic assays for the detection of antiplatelet antibody have been evaluated as potential diagnostic and predictive markers in ATP. Serum antiplatelet antibody is detected in 50% of patients (Karpatkin and Siskind, 1969; Dixon et al., 1975; Karpatkin et al., 1992), but results do not correlate with disease severity or response to treatment. Platelet-associated antibody is usually IgG but can be IgM or IgA. It is detected in a majority but not all patients with ATP and may persist in spite of apparent clinical remission. However, 99% of the total platelet IgG reflects internal stores (George and Saucerman, 1988) and is believed to result from fluid-phase pinocytosis of serum IgG by megakaryocytes. Platelet-associated IgG is increased in hypergammaglobulinemic states. Platelet-associated IgG can also result from immune-complex deposition, which results in relatively high levels of platelet surface IgG compared to classical ATP (Walsh et al., 1984; Kurata et al., 1985; Samuel et al., 1999), and can therefore be used to distinguish the two entities (Walsh et al., 1984; Kurata et al., 1985; Samuel et al., 1999). IgG can also be increased in apparent nonimmune conditions, such as sepsis and cirrhosis (Samuel et al., 1999). Platelet-associated IgG is increased in ATP and correlates with disease severity (Hymes et al., 1979; Kernoff et al., 1980). Platelet IgG is increased in many patients with Graves' disease and Hashimoto's thyroiditis (Hymes et al., 1981), and 10% of patients with ATP have associated thyroid disease (Bechgaard, 1946; Branehog et al., 1979). Platelet IgG is also increased in patients with systemic lupus erythematosus (SLE) and thrombocytopenia

(Karpatkin and Siskind, 1969; Samuel et al., 1999). Elevated antinuclear antibody (ANA) antibody is sometimes found in ATP, but does not correlate with the development of SLE (Kurata et al., 1994).

Initially, it was held that antiplatelet autoantibody was polyclonal, although this assumption has been challenged since much of the platelet-associated IgG is not autoantibody. Evidence in support of clonality of autoantibody in chronic ATP includes the findings of clonal B-cells in blood and spleen as well as clonal serum and platelet-associated autoantibody (Van der Harst et al., 1990; McMillan, 2000).

Assays of antigen-specific platelet-bound antibody have a sensitivity of 39–49%, specificity of 78–91%, and a positive predictive value of 80% (Brighton et al., 1996; Warner et al., 1999).

In summary, serum antiplatelet antibody, platelet-associated and platelet-surface IgG, as well as antigen-specific assays have been developed but their clinical utility in diagnosis and management of ATP patients remains controversial.

IMMUNOPATHOGENESIS

Mechanisms of escape from normal self-tolerance that result in autoimmunity in general and that may be applicable to ATP in particular include molecular mimicry, antigenic determinant spreading, type 1 helper T-cell (Th1)–Th2 balance, and a skewing of the cytokine microenvironment (Semple, 2002). In the context of pro-inflammatory cytokines and costimulation of Th cells, autoreactive B-cells are activated to produce platelet autoantibody, which is the primary effector of ATP.

Effector Mechanisms

The role of complement in the pathophysiology of platelet destruction is unclear. Platelet-associated C3, C4, and C9 have been detected in some ATP patients (Walsh et al., 1984; Kurata et al., 1985) and complement-dependent platelet lysis induced by autoantibody has been demonstrated *in vitro* (Cines and Schreiber, 1979; Tsubakio et al., 1986). Complement activation may function to enhance phagocytosis and induce platelet lysis *in vivo*.

Fc receptors for IgG (FcγR) on phagocytic cells of the RES, particularly in the spleen, play a pivotal role in the clearance of opsonized platelets. Clinical data and mouse models of ATP implicate the low-affinity activating receptors FcγRIIA and FcγRIIIA, which bind immune-complexed IgG, as the primary mediators of platelet clearance. FcR polymorphisms may influence disease susceptibility or severity (van der Pol and van der Winkel, 1998).

T-cell Abnormalities

While ATP is primarily antibody mediated, antiplatelet autoantibody production is regulated by T cells. Indeed, abnormal T-cell proliferation and activation is a hallmark of chronic ATP (Borkowsky and Karpatkin, 1984; Semple, 2002; 2003). T cells from ATP patients secrete IL-2 when challenged with autologous platelets *in vitro* (Semple and Friedman, 1991). Oligoclonal Th cells are detected in the peripheral blood of ATP patients. Autoreactive T cells to chemically modified GPIIb–IIIa are present in peripheral blood mononuclear cell cultures derived from ATP patients, and stimulate the *in vitro* production of IgG anti-GPIIb–IIIa antibody, which is capable of binding to normal platelets (Kuwana et al., 1998). Immunodominant epitopes have been localized to the N-terminal regions of GPIIb and GPIIIa in studies using recombinant fusion proteins (Kuwana et al., 2001). Platelet autoantibody derived from splenic B cells showed somatic hypermutation, a process that is dependent upon T-cell help (Roark et al., 2002).

Several studies have examined the Th-cytokine profiles in active ATP with inconsistent results, but most studies reveal a polarized type 1 response. Th1 cells elicit delayed-type hypersensitivity, cell-mediated immunity, and production of complement-fixing IgG responses with characteristic cytokine production of IL-2, IFN-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF)-α (Mosmann and Coffman, 1989). Expression of type 1 pro-inflammatory cytokines, such as IL-2 and IFN-γ, is observed in the peripheral blood mononuclear cells of ATP patients with active disease. ATP remission in children was associated with a polarized type 2 cytokine response (Mouzaki et al., 2002). Alternatively, a Th3 response with upregulation of the immunosuppressive cytokine TGF-β has been reported in ATP patients in remission. TGF-β could exert a bystander immune suppression effect (Andersson et al., 2000), resulting in remission, while reduction in TGF-β production by mononuclear cells is found in patients with active ATP (Andersson et al., 2002).

An additional, recently established pathogenic mechanism is direct T-cell-mediated cytotoxicity toward platelets in active chronic ATP (Olsson et al., 2003). Specific platelet lysis was observed in 6 of 8 chronic ATP patients (Olsson et al., 2003). DNA microarray analysis showed increased expression of Apo-1/fas, granzyme A, granzyme B, and perforin in ATP patients (Olsson et al., 2003), a finding consistent with activation of cytotoxic T cells. Increased expression of natural killer (NK) cell immunoglobulin-like receptors (KIRs) was observed in T cells from ATP patients in remission (Olsson et al., 2003). KIRs are known to inhibit cytotoxic and NK cell responses by binding to MHC class I (Boyington et al., 2001), and may provide an additional molecular mechanism for immunologic remission in ATP (Olsson et al., 2003).

TREATMENT

With the exception of those patients responding to splenectomy, treatment for ATP is palliative. A normal platelet count does not necessarily indicate eradication of autoimmune disease, since splenectomized women with normal platelet counts can give birth to thrombocytopenic neonates.

Numerous uncontrolled case series have evaluated various treatment modalities, as summarized in the practice guidelines from the Executive Committee of the American Society of Hematology (George et al., 1996). The goal of therapy is to achieve a "safe" platelet count (>30,000/mm) (George et al., 1996; 2002). Options for the management of symptomatic acute ATP include corticosteroids (prednisone, methylprednisolone, or high-dose decadron), IVIG (1 g/kg) or intravenous anti-D immunoglobulin (50 μg/kg) (Stasi and Provan, 2004).

Splenectomy is reserved for younger patients (generally younger than 40 years) who have had the disease for 6 months (some adult patients will remit during this interval) and respond to prednisone or IVIG, or require >10 mg of prednisone/day to maintain a safe platelet count. The response to splenectomy is due to the removal of an organ producing significant amounts of antiplatelet IgG (Karpatkin et al., 1972b; McMillan et al., 1972). Long-term durable responses (normal platelet counts) are achieved in 60–70% of patients who undergo splenectomy (Kojouri et al., 2004).

IVIG or anti-Rh (D) antibody, like corticosteroids, transiently increase the platelet count within 1 day of treatment in most patients with severe ATP and are useful as emergency therapy in addition to platelet transfusions and intravenous corticosteroids in the acutely bleeding patient. The mechanism of IVIG was initially attributed to blockade of the RES with inhibition of phagocytosis; or by anti-idiotype antibody with resultant neutralization of pathologic antiplatelet antibody. More recently, it has been established in mouse models of ATP that IVIG results in both IgG receptor FcγRIII blockade and upregulation of the inhibitory IgG receptor FcγRIIb (Clynes and Ravetch, 1995), which downregulates phagocytic activity. Both of these mechanisms are presumed to contribute to the therapeutic effect of IVIG. The salutary effect of anti-D immunoglobulin for treatment of ATP, restricted to Rh(D)-positive presplenectomy patients, is assumed to be similar to the mechanism of IVIG, although differences have been proposed on the basis of their distinct patterns of cytokine responses. A proportion of newly diagnosed ATP patients treated with anti-D as initial therapy may be spared splenectomy (Cooper et al., 2002; George et al., 2003).

There has been reported success with the anti-B-cell, anti-CD20 antibody (rituximab) in the treatment of refractory ATP (Stasi et al., 2001; Cooper et al., 2004). There are no randomized clinical trials to guide treatment of the refractory patient and no consensus of opinion. Other treatment options for the refractory ATP patient include azathioprine, vinca alkaloids, danazol, cyclosporine A, cytoxan, and combination chemotherapy.

Benefits of treatment must be carefully weighed against side effects. Ongoing and encouraging investigational approaches include the use of thrombopoietin, anti-CD154 (CD40 ligand), anti-FcγRI, the TNF inhibitor, etanercept (McMinn et al., 2003), anti-CD25, and the CD80(B7)-blocking agent, soluble cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) (Stasi and Provan, 2004).

AUTOIMMUNE THROMBOCYTOPENIC PURPURA IN PREGNANCY

ATP may complicate pregnancy and it may be difficult at times to distinguish it from benign gestational thrombocytopenia (Hart et al., 1986; Burrows and Kelton, 1993). Fifty percent of mothers with ATP infants have mild transient thrombocytopenia (Epstein et al., 1950). Five percent have platelet counts <20,000/mm (Burrows and Kelton, 1993). The duration of thrombocytopenia averages 3–4 weeks but can last up to 3 months. Bleeding complications are rare (Burrows and Kelton, 1990). ATP in pregnancy is treated with corticosteroids or IVIG. Splenectomy is usually deferred until after the pregnancy. Little correlation is observed between the mother's platelet count and that of the fetus (Scott et al., 1983).

HUMAN IMMUNODEFICIENCY VIRUS-ASSOCIATED THROMBOCYTOPENIC PURPURA

HIV-seropositive individuals may develop ATP early in the course of the infection or late in the course of the disease (Najean and Rain, 1994), and is seen in sexually-active homosexuals, hemophiliacs, and intravenous drug abusers and their heterosexual partners (Morris et al., 1982; Ratnoff et al., 1983; Walsh et al., 1984; 1985; Kurata et al., 1985; Savona et al., 1985; Karpatkin et al., 1988; 1995). The disease is clinically indistinguishable from classical ATP but differs with respect to male predominance and markedly increased platelet-associated IgG, IgM, and C3C4, and circulating immune complexes (Walsh et al., 1984; Kurata et al., 1985). Impaired platelet production is also seen with acquired immunodeficiency syndrome (AIDS). Increases in platelet count may be seen with antiretroviral therapy (Hymes et al., 1988). Patients with HIV-TP have an immunodominant epitope GPIIIa49–66 (Nardi et al., 1997). Serum anti-GPIIIa49–66 correlates inversely with platelet

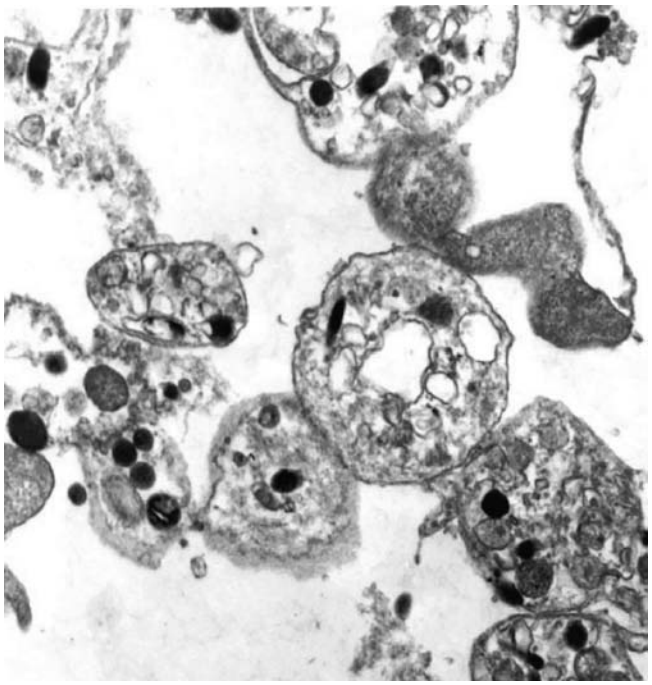


FIGURE 42.4 Electron microscopy of damaged platelets treated with anti-GPIIIa49–66 antibody. Patient sample at 4 h showing degenerating platelets and disintegration of the cell membrane. None of these changes was present in IgG controls at 4 h. Original magnification: ×40,000.

count and is enriched in circulating immune complexes (Karpatkin et al., 1995; Nardi et al., 1997). Antibody directed against the epitope induces thrombocytopenia and platelet fragmentation in mice, as well as human platelet fragmentation (Figure 42.4). Platelet fragmentation occurs by a unique complement-independent mechanism triggered by antibody-induced production of reactive oxygen species via both platelet NADPH oxidase and 12-lipoxygenase pathways (Nardi et al., 2001; 2004). Platelet destruction is likely to be due to a combination of platelet fragmentation and clearance by phagocytic cells. There is recent evidence that the antibody against the immunodominant epitope is secondary to molecular mimicry, i.e. cross-reactivity with HIV peptides gag and nef (Li et al., 2003).

CONCLUSION

ATP is an immunologic platelet disorder of exclusion, the etiology of which is unknown. It is unclear whether this represents a single disorder or multiple disorders. This is supported by observations that some patients have predominant increased peripheral platelet destruction whereas others have decreased platelet production, and still others have both. Antibody against platelets and megakaryocytes could explain this apparent heterogeneity—yet why one predominates in some patients and another in other patients is unclear.

In this respect, the work of Olsson et al. (2003) on the role of NK cells and cytotoxic T-cells against platelets is intriguing. They also noted a counterbalanced expression of the inhibitors of cytotoxic T- and NK cell receptors, called KIRs, in patients in remission. The role of T-cell-mediated in platelet destruction is also unclear and deserves to be studied.

Treatment responses cytotoxicity appear to be heterogeneous. Whereas most patients respond to antiphagocytic agents [steroids, danazol, IVIG, and anti-Rh(D)], others require immunosuppression with imuran, cytoxan, or cyclosporine, and still others do not appear to respond at all—yet have responded in the past. In addition, some patients will respond to steroids and not to IVIG, whereas others will respond to IVIG but not to steroids. The response to splenectomy is also variable. Some patients, particularly younger ones (under 40 years) who respond to steroids will respond to splenectomy, while older patients tend to be less responsive. Perhaps this relates to the fact that the spleen is an organ of platelet antibody production as well as platelet phagocytosis in younger patients but not in older ones. Older nonresponsive patients could have hepatic destruction of platelets, or antibody production from other organs.

The significance of platelet-associated antibody or platelet surface antibody is also unclear. These measurements are useful in distinguishing diseases with immune-complex-associated thrombocytopenia, such as SLE, HIV-1-TP or hepatitis, from classical ATP. The platelet antibody levels do not appear to be related to disease severity. However, in some situations they improve and/or disappear with successful treatment.

Finally, the etiology is also unclear. One possible clue is the frequent onset of childhood acute ATP following a viral infection, as well as the occasional stable chronic ATP adult patient who becomes severely thrombocytopenic following a viral infection. Molecular mimicry may also play a role. Recent reports of the association of Epstein–Barr virus with SLE secondary to the sharing of an EBNA-1/SLE-Ro antigen (McClain et al., 2005), cross-reactivity of *H. pylori* CagA protein with platelets (Takahashi et al., 2004), and the cross-reactivity of HIV-1-TP antibody against HIV-1-nef protein with platelet GPIIIa49–66 (Li et al., 2005) are intriguing. In the later report, induction of murine thrombocytopenia could be induced with antibody against these HIV-1 peptides.

Recent developments in our understanding of a new mechanism for platelet destruction in HIV-1-TP is intriguing. Patients with antibody against platelet integrin GPIIIa49–66 undergo oxidative damage and platelet fragmentation through the activation of platelet 12-lipoxygenase and NADPH oxidase. It will be of interest to determine whether other platelet immunologic disorders associated

with circulating immune complexes might have a similar mechanism.

References

Andersson, P., Stockelberg, D., Jacobsson, S., and Wadenvik, H. (2000). A transforming growth factor-beta-1-mediated bystander immune suppression could be associated with remission of chronic idiopathic thrombocytopenic purpura. *Ann. Hematol. 79*, 507–513.

Andersson, P., Olsson, A., and Wadenvik, H. (2002), Anti-GPIIb/IIIa (CD41) monoclonal antibody-induced platelet activation requires Fc receptor-dependent cell–cell interaction. *Br. J. Haematol. 116*, 862–867.

Balduini, C., Bertolino, G., Noris, P., Piovella, F., Sinigaglia, F., Bellotti, V., Samaden, A., Torti, M., and Mazzini, G. (1992). Defect of platelet aggregation and adhesion by autoantibodies against glycoprotein IIIa. *Thromb. Haemost. 68*, 208–213.

Ballem, P., Segal, G., Stratton, J., Gernsheimer, T., Adamson, J., and Slichter, S.J. (1987). Mechanisms of thrombocytopenia in chronic autoimmune thrombocytopenic purpura: evidence of both impaired platelet production and increased platelet clearance. *J. Clin. Invest. 80*, 33–40.

Beardsley, D., Spiegel, J., Jacobs, M., Handin, R., and Lux, S. (1984) Platelet membrane glycoprotein IIIa contains target antigens that bind anti-platelet antibodies in immune thrombocytopenias. *J. Clin. Invest. 74*, 1701–1707.

Bechgaard, P. (1946). Tendency to hemorrhage in thyrotoxicosis. *Acta Med. Scand. 124*, 79–91.

Borkowsky, W. and Karpatkin, S. (1984). Leukocyte migration inhibition of buffy coats from patients with autoimmune thrombocytopenic purpura (ATP) when exposed to normal platelets. *Blood 63*, 83–87.

Boyington, J., Brooks, A., and Sun, P. (2001). Structure of killer cell immunoglobulin-like receptors and their recognition of the class I MHC molecules. *Immunol. Rev. 181*, 66–78.

Branehog, I., Kutti, J., Ridell, B., Swolin, B., and Weinfeld, A. (1975) The relation of thrombokinetics to bone marrow megakaryocytes in idiopathic thrombocytopenic purpura. *Blood 45*, 551.

Branehog, I., Olsson, K., Weinfield, A., and Domellof, L. (1979). Association of hypothroidism with idiopathic thrombocytopenic purpura and hemolytic anemia. *Acta Med. Scand. 205*, 125–131.

Brighton, T., Evans, S., Castaldi, P., Chesterman, C., and Chong, B. (1996). Prospective evaluation of the clinical usefulness of an antigen-specific assay (MAIPA) in idiopathic thrombocytopenic purpura and other immune thrombocytopenias. *Blood 88*, 194–201.

Burrows, R. and Kelton, J. (1990). Low fetal risks in pregnancy associated with idiopathic thrombocytopenic purpura. *Am. J. Obstet. Gynecol. 162*, 731–734.

Burrows, R. and Kelton, J. (1993). Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N. Engl. J. Med. 329*, 1463–1466.

Chang, M., Nakagawa, P., Williams, S., Schwartz, M., Imfeld, K., Buzby, J., and Nugent, D. (2003). Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal autoantibodies inhibit megakaryocytopoiesis *in vitro*. *Blood 102*, 887–895.

Cines, D. and Schreiber, A. (1979) Immune thrombocytopenia: use of a Coombs antiglobulin test to detect IgG and C3 on platelets. *N. Engl. J. Med. 300*, 106–111.

Clynes, R. and Ravetch, J. (1995) Cytotoxic antibodies trigger inflammation through Fc receptors. *Immunity 3*, 21–26.

Cohen, Y., Djulbegovic, B., Shamai-Lubovitz, O., and Mozes, B. (2000). The bleeding risk and natural history of idiopathic thrombocytopenic purpura in patients with persistent low platelets. *Arch. Intern. Med. 160*, 1630–1638.

Cohn, J. (1976). Thrombocytopenia in childhood: An evaluation of 433 patients. S*cand. J. Haematol. 16*, 226–240.

Cooper, N., Woloski, B., Fodero, E., Novoa, M., Leber, M., Beer, J.H., and Bussel, J. (2002). Does treatment with intermittent infusions of intravenous anti-D allow a proportion of adults with recently diagnosed immune thrombocytopenic purpura to avoid splenectomy? *Blood 99*, 1922–1927.

Cooper, N., Stasi, R., Cunningham-Rundles, S., Feuerstein, A., Leonard, J., Amadori, S., and Busel, J. (2004). The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. *Br. J. Haematol. 125*, 232–239.

Dixon, R., Rosse, W., and Ebbert, L. (1975). Quantitative determination of antibody in idiopathic thrombocytopenia purpura. *N. Engl. J. Med. 292*, 230–235.

Epstein, R., Lozner, E., Coffey, T.S., and Davidson, C.S. (1950). Congenital thrombocytopenic purpura: purpura hemorrhagica in pregnancy and in the newborn. *Am. J. Med. 9*, 44–56.

Franchini, M. and Veneri, D. (2004). *Helicobacter pylori* infection and immune thrombocytopenic purpura: an update. *Helicobacter 9*, 342–346.

Frederikson, H. and Schmidt, K. (1999). The incidence of idiopathic thrombocytopenic purpura in adults increases with age. *Blood 94*, 909–913.

Fujisawa, K., O'Toole, T., Tani, P., Loftus, J.C., Plow, E.F., Ginsberg, M.H., and McMillan, R. (1991). Autoantibodies to the presumptive cytoplasmic domain of platelet glycoprotein IIIa in patients with chronic immune thrombocytopenic purpura. *Blood 77*, 2207–2213.

Fujisawa, K., Tani, P., and McMillan, R. (1993). Platelet-associated antibody to glycoprotein IIb/IIIa from chronic immune thrombocytopenic purpura patients often binds to divalent cation-dependent antigens. *Blood 81*, 1284–1289.

Garg, S., Amorosi, E., and Karpatkin, S. (1971). Use of the megathrombocyte as an index of megakaryocyte number. *N. Engl. J. Med. 283*, 11–17.

Gasbarrini, A., Franceschi, F., Tartaglione, R., Landolfi, R., Pola, P., and Gasbarrini, G. (1998). Regression of autoimmune thrombocytopenia after eradication of *Helicobacter pylori*. *Lancet 352*, 878.

George, J.N. and Saucerman, S. (1988). Platelet IgG, IgA, IgM and albumin: Correlation of platelet and plasma concentrations in normal subjects and in patients with ITP or dysproteinemia. *Blood 72*, 362–365.

George, J., Woolf, S., Raskob, G., Wasser, J., Aledort, L., Ballem, P., Blancehtto, V.S., Bussel, J.B., Cines, D.B., Kelton, J.G., Lichtin, A.E., McMillan, R., Okerbloom, J.A., Regan, D.H., and Warrier, J. (1996) Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood 88*, 3–70.

George, J.N., Raskob, G., Vesely, S., Moore, D., Lyons, R., Lobos, E., Towell, B., Klug, P., and Guthrie, T. (2003) Initial management of immune thrombocytopenic purpura in adults: a randomized controlled trial comparing intermittent anti-D with routine care. *Am. J. Hematol. 74*, 161–9.

Harker, L. (1970) Thrombokinetics in idiopathic thrombocytopenic purpura. *Br. J. Haematol. 19*, 95–104.

Harrington, W., Minnich, V., Hollingsworth, J., and Moore, C.V. (1951) Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. *J. Lab. Clin. Med. 38*, 1–10.

Hart, D., Dunetz, C., Nardi, M., Porges, R., Weiss, A., and Karpatkin, M. (1986) An epidemic of maternal thrombocytopenia associated with elevated antiplatelet antibody. Platelet count and anti-platelet antibody in 116 consecutive pregnancies: relationship to neonatal platelet count. *Am. J. Obstet. Gynecol. 154*, 878–883.

Hymes, K., Shulman, S., and Karpatkin, S. (1979) A solid-phase radioimmunoassay for bound anti-platelet antibody: studies on 45 patients with autoimmune platelet disorders. *J. Lab. Clin. Med. 94*, 639–648.

Hymes, K., Blum, M., Lackner, H., and Karpatkin, S. (1981). Easy bruising, thrombocytopenia and elevated platelet immunoglobulin G in Graves' disease and Hashimoto's thyroiditis. *Ann. Intern. Med. 94*, 27–30.

Hymes, K., Greene, B., and Karpatkin, S. (1988) The effect of azidothymidine on HIV-related thrombocytopenia. *N. Engl. J. Med. 318*, 516–517.

Jarque, I., Andreu, R., Llopis, I., De la Rubia, J., Gomis, F., Senent, L., Jiminez, C., Martin, G., Martinez, J., Sanz, G., Ponce, J., and Sanz, M. (2001) Absence of platelet response after eradication of *Helicobacter pylori* infection in patients with chronic idiopathic thrombocytopenic purpura. *Br. J. Haematol. 115*, 1002–1003.

Jillella, A., Kallab, A., and Kutlar, A. (2000) Autoimmune thrombocytopenia following autologous hematopoietic cell transplantation: review of literature and treatment options. *Bone Marrow Transplant. 26*, 925–927.

Karpatkin, S. (1978). Heterogeneity of human platelets. IV. Correlation of platelet function with platelet volume. *Blood 51*, 307–316.

Karpatkin, S. (1997). Autoimmune (idiopathic) thrombocytopenic purpura. *Lancet 349*, 1531–1536.

Karpatkin, S. and Siskind, G. (1969). *In vitro* detection of platelet antibody in patients with idiopathic thrombocytopenic purpura and systemic lupus erythmatosus. *Blood 33*, 795.

Karpatkin, S., Strick, N., Karpatkin, M., and Siskind, G. (1972a). Cumulative experience on the detection of anti-platelet antibody in 234 patients with idiopathic thrombocytopenic purpura, systemic lupus erythematosis and other clinical disorders. *Am. J. Med. 52*, 776–785.

Karpatkin, S., Strick, N., and Siskind, G. (1972b). Detection of splenic antiplatelet antibody synthesis in idiopathic autoimmune thrombocytopenic purpura. *Br. J. Haematol. 23*, 167–76.

Karpatkin, S., Nardi, M., and Hymes, K. (1988) Anti-human immunodeficiency virus type a antibody complexes on platelets of seropositive thrombocytopenic homosexuals and narcotic addicts. *Ann. Intern. Med. 109*, 190–193.

Karpatkin, S., Xia, J., Patel, J., and Thorbecke, G. (1992). Serum platelet-reactive IgG of ATP patients is not $F(ab')_2$ mediated and a function of storage. *Blood 80*, 3164–3172.

Karpatkin, S., Nardi, M.A., and Hymes, K.B. (1995) Sequestration of antiplatelet GPIIIa antibody in rheumatoid factor immune complexes of human immunodeficiency virus 1 thrombocytopenic patients. *Proc. Natl. Acad. Sci. USA 92*, 2263–2267.

Kekomaki, R., Dawson, B., McFarland, J., and Kunicki, T. (1991) Localization of human platelet autoantigens to the cysteine-rich region of glycoprotein. *J. Clin. Invest. 88*, 847–854.

Kernoff, L., Blake, K., and Shakelton, D. (1980). Influence of the amount of platelet-bound IgG on platelet survival and site of sequestration in autoimmune thrombocytopIenia. *Blood 55*, 730–733.

Kojouri, K., Vesely, S., Terrell, D., and George, J. (2004). Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications *Blood 104*, 2623–3264.

Kurata, Y., Curd, J.G., Tamerius, J.D., and McMillan, R. (1985). Platelet-associated complement in chronic ITP. *Br. J. Haematol. 60*, 723–733.

Kurata, Y., Miyagawa, S., Kosugi, S., Kashiwagi, H., Honda, S., Mitzutani, H., Tomiyama, Y, Kanayama, Y., and Matsuzawa, Y. (1994). High titer anti-nuclear antibodies, anti-SSA/RO antibodies with idiopathic thrombocytopenic purpura. *Thromb. Haemost. 71*, 184–187.

Kuwana, M., Kaburaki, J., and Ikeda, Y. (1998). Autoreactive T cells to platelet GPIIb-III in immune thrombocytopenic purpura: role in the production of antiplatelet autoantibody. *J. Clin. Invest. 102*, 1393–1402.

Kuwana, M., Kaburaki, J., Kitasato, H., Kato, M., Kawai, S., Kawakami, Y., and Ikeda, Y. (2001). Immunodominant epitopes on glycoprotein IIb-IIIa recognized by autoreactive T cells in patients with immune thrombocytopenic purpura. *Blood 98*, 130–139.

Li, L., Nardi, M., and Karpatkin, S. (2003). Role of molecular mimickry to HIV-1 peptides in HIV-1 related thrombocytopenia. *Blood 102*, 125A.

Lim, S. and Ifthikharuddin, J. (1994). Autoimmune thrombocytopenic purpura complicating lymphoproliferative disorders. *Leuk. Lymphoma 15*, 61–64.

McLain, M.T., Heinlen, L.D., Dennis, G.J., Roebuck, J., Harley, J.B., and James, J.A. (2005). Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat. Med. 11*, 85–88.

McMillan, R. (1990) Antigen-specific assays in immune thrombocytopenia. *Transfus. Med. Rev. 4*, 136–143.

McMillan, R. (2000) Autoantibodies and autoantigens in chronic immune thrombocytopenia purpura. *Sem. Thromb. Hemost. 37*, 239–248.

McMillan, R., Longmire, R., Yelenosky, R., Smith, R.S., and Craddock, C.G. (1972). Immunoglobulin synthesis *in vitro* by splenic tissue in idiopathic thrombocytopenic purpura. *N. Engl. J. Med. 286*, 681–684.

McMillan, R., Luiken, G., Levy, R., Yelenosky, R., and Longmire, R.L. (1978) Antibody against megakaryocytes in idiopathic thrombocytopenic purpura. *JAMA 239*, 2460–2462.

McMillan, R., Bowditch, R., Tani, P., Anderson, H., and Goodnight, S. (1996). A non-thrombocytopenic bleeding disorder due to an IgG4 kappa anti-GPIIb/IIIa autoantibody. *Br. J. Haematol. 4*, 747–749.

McMillan, R., Wang, L., Lopez-Dee, J., Jiu, S., and Loftus, J. (2002). Many αIIIβ3 autoepitopes in chronic immune thrombocytopenic purpura are localized to αIIb between amino acids L1 and Q459. *Br. J. Haematol. 118*, 1132–1136.

McMillan, R., Wang, L., Tomer, A., Nichol, J., and Pistillo, J. (2004). Suppression of *in vitro* megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. *Blood 103*, 1364–1369.

McMinn, J., Cohen, S., Moore, J., Lilly, S., Parkhurst, J., Tarantino, M., Terrell, D., and George, J. (2003). Complete recovery from refractory immune thrombocytopenic purpura in three patients treated with etanercept. *Am. J. Haematol. 73*, 135–140.

Michael, M., Cooper, N., Jean, C., Frissora, C., and Bussel, J. (2004). Does *Helicobacter pylori* initiate or perpetuate immune thrombocytopenic purpura? *Blood 103*, 890–896.

Morris, L., Distenfeld, A., Amorosi, E., and Karpatkin, S. (1982). Autoimmune thrombocytopenic purpura in homosexual men. *Ann. Intern. Med. 96*, 714–717.

Mosmann, T. and Coffman, R. (1989). Heterogeneity of cytokine secretion patterns and functions of helper T cells TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol. 7*, 145–173.

Mouzaki, A., Theodoropoulou, M., Gianakopoulos, I., Vlaha, V., Kyrtsonis, M.C., and Maniatis, A. (2002). Expression patterns of Th1 and Th2 cytokine genes in childhood idiopathic thrombocytopenic purpura (ITP) at presentation and their modulation by intravenous immunoglobulin G (IVIg) treatment: their role in prognosis. *Blood 100*, 1774–1779.

Najean, Y. and Rain, J.-D. (1994). The mechanism of thrombocytopenia in patients with HIV. *J. Lab. Clin. Med. 123*, 415–420.

Najean, Y., Ardaillou, N., Dresch, C., and Bernard, J. (1967). The platelet destruction site in thrombocytopenic purpura. *Br. J. Haematol. 13*, 409–426.

Nardi, M.A., Liu, L.X., and Karpatkin, S. (1997). GPIIIa-(49–66) is a major pathophysiologically relevant antigenic determinant for anti-platelet GPIIIa of HIV-1-related immunologic thrombocytopenia. *Proc. Natl. Acad. Sci. USA 94*, 7589–7594.

Nardi, M., Tomlinson, S., Greco, M., and Karpatkin, S. (2001). Complement-independent, peroxide induced antibody lysis of platelets in HIV-1-related immune thrombocytopenia. *Cell 106*, 551–561.

Nardi, M., Feinmark, S., Liang, H., Li, Z., and Karpatkin, S. (2004). Complement-independent Ab-induced peroxide lysis of platelets requires 12-lipoxygenase and a platelet NADPH oxidase pathway. *J. Clin. Invest. 113*, 973–980.

Neylon, M., Saunders, P., Howard, M., Proctor, S., Taylor, P., and Group, N.R.H. (2003). Clinically significant newly presenting autoimmune thrombocytopenic purpura in adults: a prospective study of a population-based cohort of 245 patients. *Br. J. Haematol. 122*, 966–974.

Nomura, S., Matsuzakai, T., Ozaki, Y., Yamaoka, M., Yoshimura, C., Katsura, K., Xie, G.L., Kagawa, H., Ishida, T., and Fukuhara, S.C. (1998). Clinical significance of HLA-DRB10410 in Japanese patients with idiopathic thrombocytopenic purpura. *Blood 91*, 3616–3622.

Nomura, S., Dan, K., Hotta, T., Fujimara, K., and Ikeda, Y. (2002). Effects of pegylated recombinant human megakaryocyte growth and development factor in patients with idiopathic thrombocytopenic purpura. *Blood 100*, 728–730.

Olsson, B., Andersson, P., Jernas, M., Jacobsson, S., Carlsson, B., Carlsson, L., and Wadenvik, H. (2003). T-cell mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. *Nature 9*, 1123–1124.

Pfueller, S., David, R., Firkin, B., Bilston, R., Cortizo, W., and Raines, G. (1990). Platelet aggregating IgG antibody to platelet surface glycoproteins associated with thrombosis and thrombocytopenia. *Br. J. Haematol. 74*, 336–341.

Portielje, J., Westendorp, R., Kluin-Nelemans, H., and Brand, A. (2001). Morbidity and mortality in adults with idiopathic thrombocytopenic purpura. *Blood 97*, 2549–2583.

Rabinowitz, Y. and Dameshek, W. (1960). Systemic lupus erythematosus after "idiopathic" thrombocytopenia purpura: a review: a study of systemic lupus erythematosus occuring after 78 splenectomies for "idiopathic" thrombocytopenic purpura with a review of the pertinent literature. *Ann. Intern. Med. 52*, 1–28.

Ratnoff, O., Menitove, J., Aster, R., and Lederman, M. (1983). Coincident classic hemophilia and "idiopathic" thrombocytopenic purpura in patients under treatment with concentrates of anti-hemophilic factor (factor VIII). *N. Engl. J. Med. 308*, 439–442.

Roark, J.H., Bussel, J.B., Cines, D.B., and Siegel, D.L. (2002). Genetic analysis of autoantibodies in idiopathic thrombocytopenic purpura reveals evidence of clonal expansion and somatic mutation. *Blood 100*, 1388–1398.

Samuel, H., Nardi, M., Karpatkin, M., Hart, D., Belmont, M., and Karpatkin, S. (1999). Differentiation of autoimmune thrombocytopenia (ATP) from thrombocytopenia associated with immune complex disease: Systemic lupus erythomatosis (SLE), hepatitis-cirrhosis, and HIV-1 infection by platelet and serum immunologic measurements. *Br. J. Haematol. 105*, 1086–1091.

Savona, S., Nardi, M., and Karpatkin, S. (1985). Thrombocytopenic purpura in narcotics addicts. *Ann. Intern. Med. 102*, 737–741.

Scott, J.R., Rote, N.S., and Cruikshank, D.P. (1983). Antiplatelet antibodies and platelet counts in pregnancies complicated by autoimmune thrombocytopenic purpura. *Am. J. Obstet. Gynecol. 145*, 932–939.

Semple, J. (2002). Immune pathophysiology of autoimmune thrombocytopenic purpura. *Blood Rev. 16*, 9–12.

Semple, J. (2003). T cell cytokine abnormalities in patients with autoimmune thrombocytopenic purpura. *Transfusion Apheresis Sci. 28*, 237–242.

Semple, J. and Friedman, J. (1991). Enhanced antiplatelet lymphocyte reactivity in patients with autoimmune thrombocytopenia (AITP). *Blood 78*, 2619–2625.

Shulman, N., Marder, V., and Weinrach, R. (1965). Similarities between known anti-platelet antibodies and the factor responsible for thrombocytopenia in idiopathic purpura. Physiologic, serologic and isotopic studies. *Ann. N. Y. Acad. Sci. 124*, 499.

Spaet, T. (1952). Vascular factors in the pathogenesis of hemorrhagic syndromes. *Blood 7*, 641–653.

Stasi, R. and Provan, D. (2004). Management of immune thrombocytopenic purpura in adults. *Mayo Clin. Proc. 79*, 504–522.

Stasi, R., Stipa, E., Masi, M., Oliva, F., Sciarra, A., Perrotti, A, Olivieri, M., Zaccari, G., Gandolfo, G.M., and Galli, M. (1994). Prevalence and clinical significance of elevated antiphospholipid antibodies in patients with idiopathic thrombocytopenic purpura. *Blood 84*, 4203–4208.

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*, 952–957.

Takahashi, T., Yujiri, T., Shinohara, K., Inoue, Y., Sato, Y., Fuji, Y., Okubo, M., Zaitsu, Y., Ariyoshi, K., Nakamura, Y., Nawata, R., Oka, Y., Shirai, M., and Tanizawa, Y. (2004). Molecular mimicry by *Helicobacter pylori* CagA protein may be involved in the pathogenesis of *H. pylori*-associated chronic idiopathic thrombocytopenic purpura. *Br. J. Haematol. 124*, 91–96.

Tsubakio, T., Tani, P., Curd, J.G., and McMillan, R. (1986). Complement activation *in vitro* by antiplatelet antibodies in chronic immune thrombocytopenic purpura. *Br. J. Haematol*. *63*, 293–300.

van der Harst, D., de Jong, D., Limpens, J., Kluin, P., Rozier, Y., van Ommen, G., and Brand, A. (1990). Clonal B-cell populations in patients with idiopathic thrombocytopenic purpura. *Blood 76*, 2321–2326.

van der Pol, W. and van de Winkel, J.G. (1998). IgG receptor polymorphisms: risk factors for disease. *Immunogenetics 48*, 222–232.

van Leeuwen, E., van der Ven, J., Engelfriet, C.P., and van dem Borne, A.E. (1982) Clonal B-cell populations in patients with idiopathic thrombocytopenic purpura. *Blood 59*, 23–26.

Varon, D. and Karpatkin, S. (1983). A monoclonal anti-platelet antibody with decreased reactivity for autoimmune thrombocytopenic platelets. *Proc. Natl. Acad. Sci. USA 80*, 6992.

Varon, D., Gitel, S., Varon, N., Lahav, J., Dardik, R., Klepfish, A., and Berribi, A. (1992) Immune Bernard Soulier-like syndrome associated with anti-glycoprotein-IX antibody [letter]. *Am. J. Hematol. 41*, 67–68.

Walker, R. and Walker, W. (1984). Idiopathic thrombocytopenia, initial illness and long term follow-up. *Arch. Dis. Child. 59*, 316–322.

Walsh, C., Nardi, M., and Karpatkin, S. (1984). On the mechanism of thrombocytopenic purpura in sexually-active homosexual men. *N. Engl. J. Med. 311*, 635–639.

Walsh, C., Kriegal, R., Lennette, E., and Karpatkin, S. (1985). Thrombocytopenia in homosexual patients. Prognosis, response to therapy, and prevalence of antibody to the retrovirus associated with the acquired immunodeficiency syndrome. *Ann. Intern. Med. 103*, 542–545.

Warner, M., Moore, J., Warkentin, T., Santos, A., and Kelton, J. (1999). A prospective study of protein-specific assays used to investigate idiopathic thrombocytopenic purpura. *Br. J. Haematol. 104*, 442–447.

Zucker-Franklin, D. and Karpatkin, S. (1977). Red-cell and platelet fragmentation in idiopathic autoimmune thrombocytopenic purpura. *N. Engl. J. Med. 297*, 517–523.

CHAPTER

43

Neutropenia

PARVIZ LALEZARI

The Biomedical Research Institute, and Departments of Medicine and Pathology, Montefiore Medical Center and Albert Einstein College of Medicine, New York, New York, USA

HISTORIC BACKGROUND

In 1926, Doan observed that sera from some patients agglutinated leukocytes from other individuals. Subsequent work by Dausset and Nenna (1952), Moeschlin and Wagner (1952), and Goudsmit and van Loghem (1953) laid the foundation for leukocyte immunology. Further progress in the field came with the discovery that leukocyte antibodies develop during pregnancy (Payne and Rolfs, 1958; van Rood et al., 1959), and that fetal–maternal neutrophil incompatibility causes neonatal neutropenia (Lalezari et al., 1960). Cooperative research on leukocyte immunology, initiated in 1964, then led to the discovery of the HLA system. The finding that certain leukocyte antigens were exclusively expressed on neutrophils (Lalezari and Bernard, 1966) separated immunology of neutrophils from histocompatibility, where the cells of interest are the lymphocytes.

In early reports on autoimmune neutropenia, Moeschlin and Wagner (1952) and Butler (1958) showed that infusion of plasma from neutropenic patients into normal recipients caused leukopenia. Boxer and Stossel (1974) designed novel techniques for detection of neutrophil antibodies, and autoantibodies in autoimmune neutropenia of infancy were shown to be directed against neutrophil-specific antigens (Lalezari et al., 1975).

Since the first edition of *The Autoimmune Diseases* two decades ago, new information has become available on the structure, function, and genetic control of neutrophil-specific antigens, the main targets of neutrophil autoimmunity. New serologic methods for antibody detection have been developed, and the use of colony-stimulating factors has had major impact on the management of the disease.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

The levels of blood cells in the circulation are determined by the rate of their production and destruction. Neutrophil levels are also influenced by their large marginal pool. Neutropenia is defined by the fall of absolute neutrophil and band forms below 1.5×10^9/L. Immunologically-induced neutropenias are probably as common as the more familiar autoimmune disorders of red cells and platelets, with clinical manifestations varying from being asymptomatic to severe forms complicated by overwhelming sepsis. Most patients develop intermittent mucocutaneous infections. Splenomegaly is an exception rather than the rule, and the hematologic profile is that of selective absence or reduction in the number of neutrophils, while monocytes, eosinophils, and basophils are either normal or increased. Lymphocyte counts are normal or occasionally reduced. Platelets are normal, and a mild anemia together with hypergammaglobulinemia may exist due to persistent infections in chronic

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

forms. Bone marrow shows myeloid hyperplasia with a distinctly diminished number of mature cells, resembling "maturation arrest." Autoimmune neutropenia is divided into primary and secondary forms, depending on whether or not neutropenia is part of a complex autoimmune disease. In the primary forms, "autoimmune neutropenia of infancy (AINI)" is distinct from the adult forms.

Autoimmune Neutropenia of Infancy

Since the first reports (Boxer et al., 1975; Lalezari et al., 1975), many cases with identical features have been reported (Bux et al., 1998; McCullough et al., 1988). AINI is characterized by a severe neutropenia diagnosed when infants are about 5–7 months old. Neutropenia is commonly associated with bacterial or fungal infections, and spontaneous recovery within 1–4 years is the rule. Diagnosis is made by demonstrating neutrophil-bound and circulating antineutrophil antibodies. In many cases, the antibodies are directed against neutrophil-specific antigens (Lalezari et al., 1986). The cause of AINI remains unclear. The disease may represent a "physiologic state" where the autologous antigens, or the antigens shared with the mother, provoke an immune response in an infant in whom the immune regulatory system is underdeveloped. The process reverses when the child's regulatory mechanism reaches maturity. A long-term follow-up of AINI has confirmed that affected children remain immunologically normal after recovery. On rare occasions, patients remain chronically and severely neutropenic. These patients represent a subgroup of true autoimmune disease, similar to the chronic forms seen in adults and adolescents.

Primary Autoimmune Neutropenias of Adults and Adolescents

Neutropenia in adults and adolescents is often a chronic and debilitating disease with a preponderance in females. In pregnant women, the infants may develop transient neutropenia (Stefanini et al., 1958). The acute forms are relatively rare and are self-limited. Little information is available on the specificities of the antibodies in the adult forms of autoimmune neutropenia. In a report by McCullough et al. (1988), the antibody had partial specificity for NA1, the first neutrophil-specific antigen. We documented NA1 specificity in a 73-year-old man who had acute neutropenia. The patient had a past history of sarcoidosis and had not received transfusion of blood or blood products. He suddenly developed fever, pharyngitis, and a severe gingivitis. His leukocyte count was below 800/μl with absent neutrophils. Platelets were normal but he had a mild anemia. All other laboratory data were normal except for a positive test for antinuclear antibodies. Bone marrow was hyperplastic but mature neutrophils were absent. After 15 days of persistent severe neutropenia, treatment with prednisone was initiated and continued for 3.5 months. Within 10 days after the start of treatment, the neutrophil count rose to 12,000/μl and remained normal. The blood sample obtained before prednisone treatment contained a strong agglutinating antibody that reacted with neutrophils from all 28 NA1-positive donors and none of the 12 NA1-negative donors. The blood samples obtained 3 and 6 months after recovery did not react with neutrophils from any of 30 donors tested. The patient's neutrophils, obtained after recovery, were typed as NA1/NA2 and reacted strongly with the autologous serum obtained during the neutropenic phase, but not with the recovery samples. It is of interest that the target of the autoantibody was NA1 and not NA2, the allele also expressed.

Secondary Autoimmune Neutropenias

Association of neutropenia with autoimmune hemolytic anemia or thrombocytopenia is not uncommon, as in Evans syndrome (Evans et al., 1951). A special combination, which we have designated "alternating autoimmune hemocytopenia," is characterized by being multiphasic. Initially, cytopenia is limited to either thrombocytopenia, neutropenia or hemolytic anemia, and responds to treatment. After an interval, which may last from months to years, the autoimmune process recurs, this time involving another cell type. Alternation between neutropenia, hemolytic anemia, and thrombocytopenia continues for several years, and eventually a combination prevails that is severe and resistant to treatment.

Nonhematologic autoimmune disorders associated with neutropenia include systemic lupus erythematosus (SLE), Sjögren syndrome, Graves' disease, rheumatoid arthritis, lymphoproliferative disorders (e.g., T-cell and hairy cell leukemia), Felty syndrome, common variable immunodeficiency, and immune complex diseases. Coppo et al. (2004) have reported 9 patients with secondary neutropenia who had antineutrophil cytoplasmic antibodies (ANCAs). Many infectious diseases are associated with neutropenia, which may be due to myelosuppression, especially in infants who have a small bone marrow reserve. In viral diseases such as mycoplasma pneumonia and infectious mononucleosis, cold-reacting antibodies are likely to be a contributory factor.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

NA and NB neutrophil-specific antigens are often the target of autoantibodies in autoimmune neutropenias. These antigens are anchored to the outer leaflet of the lipid bilayer

of neutrophils via a glycosyl phosphatidyl inositol structure. The NA gene has been mapped to chromosome 1q22 and is the low-affinity receptor for IgG (FcγRIII) (Ory et al., 1989). The alleles of the NA gene are NA1, NA2, and SH (Bux et al., 1997). Some rare individuals lack both NA1 and NA2 and are called NA^{null}. It is not clear whether NA^{null} represents the lack of the NA gene or a mutant that fails to react with the currently available typing reagents. The NB antigen is expressed on CD177 glycoprotein (Stroncek et al., 2001) and its gene is located on chromosome 19 at 19q13.2 (Kissel et al., 2001). Recently, a glycoprotein called PRV-1 has been identified and has been shown to be overexpressed in polycythemia vera, in some essential thrombocythemic patients, and during pregnancy (Temerinac et al., 2000). Caruccio et al. (2004) have provided evidence that PRV-1, which belongs to the LY6/snake toxin gene and differs from NB1 in four nucleotides, is an allele of NB1, although the possibility of a gene duplication has not been excluded. The i and I antigens, which are known to react with cold agglutinins, are also expressed on neutrophils and platelets, and in high titers their antibodies can cause neutropenia and thrombocytopenia (Lalezari and Murphy, 1967; Markenson et al., 1975). The antigens that react with ANCA (Hagen et al., 1993) and may be involved in secondary autoimmune neutropenias are intracellular neutrophil proteins, such as proteinase 3, elastase, myeloperoxidase, lysozyme lactoferrin, catepsin G, azurocidin, and bactericidal/permeability-increasing protein.

MECHANISMS OF CELL DESTRUCTION

Most information on the mechanisms of neutrophil destruction in immunologic diseases is based on laboratory observations, and the relevance to *in vivo* events has not been established. Neutrophils usually respond to their antibodies by agglutination, which, unlike with red cells, is an active process requiring living cells. Neutrophils also aggregate in the presence of the activated complement and adhere to endothelial cells (Jacob et al., 1980). Some neutrophil antibodies are known to activate the complement system, and thereby initiate complement-induced neutrophil aggregation. Thus, the *in vivo* neutrophil aggregation may be a mechanism for neutrophil removal in some autoimmune neutropenias.

Boxer and Stossel (1974) have shown that neutrophils coated with auto- or allo-antibodies can stimulate phagocytic cells. Leukophagocytosis is seen in the spleen of some patients with autoimmune neutropenia and indicates that this organ is a location for the clearance of opsonized neutrophils. Using ^{111}In-radiolabeling techniques, McCullough et al. (1988) have shown that neutrophils are not cleared at any selective site, but are removed by phagocytic cells distributed in many tissues. Cytotoxicity, either complement-mediated or antibody-dependent cell-mediated, has been shown *in vitro* by dye exclusion or the release of ^{51}Cr. Inhibition of colony-forming unit (CFU)-C colonies by lymphocytes from neutropenic patients has been demonstrated in Felty syndrome, rheumatoid disorders, and SLE. The mechanism of this suppression is not clear. Neutrophils bind circulating immunoglobulins. This binding, especially when high levels of immune complexes exist, may not be innocuous and may lead to neutropenia, either by complement activation or by phagocytosis. Many patients with known immune complex diseases, however, are not neutropenic. Neutropenia in acquired immune deficiency syndrome (AIDS) may be due to myelosuppression or to immune complexes. A positive test for a high level of neutrophil-bound immunoglobulins is a common finding in AIDS patients.

Many drugs cause dose-dependent neutropenia due to myelotoxicity and interference with protein synthesis and cell replication. Some drug-induced neutropenias are not dose related and are considered to be immunologic in origin. In rare cases, the antibodies or the suppressor cells are directed against progenitor cells, causing pure white cell aplasia (Levitt, 1983).

Finally, alteration of neutrophil functions may result from reaction with autoantibodies. Thus far, the functional consequences of the binding of autoantibodies to Fc receptors or to NB1 have remained unknown. The report by Kramer et al. (1980) exemplifies the inhibition of neutrophil motility by an IgG autoantibody, causing a non-neutropenic clinical condition indistinguishable from chronic neutropenic states. Thus, neutrophil dysfunction may explain the severity of clinical manifestations in cases where symptoms are disproportionate to the number of neutrophils.

LABORATORY DIAGNOSIS

Techniques available for detection of neutrophil antibodies have been summarized elsewhere (McCullogh et al., 1988). In autoimmune neutropenia, as in other autoimmune diseases, direct examination of the target cells should be most relevant. Because of limitations in obtaining adequate number of neutrophils, however, it is often necessary to use indirect techniques. One exception may be the immunofluorescence (IF) test (Verheugt et al., 1977), which requires fewer cells. In addition, by using labeled antiglobulin reagents and flow cytometry, IF allows determination of the class and subclass of antibodies. Despite these technical advances, the tests for autoimmune neutropenia have many shortcomings. In the IF, as it is used with microscopic aid, neutrophils should be differentiated from the frequently present monocytes that give strong reactions with dye-labeled antihuman immunoglobulin reagents. In addition,

neutrophils of patients who receive granulocyte–colony-stimulating factor (G-CSF) treatment overexpress Fc receptors, leading to a nonspecific positive direct IF.

Among the indirect tests, reliable neutrophil agglutination can be obtained only in the presence of EDTA, which prevents spontaneous neutrophil aggregation. Unfortunately, the EDTA-dependent agglutination test is more sensitive for detection of alloantibodies, and autoantibodies may be missed if the required incubation period is shortened. Cytotoxic antibodies are less useful, because sera from many non-neutropenic, normal individuals produce a positive reaction, and the results can be considered positive only if high titers are obtained. Enthusiasm for the tests based on opsonization and inhibition of phagocytosis is tempered by their complexity. Moreover, examples of strong agglutinating antibodies have been found in severe cases of alloimmune neonatal neutropenias that have failed to cause neutrophil opsonization. The quantitative immunoglobulin assays also have limitations because of the presence of large amounts of immunoglobulins on normal neutrophils, and potential difficulties in distinguishing specific antibodies. MAIGA (monoclonal antibody immobilization of granulocyte antigens) has been a successful method for defining neutrophil antigens (Metcalfe and Waters, 1992). Unfortunately, unlike hemoglobin in hemolytic anemia, simple and reliable markers for *in vivo* neutrophil lysis are not available. For this purpose, measurement of the released neutrophil lysozyme (Boxer et al., 1975) has had limited clinical value.

Considering the difficulties in detecting neutrophil antibodies, multiple techniques must often be used. The observations on a possible role for ANCA in neutropenia suggest that in secondary cases, and when antibodies to surface antigens are not found, tests for ANCA should be included in the diagnostic work-up.

TREATMENT

The introduction of CSFs has changed the outcome of all neutropenias, including autoimmune forms. Many patients with primary autoimmune neutropenia, especially children, tolerate the condition well and need antibiotics and other treatments only for the management of intercurrent infections.

Splenectomy and steroid therapy are not as effective as they are in autoimmune hemolytic anemia and thrombocytopenia. These differences may reflect pathophysiologic features unique to neutrophils, especially the multiplicity of mechanisms involved in their destruction. It may be reasoned that neutrophil destruction by cytotoxicity, aggregation, and embolization is unlikely to be affected by splenectomy. Steroids are not fully effective when phagocytosis by macrophages plays an essential role. Neutropenic patients often have chronic infection, which activates macrophages. Activated macrophages are known to be more destructive and less susceptible to inhibition by steroids. Despite these shortcomings, and before the introduction of CSFs, when there were no alternative treatments, most severe cases of chronic neutropenia were treated with steroids and some with splenectomy. Some patients were subjected to plasmaphereses with doubtful results, and in some cases, granulocytes obtained from compatible normal donors were transfused. In one example, repeated transfusions of NA2-negative neutrophils controlled the lung and brain abscesses in a 12-year-old boy who had a severe neutropenia due to anti-NA2 autoantibody. Unfortunately, after multiple transfusions, the patient developed resistance and succumbed to infection. Another modality is intravenous gamma-globulin, which temporarily reverses neutropenia (Bussel et al., 1988). These treatments should be considered in rare cases where G-CSF is not effective.

The effectiveness of G-CSF may be due to increased neutrophil production and mobilization of the marginal pool, which may result in consumption of the antibodies by mass action. Plausible though this hypothesis may be, alternative mechanisms such as an increase in the expression of neutrophil antigens or suppression of the lymphoid system cannot be excluded. The dose of G-CSF must be individualized. Most patients do not require more than 2 to 3 subcutaneous injections at $2.5 \pm 1\,\mu g/kg$. The treatment should be given only for control of complications and in preparation for surgical procedures.

PERSPECTIVES AND FUTURE DIRECTIONS

CSFs have become the treatment of choice for neutropenias regardless of their etiology. Because of this, and for economic considerations, an increasing number of physicians avoid laboratory work-up. Consequently, availability of clinical material for research has noticeably diminished, and in the past two decades few new antigens have been discovered and it has not been possible to confirm several antigens described by single examples. By contrast, the shift of research to molecular biology has led to much progress in our understanding of the molecular genetics of neutrophil antigens. Further research on the function of neutrophil antigens, especially on the possible role of NB1/PRV-1 glycoprotein in hematopoiesis, is likely to reveal that autoantibodies against these antigens have consequences beyond the obvious numerical reduction of neutrophils. At the clinical level, more research on secondary autoimmune neutropenias and the potential role of ANCAs and their targets may produce valuable new information.

References

Boxer, L.A. and Stossel, T.P. (1974). Effects of anti-human neutrophil antibodies *in vitro*. Quantitative studies. *J. Clin. Invest. 53*, 1534–1545.

Boxer, L.A., Greenberg, M.S., Boxer, G.J., and Stossel, T.P. (1975). Autoimmune neutropenia. *N. Engl. J. Med. 293*, 748–753.

Bussel, J.B., Lalezari, P., and Fikrig, S. (1988). Intravenous treatment with gamma-globulin of autoimmune neutropenia of infancy. *J. Pediatr 112*, 298–301.

Butler, J.J. (1958). Chronic idiopathic immuno-neutropenia. *Am. J. Med. 24*, 145–152.

Bux, J., Stein, E.L., Bierling, P., Fromont, P., Clay, M., Stroncek, D., and Santoso, S. (1997). Characterization of a new alloantigen (SH) on the human neutrophil Fc gamma receptor IIIb. *Blood 89*, 1027–1034.

Bux, J., Bejhrens, G., Jaeger, G., and Welte, K. (1998). Diagnosis and clinical course of autoimmune neutropenia in infancy: analysis of 240 cases. *Blood 91*,181–186.

Caruccio, L., Walkovich, K., Bettinetti, M., Schuller, R., and Stroncek, D. (2004). CD177 polymorphisms: correlation between high-frequency single nucleotide polymorphisms and neutrophil surface protein expression. *Transfusion 44*, 77–82.

Coppo, P., Ghez, D., Fuentes, V., Bengoufa, D., Oksenhendler, E., Tribout, B., Clauvel, J.P., and Lassoued, K. (2004). Antineutrophil cytoplasmic antibodies-associated neutropenia. *Eur. J. Intern. Med. 7*, 451–459.

Dausset, J. and Nenna. A. (1952). Presence of leuko-agglutinin in the serum of a case of chronic agranulocytosis. *C. R. Soc. Biol. Fil*. 146, 1539–1541.

Doan, C.A. (1926). The recognition of a biologic differentiation in the white blood cells with a specific reference to blood transfusion. *JAMA 86*, 1593–1597.

Evans, R.S., Takahashi, K., Duane, R.T., Payner, R., and Liu, C. (1951). Primary thrombocytopenic purpura and acquired hemolytic anemia; evidence for a common etiology. *Arch. Intern. Med. 87*, 48–65.

Goudsmit, R. and van Loghem, J.J. (1953). Studies on the occurrence of leukocyte-antibodies. *Vox Sang. 3*, 58–67.

Hagen, E.C., Ballieux, B.E., van Es, L.A., Daha, M.R., and van der Woude, F.J. (1993). Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenetic consequences. Blood 80, 1996–2002.

Jacob, H.S., Craddock, P.R., Hammerschmidt, D.E., and Moldov, C.F. (1980). Complement-induced granulocyte aggregation: an unsuspected mechanism of disease. *N. Engl. J. Med. 302*, 789–794.

Kissel, K., Santoso, S., Hofmann, C., Stroncek, D., and Bux, J. (2001). Molecular basis of the neutrophil glycoprotein NB1 (CD177) involved in the pathogenesis of immune neutropenias and transfusion reactions. *Eur. J. Immunol. 31*, 1301–1309.

Kramer, N., Perez, H.D., and Goldstein, I.M. (1980). An immunoglobulin (IgG) inhibitor of polymorphonuclear leukocyte motility in a patient with recurrent infection. *N. Engl. J. Med. 303*, 1253–1258.

Lalezari, P. and Bernard, G.E. (1966). An isologous antigen-antibody reaction with human neutrophils, related to neonatal neutropenia. *J. Clin. Invest. 45*, 1741–1750.

Lalezari, P. and Murphy, G.B. (1967). In Curtoni et al. (eds), *Histocompatibility Testing* (p. 421). Baltimore: Williams & Wilkins.

Lalezari, P., Nussbaum, M.G., Gelman, S., and Spaet, T.H. (1960). Neonatal neutropenia due to maternal isoimmunization. *Blood 15*, 236–243.

Lalezari, P., Jiang, A.F., Yegon, L., and Santorineau, M. (1975). Chronic autoimmune neutropenia due to anti-NA2 antibody. *N. Engl. J. Med. 293*, 744–747.

Lalezari, P., Khorshidi, M., and Petrosova, M. (1986). Autoimmune neutropenia of infancy. *J. Pediatr. 109*, 764–769.

Levitt, L., Ries, C.A., and Greenberg, P.L. (1983). Pure white-cell aplasia. Antibody-mediated autoimmune inhibition of granulopoiesis. *N. Engl. J. Med. 308*, 1141–1146.

Markenson, A.L., Lalezari, P., and Markenson, J.A. (1975). In Proceedings of the 18th Congress of the American Society of Hematology, Dallas (p. 174), Grune & Stratton, Orlando, Fl., USA.

McCullough, J. et al. (1988). In *Granulocyte Serology. A Clinical and Laboratory Guide* (pp. 90–112, 113–124 and 134–135). Chicago: ASCP Press.

Metcalfe, P. and Waters, A.H. (1992). Location of the granulocyte-specific antigen LAN on the Fc-receptor III. *Transfus. Med. 2*, 283–287.

Moeschlin, S. and Wagner, K. (1952). Agranulocytosis due to the occurrence of leukocyte-agglutinins (pyramidon and cold agglutinins). *Acta Hematol. 8*, 29–41.

Ory, P.A., Clark, M.R., Kwoh, E.E., Clarkson, S.B., and Goldstein, I.M. (1989). Sequences of complementary DNAs that encode the NA1 and NA2 forms of Fc receptor III on human neutrophils. *J. Clin. Invest. 84*, 1688–1691.

Payne, R. and Rolfs, M.R. (1958). Fetomaternal leukocyte incompatibility. *J. Clin. Invest. 37*, 1756–1763.

Rodey, G.E. and Lalezari, P. (2000). In Hoffman, R., et al. (eds), *Hematology, Basic Principles and Practice*, 3rd edn (pp. 2220–2241). New York: Churchill and Livingstone.

Stefanini, M., Mele, R.H., and Skinner, D. (1958). Transitory congenital neutropenia: a new syndrome. *Am. J. Med. 25*, 749–758.

Stroncek, D.F., Kissel, K., von dem Borne, A., and Bux, J. (2001). *Protein Reviews on the Web (PROW) 3*, 19–24.

Temerinac, S., Klippel, S., Strunck, E., W., Lubbert, M., Lange, W., Azemar, M., Meinhardt, G., Schaefer, H.E., and Pahl, H.L. (2000). Cloning of PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in polycythemia rubra vera. *Blood 95*, 2569–2576.

van Rood, J.J. and van Leeuwen, Eernisse, J.G. (1959). Leucocyte antibodies in sera of pregnant women. *Vox Sang. 4*, 427–444.

Verheugt, F.W.A., von dem Borne, A.E., Decary, F., and Engelfriet, C.P. (1977). The detection of granulocyte alloantibodies with an indirect immunofluorescence test. *Br. J. Haematol. 36*, 533–544.

CHAPTER

44

Aplastic Anemia

ROBERT A. BRODSKY*
**John Hopkins Medicine, Division of Hematology*

RICHARD JONES†
†The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Division of Hematologic Malignancies, Baltimore, Maryland, USA

Aplastic anemia may be congenital or acquired. The acquired form represents over 90% of cases and usually results from an autoimmune attack against hematopoietic stem cells. Congenital forms of aplastic anemia are not due to autoimmunity, but it is important to recognize these disorders since they do not respond to immunosuppressive therapy. Congenital aplastic anemia tends to present in the first decade of life and is often, but not always, associated with other physical anomalies. Fanconi anemia, the most common form of congenital bone marrow failure, predisposes to cancer and is frequently associated with other congenital abnormalities, including short stature, upper limb anomalies, hypogonadism, and café-au-lait spots (Bagby, 2003). Dyskeratosis congenital (DKC) is another congenital bone marrow failure disorder that can be either X-linked recessive, autosomal dominant, or autosomal recessive (Dokal and Vulliamy, 2003). The X-linked recessive form results from mutations in the gene DKC1 whose gene product, dyskerin, is important for stabilizing telomerase. The resulting telomerase deficiency leads to bone marrow failure and premature aging. The autosomal-dominant form of DKC results from hTERC gene mutations, the RNA component of telomerase. This chapter will focus on the acquired form of aplastic anemia.

HISTORIC BACKGROUND

The earliest case description of acquired aplastic anemia was by Dr Paul Ehrlich in 1888. He described a young woman who died following an abrupt illness characterized by severe anemia, bleeding, high fever, and a markedly hypocellular bone marrow. The term aplastic anemia was first introduced in 1904 by Chauffard. Until the early 1970s, most patients with severe aplastic anemia died within a year of diagnosis. The advent of potent immunosuppressive treatments, particularly antithymocyte globulin (ATG) and allogeneic bone marrow transplantation (BMT), markedly improved the outcome of these patients and prompted vigorous clinical and laboratory investigation. These studies have generated important insight into hematopoietic stem cell biology, immunology, and autoimmunity. A patient with aplastic anemia became the first long-term survivor after receiving an allogeneic BMT in 1972. Today, the majority of patients survive this potentially devastating autoimmune disorder.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Aplastic anemia manifests as pancytopenia in conjunction with a hypocellular bone marrow. The disease may present abruptly (over days) or insidiously (over weeks to

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

months). The most common clinical manifestations reflect the low blood counts and include dyspnea on exertion, fatigue, easy bruising, petechia, epistaxis, gingival bleeding, heavy menses, headaches, and fever. The primary diagnostic procedure, in addition to a complete blood count, leukocyte differential, and reticulocyte count is the bone marrow aspirate and biopsy (>1 cm core). Flow cytometry on peripheral blood and cytogenetics should be performed on the bone marrow aspirate to rule out paroxysmal nocturnal hemoglobinuria (PNH) (Hall and Rosse, 1996; Brodsky et al., 2000) and hypoplastic myelodysplastic syndromes (hMDS). Patients under the age of 40 years should be screened for Fanconi anemia using the clastogenic agents diepoxybutane or mitomycin C (Bagby, 2003).

A hypocellular bone marrow is required for the diagnosis of aplastic anemia. However, most patients will have residual pockets of ongoing hematopoiesis; thus, an adequate biopsy is essential for establishing the diagnosis, as spicules from the aspirate may be surprisingly cellular given the degree of hypocellularity on the biopsy. Dyserythropoiesis is not uncommon in aplastic anemia, especially in cases with coincidental small-to-moderate PNH populations; however, a small percentage of myeloid blasts, or dysplastic features in the myeloid or megakaryocyte lineages, is more typical of hMDS. CD34 is expressed on early hematopoietic progenitors and the number of $CD34^+$ cells has also been used to help discriminate between aplastic anemia and hMDS. In aplastic anemia the percentage of cells expressing CD34 is usually <0.1%; in hMDS the CD34 count is either normal (0.5–1.0%) or elevated (Orazi et al., 1997).

As with other autoimmune diseases, there is a wide spectrum of disease severity in aplastic anemia. The prognosis in aplastic anemia is proportional to the quantitative depression of peripheral blood counts, most notably the absolute neutrophil count. Accordingly, aplastic anemia is classified as nonsevere, severe, and very severe based largely upon the degree of neutropenia (Table 44.1). The 2-year mortality rate with supportive care alone for patients with severe or very severe aplastic anemia approaches 80% (Camitta et al., 1979), with invasive fungal infections and overwhelming bacterial sepsis being the most frequent causes of death. Nonsevere aplastic anemia is seldom life-threatening and in many instances requires no therapy. Although some cases of nonsevere aplastic anemia will progress, many will remain stable for years, and some may spontaneously improve.

Epidemiologic data are sparse. Precise estimates of incidence and prevalence of aplastic anemia are confounded by its rarity, imprecision in establishing the diagnosis, and difficulty in identifying the onset of the disorder. The best estimates of incidence are from case–control studies, which report an incidence of 2 cases in a million inhabitants in Europe (Kaufman et al., 1991) and Israel (Modan et al., 1975), but the incidence may be two- to three-fold higher in South-East Asia (Szklo et al., 1985; Issaragrisil et al., 1997b). The disease most commonly affects children and young adults but may occur at any age. Acquired aplastic anemia has been associated with drugs, benzene exposure, insecticides, viruses, and other agents. However, over 80% of cases are classified as idiopathic since no etiologic agent is identified (Issaragrisil et al., 1997b; Bacigalupo et al., 2000b). A population-based case–control study of aplastic anemia in Thailand found that drugs, the most commonly implicated etiology, explain only 5% of newly diagnosed cases (Issaragrisil et al., 1997b).

AUTOIMMUNE FEATURES AND PATHOGENIC MECHANISMS

Aplastic anemia was originally thought to result from a quantitative deficiency of hematopoietic stem cells precipitated by a direct toxic effect on stem cells. However, attempts to treat aplastic anemia by simple transfusion of bone marrow from an identical twin failed to reconstitute hematopoieisis in most patients. Retransplant of many of these patients following a high-dose cyclophosphamide preparative regimen was successful, suggesting that the pathophysiology of aplastic anemia was more intricate (Champlin et al., 1984; Hintererger et al., 1997).

In the late 1960s, Mathé et al. (1970) were among the first to postulate an immune basis for aplastic anemia. They performed BMT in patients with aplastic anemia using partially mismatched donors after administering antilymphocyte globulin as an immunosuppressive conditioning

TABLE 44.1 Aplastic anemia: diagnosis and definitions. Bone marrow cellularity must be <25% to diagnose aplastic anemia

Peripheral blood counts	Nonsevere aplastic anemia (not meeting criteria for severe disease)	Severe aplastic anemia (any 2 of 3 criteria below)	Very severe aplastic anemia (meets criteria for severe disease and absolute neutrophils <200)
Absolute neutrophils		<500/μl	<200/μl
Platelets		<20,000/μl	
Reticulocyte count		<1.0% corrected or <60,000/μl	

regimen. Although the patients failed to engraft, the investigators witnessed autologous recovery of hematopoiesis in some patients. This suggested that functional hematopoietic stem cells existed in aplastic anemia patients and that the immune system was somehow suppressing their growth and the differentiation of hematopoietic stem cells. The response to immunosuppressive therapy was the first clear evidence that aplastic anemia was truly an autoimmune disease. Additionally, there appears to be an underlying genetic predisposition to acquired aplastic anemia, as evidenced by the over-representation of the HLA-DR2 subtypes (Nimer et al., 1994).

The first laboratory experiments suggesting an autoimmune pathophysiology were co-culture experiments showing that T lymphocytes from aplastic patients inhibited hematopoietic colony formation *in vitro* (Nissen et al., 1980; Hoffman et al., 1997). Since then, it has been shown that the immune destruction of hematopoietic stem cells in aplastic anemia is mediated by cytotoxic T cells, and involves inhibitory type 1 helper T-cell (Th1) cytokines and the Fas-dependent cell death pathway. The cytotoxic T cells are usually more conspicuous in the bone marrow than in the peripheral blood (Zoumbos et al., 1985; Maciejewski et al., 1994; Melenhorst et al., 1997b) and overproduce interferon (IFN)-γ and tumor necrosis factor (TNF) (Nakao et al., 1992; Nistico and Young, 1994). TNF and IFN-γ are direct inhibitors of hematopoiesis and appear to upregulate Fas expression on $CD34^+$ cells (Maciejewski et al., 1995). Immortalized $CD4^+$ and $CD8^+$ T-cell clones from some aplastic anemia patients have been shown to secret Th1 cytokines and are capable of killing autologous CD34 cells (Nakao et al., 1997; Zeng et al., 2001). Recently, evidence for a humoral autoimmune response in aplastic anemia has also been reported; 7 of 18 aplastic anemia patients were found to have autoantibodies against kinectin, a 1300 amino acid molecule expressed on human hematopoietic cells, as well as liver, ovary, testis, and brain cells (Hirano et al., 2003).

Studies examining T-cell diversity using complementarity-determining region (CDR3) spectratyping have further implicated the immune system in aplastic anemia. Several groups have found limited heterogeneity of the T cell receptor β chain (Vβ) in aplastic anemia, suggesting that there is oligoclonal or even monoclonal expansion of T-cells in response to a specific antigen (Manz et al., 1997; Melenhorst et al., 1997a; Zeng et al., 1999; 2001). Thus, it is likely that, regardless of the inciting event in aplastic anemia, damage to hematopoietic progenitors initiates an immune process that inhibits hematopoiesis.

An intriguing association exists with hepatitis and is seen in about 1% of newly diagnosed cases. Virtually all cases of the hepatitis–aplastic anemia syndrome are seronegative for known hepatitis viruses (non-A through -G hepatitis). The disease predominantly affects young males, with a precipitous onset of severe pancytopenia occurring within 2–3 months after the onset of hepatitis (Brown et al., 1997; Hagler et al., 1975). Moreover, aplastic anemia has been reported to occur in up to 30% of patients following orthotopic liver transplantation for seronegative hepatitis (Tzakis et al., 1988; Cattral et al., 1994). The aplastic anemia in the hepatitis/aplastic anemia syndrome is autoimmune since most cases respond to immunosuppressive therapy; however, it remains unclear whether the hepatitis results from an undiscovered virus or whether this too is autoimmune.

ENVIRONMENTAL INFLUENCES

The medical literature is replete with case reports of environmental exposures, most notably benzene, causing aplastic anemia. However, rigorous epidemiologic studies supporting an association between environmental toxins and aplastic anemia are lacking. A major confounder is that benzene and other toxins also predispose to MDS and leukemia. The older literature was unlikely to have been able to distinguish the different types of marrow failure, such as aplastic anemia, MDS, and hypoplastic leukemia, leading to an overestimation of the association between benzene and aplastic anemia. While the magnitude of the risk remains uncertain, benzene is probably not a major risk factor for aplastic anemia in countries with modern standards of industrial hygiene. A large case–controlled study in Thailand employing modern diagnostic and epidemiologic methods found that individuals of lower economic status and younger age are at greater risk than their counterparts in other countries following exposure to solvents, glues, and hepatitis A (likely a surrogate marker). Grain farmers were also found to have higher risk of developing aplastic anemia (relative risk, 2.7) regardless of whether or not they used insecticides (Issaragrisil et al., 1997a). These same investigators noted marked differences in incidence between northern and southern rural regions of Thailand and among Bangkok suburbs, implicating potential environmental factors causing the disease (Issaragrisil et al., 1999).

ANIMAL MODELS

Animal models of bone marrow failure exist, but none fully approximate the human disease of acquired aplastic anemia. Busulfan, benzene, and irradiation have all been used to establish models of marrow failure (Knospe et al., 1971; Morley and Blake, 1974; Hak, 1980). All three agents lead to pancytopenia and a hypocellular marrow but the marrow failure is due to stem cell injury and damage to the microenvironment rather than autoimmune-mediated suppression of hematopoiesis.

TREATMENT AND OUTCOME

There are three principle therapies for severe aplastic anemia: 1) allogeneic bone marrow (stem cell) transplantation (BMT); 2) immunosuppressive therapy with antithymocyte globulin and cyclosporine (ATG/CSA); and 3) high-dose cyclophosphamide without BMT. Allogeneic BMT is the treatment of choice for young patients with aplastic anemia who have an HLA-matched sibling donor. Unfortunately, this approach is available to a minority of patients since less than 30% of patients have a matched sibling donor. The most important factors that influence outcome following allogeneic BMT for aplastic anemia are the age of the patient and the type of allograft (HLA-matched sibling versus unrelated or mismatched donors) (Figure 44.1). In patients younger than 30 years, the cure rate after an HLA-matched sibling BMT ranges from 70 to 90% (Horowitz, 2000; Storb et al., 2001; Ades et al., 2004). However, with increasing age over 30 years, the risk for graft-versus-host disease (GVHD) steadily increases, leading to reduced survival.

Transplant-related mortality approaches 50% in patients over the age of 40 years (Doney et al., 1997; Horowitz, 2000). Late BMT-related complications, including chronic GVHD, occur in up to one-third of patients, with over 40% requiring immunosuppressive therapy for their GVHD at 3 years (Storb et al., 2001). Thus, despite the high probability of curing severe aplastic anemia with BMT, many patients develop long-term complications from the procedure.

BMT from unrelated donors or mismatched donors for patients with severe aplastic anemia is usually reserved for patients who do not respond to immunosuppressive therapy; the transplant-related mortality and the risk of GVHD are roughly double with this approach. The best results are seen in patients younger than 21 years with a disease duration of under 1 year (Deeg et al., 2001).

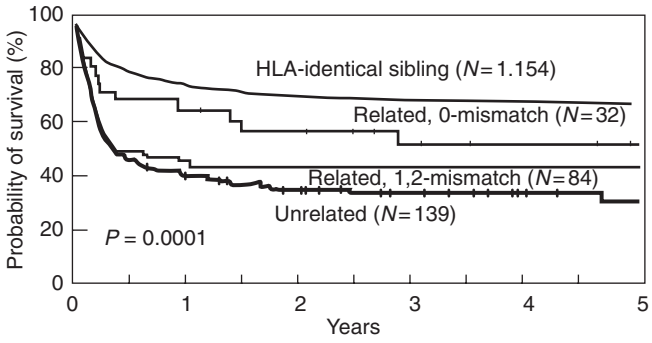


FIGURE 44.1 Survival after allogeneic bone marrow transplantation for severe aplastic anemia. Data for HLA-identical siblings and related match/mismatched transplants are from the International Bone Marrow Transplant Registry (IBMTR). Data for unrelated donor transplants are from the European Bone Marrow Transplant registry (EBMT), Fred Hutchinson Cancer Research Center, IBMTR, and the International Marrow Unrelated Search and Transplant (IMUST) study group. Survival curves are not adjusted for varying patient, disease, and transplant regimen characteristics.

Courtesy of the National Bone Marrow Donor Program.

Immunosuppressive Therapy with Antithymocyte Globulin/Cyclosporine

The combination of ATG/CSA leads to 5-year survival rates comparable to BMT, but most of these patients are not cured of their disease (Bacigalupo et al., 1995; Marsh et al., 1999). Response rates to ATG/CSA range between 60 and 80%, but in contrast to BMT, most patients do not acquire normal blood counts (Frickhofen et al., 1991; Rosenfeld et al., 1995). Another limitation of this approach is that many patients relapse, become dependent on CSA, or develop a secondary clonal disease such as PNH or MDS (Bacigalupo et al., 2000a; Rosenfeld et al., 2003). These late events often lead to substantial morbidity and mortality. Therapists at The National Institute of Health treated 122 patients (median age, 35 years) with the combination of ATG/CSA and methylprednisolone (Rosenfeld et al., 2003). The response rate was 58% and actuarial survival at 7 years was 55%; 13% of patients died within 3 months of treatment, most from fungal infections (Figure 44.2). The relapse rate for responders was 40% and at 8 years the actuarial rate for developing MDS was 15%.

High-Dose Cyclophosphamide Without Bone Marrow Transplantation

The first successful human allogeneic BMT, reported in 1972 by Thomas et al. in a patient with aplastic anemia, employed high-dose cyclophosphamide, and this remains (often in conjunction with ATG) the most commonly employed conditioning regimen for aplastic anemia (May et al., 1993; Storb et al., 2001). Interestingly, complete autologous recovery occurs in up to 20% of patients undergoing allogeneic BMT for aplastic anemia when cyclophosphamide alone is the conditioning regimen (Thomas et al., 1976; Sensenbrenner et al., 1977; Gmur et al., 1979; Weitzel et al., 1988). The unique pharmacology of cyclophosphamide is responsible for these occurrences. As a prodrug, cyclophosphamide is converted to 4-hydroxycyclophosphamide and its tautomer aldophosphamide in the liver. These compounds diffuse into the cell and are converted to the active compound phosphoramide mustard, or they are inactivated by aldehyde dehydrogenase to form the inert carboxyphosphamide. Lymphocytes have low levels of aldehyde dehydrogenase and are rapidly killed by high doses of cyclophosphamide; hematopoietic stem cells possess high levels of aldehyde dehydrogenase and are resistant to cyclophosphamide (Hilton, 1984; Kastan et al., 1990; Jones

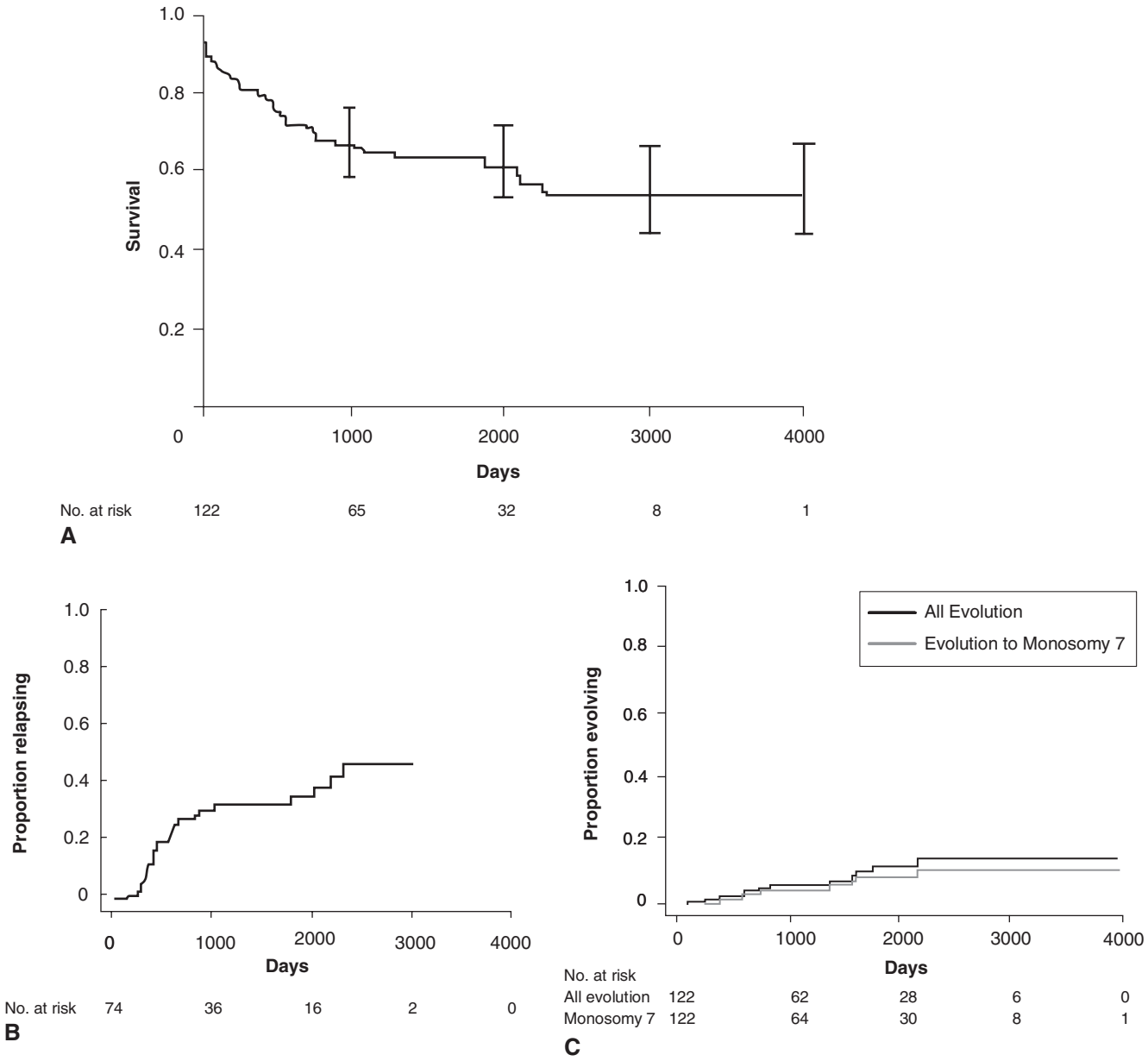


FIGURE 44.2 *A*, Survival probability for 122 patients with severe aplastic anemia following treatment with antithymocyte globulin and cyclosporine. *B*, Probability of relapse in 74 patients with aplastic anemia classified as responders at 3 months after treatment with antithymocyte globulin and cyclosporine. *C*, Proportion of patients experiencing clonal evolution.

Reproduced from Rosenfeld et al. (2003), with permission.

et al., 1995). Thus, high-dose cyclophosphamide is highly immunosuppressive, but not myeloablative, allowing endogenous hematopoietic stem cells to reconstitute hematopoiesis. With this background, high-dose cyclophosphamide without BMT was used successfully in aplastic anemia patients who lacked appropriate donors (Baran et al., 1976; Brodsky et al., 1996; 2001; Tisdale, 2000; Jaime-Perez et al., 2001). Based on these early promising studies, high-dose cyclophosphamide without BMT is now considered first-line therapy for severe aplastic anemia by some centers, but others have raised concerns about the toxicity of this approach (Figure 44.3).

High-dose cyclophosphamide has also been shown to salvage many patients who relapse or fail to respond to ATG/CSA (Brodsky et al., 2004). Similar to BMT, the early toxicity of high-dose cyclophosphamide may exceed that of ATG/CSA; however, the quality and duration of response appear to be superior.

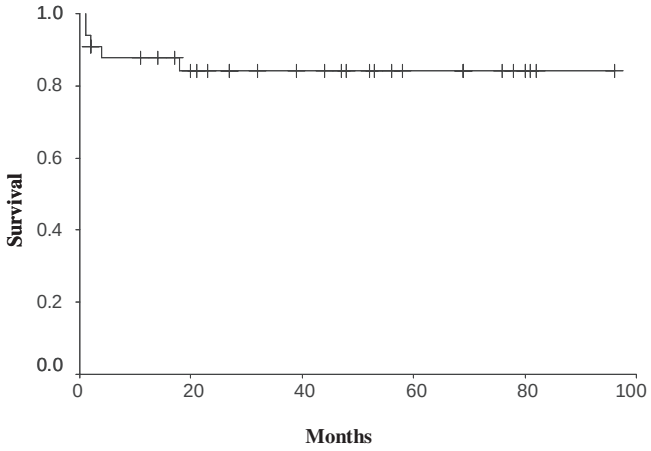


FIGURE 44.3 Survival probability following high-dose cyclophosphamide without bone marrow transplantation in 33 patients with severe aplastic anemia (SAA) (median age 42 years) followed at Johns Hopkins and Drexel University.

Clonality

Survivors of aplastic anemia are at high risk of clonal progression following immunosuppressive therapy, but not after allogeneic BMT (Socie et al., 1993). PNH and MDS are the most common clonal disorders to evolve from aplastic anemia (Tichelli et al., 1988; de Planque et al., 1989; Nagarajan et al., 1995). Even before the widespread use of immunosuppressive therapy, 5% of patients progressed to clonal hematopoiesis. This suggests that the increase in MDS and PNH following immunosuppressive therapy is not caused by the immunosuppression; rather, the increased survival following immunosuppressive therapy may allow time for these underlying clones to expand (Moore and Castro-Malaspina, 1991; Mukhina et al., 2001).

PNH results from the expansion of an abnormal hematopoietic stem cell that harbors a somatic mutation of the X-linked gene, termed PIGA (Rosse, 1997; Moyo, 2004). The product of the PIGA gene is required for the first step in the biosynthesis of glycosylphosphatidylinositol (GPI) anchor biosynthesis; consequently, PNH cells are deficient in GPI-anchored proteins. Several GPI-anchored proteins (CD59 and CD55) protect cells from complement-mediated destruction, and their absence explains the hemolytic anemia associated with PNH. However, it is unclear why the defective PNH stem cell and its progeny displace normal hematopoiesis, despite the fact that PNH cells are more vulnerable to complement-mediated destruction. One hypothesis to explain the relationship between aplastic anemia and PNH is that PNH cells resist the immune attack in aplastic anemia, ostensibly due to the lack of GPI-anchored proteins, but direct data supporting this hypothesis are still lacking.

MDS also commonly arises in aplastic anemia patients treated with immunosuppressive therapy (Tichelli et al., 1988; Socie et al., 1993). In a retrospective review of children with severe aplastic anemia, 11 of 86 patients who received immunosuppressive therapy developed MDS (Ohara et al., 1997). Up to 15% of adult patients with aplastic anemia will also develop MDS following immunosuppressive therapy with monosomy 7 being the most common chromosomal abnormality (Rosenfeld et al., 2003).

Although it is unclear why patients with aplastic anemia are predisposed to develop PNH and MDS, it is possible that all three diseases have a common pathogenesis. Damage to a hematopoietic progenitor cell that leads to an autoimmune response directed against the bone marrow in aplastic anemia (Thomas and Storb, 1984) could also produce genetic mutations (PNH/MDS) that give these hematopoietic progenitors a relative growth advantage. Over time, an abnormal clone arising from a slowly proliferating, transformed progenitor cell could eventually become dominant. The finding that almost 70% of patients with aplastic anemia have detectable PNH cells at diagnosis supports this hypothesis (Mukhina et al., 2001). Alternatively, prolonged immune attack against hematopoietic stem cells may lead to DNA damage, ultimately leading to clonal disorders.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Only recently has acquired aplastic anemia been recognized as an autoimmune disease. Studies into the biology of aplastic anemia have been inherently difficult. In patients with active disease, the targets of the immune attack, hematopoietic stem cells, are exceedingly rare; these cells probably represent <0.1% of bone marrow cells in normal individuals and are even less frequent in patients with aplastic anemia. BMT restores hematopoiesis from the donor, making it impossible to study the affected host stem cells and the immune system. The clinical response of aplastic anemia to immunosuppressive therapy provided the first data suggesting that aplastic anemia was immune mediated. More recently, it has been shown that the bone marrow in aplastic anemia is infiltrated with lymphocytes that secrete Th1 cytokines. Skewing of the Vβ repertoires demonstrated by CDR3 spectratyping further suggests that this is an antigen-driven autoimmune disorder. Moreover, newer methodologies are beginning to allow the study of the rare target cell populations in aplastic anemia. In fact, emerging data suggest that the most primitive hematopoietic stem cells may not be the target of the immune attack in aplastic anemia, and that these cells persist in many patients. Rather, the immune attack in aplastic anemia may be against more committed myeloid or "low-quality" (Van Zant et al., 1997) stem cells, while the most primitive hematopoietic stem

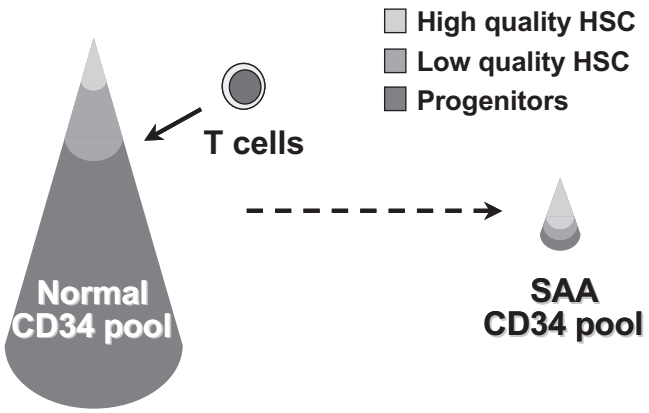


FIGURE 44.4 Model depicting the pathophysiology of bone marrow failure in acquired aplastic anemia. Autoagressive lymphocytes lyse $CD34^+$ bone marrow progenitor cells but seem to spare more immature $CD34^+$ cells, known as high-quality stem cells.

cells elude or are resistant to attack (Figure 44.4). There are a number of potential mechanisms by which primitive hematopoietic stem cells could evade immune attack. Data suggest that the antigens targeted in aplastic anemia are presented by HLA class II molecules (Nakao et al., 1997; Zeng et al., 2001). Primitive hematopoietic stem cells appear to express little or no HLA class II (Rusten et al., 1994; Van Zant et al., 1997), which may account for their relative invisibility to autoreactive effector lymphocytes. In addition, the Fas–Fas ligand plays an important role in the pathophysiology of aplastic anemia (Maciejewski et al., 1995), and Fas appears to be absent on primitive hematopoietic stem cells with its expression increasing as they differentiate (Nagafuji et al., 1995; Stahnke et al., 1998). Recent advances in the understanding of the immunobiology of aplastic anemia make it very likely that future editions of this textbook will fully define the basis of the autoimmune attack in this disease.

References

Ades, L., Mary, J.Y., Robin, M., Ferry, C., Porcher, R., Esperou, H., Ribaud, P., Devergie, A., Traineau, R., Gluckman, E., and Socie, G. (2004). Long-term outcome after bone marrow transplantation for severe aplastic anemia. *Blood 103*, 2490–2497.

Bacigalupo, A., Broccia, G., Corda, G., Arcese, W., Carotenuto, M., Gallamini, A., Locatelli, F., Mori, P.G., Saracco, P., Todeschini, G., Coser, P., Iacopino, P., Van Lint, M.T., and Gluckman, E. (1995). Antilymphocyte globulin, cyclosporin, and granulocye colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): A pilot study of the EMBT SAA Working Party. *Blood 85*, 1348–1353.

Bacigalupo, A., Bruno, B., Saracco, P., Di Bona, E., Locasciulli, A., Locatelli, F., Gabbas, A., Dufour, C., Arcese, W., Testi, G., Broccia, G., Carotenuto, M., Coser, P., Barbui, T., Leoni, P., and Ferster, A. (2000a). Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMY study on 100 patients. *Blood 95*, 1931–1934.

Bacigalupo, A., Oneto, R., Bruno, B., Socie, G., Passweg, J., Locasciulli, A., Van Lint, M.T., Tichelli, A., McCann, S., Marsh, J., Ljungman, P., Hows, J., Marin, P., and Schrezenmeier, H. (2000b). Current results of bone marrow transplantation in patients with acquired severe aplastic anemia. Report of the European Group for Blood and Marrow Transplantation. On behalf of the Working Party on Severe Aplastic Anemia of the European Group for Blood and Marrow Transplantation. *Acta Haematol. 103*, 19–25.

Bagby, G.C., Jr. (2003). Genetic basis of Fanconi anemia. *Curr. Opin. Hematol. 10*, 68–76.

Baran, D.T., Griner, F., and Klemperer, M.R. (1976). Recovery from aplastic anemia after treatment with cyclophosphamide. *N. Engl. J. Med. 295*, 1522–2523.

Brodsky, R.A., Sensenbrenner, L.L., and Jones, R.J. (1996). Complete remission in acquired severe aplastic anemia following high-dose cyclophosphamide. *Blood 87*, 491–494.

Brodsky, R.A., Mukhina, G.L., Li, S., Nelson, K.L., Chiurazzi, P.L., Buckley, J.T., and Borowitz, M.D. (2000). Improved detection and characterization of paroxysmal nocturnal hemoglobinuria using fluorescent aerolysin. *Am. J. Clin. Pathol. 114*, 459–466.

Brodsky, R.A., Sensenbrenner, L.L., Smith, B.D., Dorr, D., Seaman, P.J., Karp, J.E., Brodsky, I., and Jones, R.J. (2001). Durable treatment-free remission following high-dose cyclophosphamide for previously untreated severe aplastic anemia. *Ann. Intern. Med 135*, 477–483.

Brodsky, R.A., Chen, A.R., Brodsky, I., and Jones, R.J. (2004). High-dose cyclophosphamide as salvage therapy for severe aplastic anemia. *Exp. Hematol. 32*, 435–440.

Brown, K.E., Tisdale, J., Barrett, A.J., Dunbar, C.E., and Young, N.S. (1997). Hepatitis-associated aplastic anemia. *N. Engl. J. Med. 336*, 1059–1064.

Camitta, B.M., Thomas, E.D., Nathan, D.G., Gale, R.P., Kopecky, K.J., Rappeport, J.M., Santos, G., Gordon-Smith, E.C., and Storb, R. (1979). A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. *Blood 53*, 504–514.

Cattral, M.S., Langnas, A.N., Markin, R.S., Antonson, D.L., Heffron, T.G., Fox, I.J., Sorrell, M.F., and Shaw, B.W., Jr. (1994). Aplastic anemia after liver transplantation for fulminant liver failure. *Hepatology 20*, 813–818.

Champlin, R.E., Reig, S.A., Sparkes, R.S., and Galen, R.P. (1984). Bone marrow transplantation from identical twins in the treatment of aplastic anemia: implication for the pathogenesis of the disease. *Br. J. Haematol. 56*, 455–463.

de Planque, M.M., Bacigalupo, A., Wursch, A., Hows, J.M., Devergie, A., Frickhofen, N., Brand, A., and Nissen, C. (1989). Long-term follow-up of severe aplastic anemia patients treated with antithymocyte globulin. *Br. J. Haematol. 73*, 121–126.

Deeg, H.J., Amylon, I.D., Harris, R.E., Collins, R., Beatty, P.G., Feig, S., Ramsay, N., Territo, M., Khan, S.P., Pamphilon, D., Leis, J.F., Burdach, S., Anasetti, C., Hackman, R., Storer, B., and Mueller, B. (2001). Marrow transplants from unrelated donors for patients with aplastic anemia: minimum effective dose of total body irradiation. *Biol. Blood Marrow Transplant. 7*, 208–215.

Dokal, I. and Vulliamy, T. (2003). Dyskeratosis congenital: its link to telomerase and aplastic anemia. *Blood Rev. 17*, 217–255.

Doney, K., Leisenring, W., Storb, R., and Appelbaum, F.R. (1997). Primary treatment of acquired aplastic anemia: Outcomes with bone marrow transplantation and immunosuppressive therapy. *Ann. Intern. Med. 126*, 107–115.

Ehrlich, P. (1888). Ueber einem Fall von Anamie mit Bemer-kungen uber regenerative Veranderungen des Knockenmarks. *Charite-Annalen 13*, 301–309.

Frickhofen, N., Kaltwasser, J.R., Schrezenmeier, H., Raghavacher, A., Vogt, H.G., Hermann, F., Freund, M., Meusers, P., Salama, A., and Heimpel, H. (1991). Treatment of aplastic anemia with antilymphocyte globulin

and methylprednisolone with or without cyclosporine. *N. Engl. J. Med. 324*, 1297–1304.

Gmur, J., von Felten, A., Phyner, K., and Frick, P.G. (1979). Autologous hematologic recovery from aplastic anemia following high dose cyclophosphamide and HLA-matched allogeneic bone marrow transplantation. *Acta Haematol. 62*, 20–24.

Haak, H.L. (1980). Experimental drug-induced aplastic anemia. *Clin. Haematol. 9*, 621–639.

Hagler, L., Pastore, R.A., Bergin, J.J., and Wrensch, M.R. (1975). Aplastic anemia following viral hepatitis: report of two fatal cases and literature review. *Medicine 54*, 139–164.

Hall, S.E. and Rosse, W.F. (1996). The use of monoclonal antibodies and flow cytometry in the diagnosis of paroxysmal nocturnal hemoglobinuria. *Blood 87*, 5332–5340.

Hilton, J. (1984). Role of aldehyde dehydrogenase in cyclophosphamide-resistant L1210 leukemia. *Cancer Res. 44*, 5156–5160.

Hinterberger, W., Rowlings, P.A., Hinterberger-Fischer, M., Gibson, J., Jacobsen, N., Klein, J.P., Kolb, H.J., Stevens, D.A., Horowitz, M.M., and Gale, R.P. (1997). Results of transplanting bone marrow from genetically identical twins into patients with aplastic anemia [see comments]. *Ann. Intern. Med. 126*, 116–122.

Hirano, N., Butler, M.O., Bergwelt-Baildon, M.S., Maecker, B., Schultze, J.L., O'Connor, K.C., Schur, P.H., Kojima, S., Guinan, E.C., and Nadler, L.M. (2003). Autoantibodies frequently detected in patients with aplastic anemia. *Blood 102*, 4567–4575.

Hoffman, R., Zanjani, E.D., Lutton, J.D., Zalusky, R., and Wasserman, L.R. (1977). Suppression of erythroid-colony formation by lymphocytes from patients with aplastic anemia. *N. Engl. J. Med. 296*, 10–13.

Horowitz, M.M. (2000). Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. *Semin. Hematol. 37*, 30–42.

Issaragrisil, S., Chansung, K., Kaufman, D.W., Sirijirachai, J., Thamprasit, T., and Young, N.S. (1997a). Aplastic anemia in rural Thailand: its association with grain farming and agricultural pesticide exposure. Aplastic Anemia Study Group. *Am. J. Public Health 87*, 1551–1554.

Issaragrisil, S., Kaufman, D.W., Anderson, T., Chansung, K., Thamprasit, T., Sirijirachai, J., Piankijagum, A., Porapakkham, Y., Vannasaeng, S., Leaverton, P.E., Shapiro, S., Young, N.S., and The Aplastic Anemia Study Group (1997b). Low drug attributability of aplastic anemia in Thailand. *Blood 89*, 4034–4039.

Issaragrisil, S., Leaverton, P.E., Chansung, K., Thamprasit, T., Porapakham, Y., Vannasaeng, S., Piankijaum, A., Kaufman, D.W., Anderson, T.E., Shapiro, S., and Young, N.S. (1999). Regional patterns in the incidence of aplastic anemia in Thailand. The Aplastic Anemia Study Group. *Am. J. Hematol. 61*, 164–168.

Jaime-Perez, J.Z., Gonzalez-Llano, O., and Gomez-Almaguer, D. (2001). High-dose cyclophosphamide in the treatment of severe aplastic anemia in children. *Am. J. Hematol. 66*, 71.

Jones, R.J., Barber, J.P., Vala, M.S., Collector, M.I., Kaufmann, S.H., Ludeman, S.M., Colvin, O.M., and Hilton, J. (1995). Assessment of aldehyde dehydrogenase in viable cells. *Blood 85*, 2742–2746.

Kastan, M.B., Schlaffer, E., Russo, J.E., Colvin, O.M., Civin, C.I., and Hilton, J. (1990). Direct demonstration of elevated aldehyde dehydrogenase in human progenitor cells. *Blood 75*, 1947–1950.

Kaufman, D.W., Kelly, J.P., Levy, M., and Shapiro, S. (1991). *The Drug Etiology of Agranulocytosis and Aplastic Anemia*. New York: Oxford University Press.

Knospe, W.H., Blom, J., Goldstein, H.B., and Crosby, W.H. (1971). Delayed bone marrow aplasia in rats protected against lethal irradiation by allogeneic marrow transplantation. *Radiat. Res. 47*, 199–212.

Maciejewski, J.P., Hibbs, J.R., Anderson, S., Katevas, P., and Young, N.S. (1994). Bone marrow and peripheral blood lymphocyte phenotype in patients with bone marrow failure. *Exp. Hematol. 22*, 1102–1110.

Maciejewski, J.P., Selleri, C., Sato, T., Anderson, S., and Young, N.S. (1995). Increased expression of Fas antigen on bone marrow $CD34^+$ cells of patients with aplastic anaemia. *Br. J. Haematol. 91*, 245–252.

Manz, C.Y., Dietrich, P.Y., Schnuriger, V., Nissen, C., and Wodnar-Filipowicz, A. (1997). T-cell receptor beta chain variability in bone marrow and peripheral blood in severe acquired aplastic anemia. *Blood Cells Mol. Dis. 23*, 110–122.

Marsh, J., Schrezenmeier, H., Marin P., Ilhan, O., Ljungnan, P., McCann, S., Socie, G., Tichelli, A., Passweg, J., Hows, J., Raghavacher, A., Locastuilli, A., and Bacigalupo, A. (1999). Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with nonsevere aplastic anemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anaemia Working Party. *Blood 93*, 2191–2195.

Mathé, G., Amiel, J.L., Schwarzenberg, L., Choay, J., Trolard, P., Schneider, M., Hayat, M., Schlumberger, J.R., and Jasmin, C.I. (1970). Bone marrow graft in man after conditioning by antilymphocytic serum. *BMJ 2*, 131–136.

May, W.S., Sensenbrenner, L.L., Burns, W.H., Ambinder, R., Carroll, M.P., Jones, R.J., Miller, C.B., Mellits, E.D., Vogelsang, G.B., Wagner, J., Wingard, J., Yeager, A.M., and Santos, G.W. (1993). BMT for severe aplastic anemia using cyclosporin. *Bone Marrow Transplant. 11*, 459–464.

Melenhorst, J.J., Fibbe, W.E., Struyk, L., van der Elsen, P.J., Willemze, R., and Landegent, J.E. (1997a). Analysis of T-cell clonality in bone marrow of patients with acquired aplastic anemia. *Br. J. Haematol. 96*, 85–91.

Melenhorst, J.J., van Krieken, J.H. J.M., Dreef, E., Landegent, J.E., Willemze, R., and Fibbe, W.E. (1997b). T-cells selectively infiltrate bone marrow areas with residual haemopoiesis of patients with acquired aplastic anaemia. *Br. J. Haematol. 99*, 517–519.

Modan, B., Segal, S., Shani, M., and Sheba, C. (1975). Aplastic anemia in Israel: evaluation of the etiological role of chloramphenicol on a community-wide basis. *Am. J. Med. Sci. 270*, 441–445.

Moore, M.A.S. and Castro-Malaspina, H. (1991). Immunosuppression in aplastic anemia—postponing the inevitable? *N. Engl. J. Med. 324*, 1358–1360.

Morley, A. and Blake, J. (1974). An animal model of chronic aplastic marrow failure. I. Late marrow failure after busulfan. *Blood 44*, 49–56.

Moyo, V.M., Mukhina, G.L., Garret, E.S., and Brodsky, R.A. (2004). Natural history of paroxysmal nocturnal hemoglobinuria using modern diagnostic assays. *Br. J. Haematol. 126*, 133–138.

Mukhina, G.L., Buckley, J.T., Barber, J.P., Jones, R.J., and Brodsky, R.A. (2001). Multilineage glycosylphosphatidylinositol anchor deficient hematopoiesis in untreated aplastic anemia. *Br. J. Haematol. 115*, 476–482.

Nagafuji, K., Shibuya, T., Harada, M., Mizuno, S., Takenaka, K., Miyamoto, T., Okamura, T., Gondo, H., and Niho, Y. (1995). Functional expression of Fas antigen (CD95) on hematopoietic progenitor cells. Blood *86*, 883–889.

Nagarajan, S., Brodsky, R.A., Young, N.S., and Medof, M.E. (1995). Genetic defects underlying paroxysmal nocturnal hemoglobinuria that arises out of aplastic anemia. *Blood 86*, 4656–4661.

Nakao, S., Yamaguchi, M., Shiobara, S., Yokoi, T., Miyawaki, T., Taniguchi, T., and Matsuda, T. (1992). Interferon-gamma gene expression in unstimulated bone marrow mononuclear cells predicts a good response to cyclosporine therapy in aplastic anemia. *Blood 79*, 2532–2535.

Nakao, S., Takami, A., Takamatsu, H., Zeng, W., Sugimori, N., Yamazaki, H., Miura, Y., Ueda, M., Shiobara, S., Yoshioka, T., Kaneshinge, T., Yasukawa, M., and Matsuda, T. (1997). Isolation of a T-cell clone showing HLA-DRB1*0405-restricted cytotoxicity for hematopoietic cells in a patient with aplastic anemia. *Blood 89*, 3691–3699.

Nimer, S.D., Ireland, P., Meshkinpour, A., and Frane, M. (1994). An increased HLA DR2 frequency is seen in aplastic anemia patients. *Blood 84*, 923–927.

Nissen, C., Cornu, P., Gratwohl, A., and Speck, B. (1980). Peripheral blood cells from patients with aplastic anaemia in partial remission suppress growth of their own bone marrow precursors in culture. *Br. J. Haematol. 45*, 233–243.

Nistico, A. and Young, N.S. (1994). Gamma-interferon gene expression in the bone marrow of patients with aplastic anemia. *Ann. Intern. Med. 120*, 463–469.

Ohara, A., Kojima, S., Hamajima, N., Tsuchida, M., Imashuku, S., Ohta, S., Sasaki, H., Okamura, J., Sugita, K., Kigasawa, H., Kiriyama, Y., Akatsuka, J., and Tsukimoto, I. (1997). Myelodysplastic syndrome and acute myelogenous leukemia as a late clonal complication in children with acquired aplastic anemia. *Blood 90*, 1009–1013.

Orazi, A., Albitar, M., Heerema, N.A., Haskins, S., and Neiman, R.S. (1997). Hypoplastic myelodysplastic syndromes can be distinguished from acquired aplastic anemia by CD34 and PDNA immunostaining of bone marrow biopsy specimens. *Am. J. Clin. Pathol. 107*, 261–264.

Rosenfeld, S.J., Kimball, J., Vining, D., and Young, N.S. (1995). Intensive immunosuppression with antithymocyte globulin and cyclosporin as treatment for severe aplastic anemia. *Blood 85*, 3058–3065.

Rosenfeld, S., Follmann, D., Nunez, O., and Young, N.S. (2003). Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. *JAMA 289*, 1130–1135.

Rosse, W.F. (1997). Paroxysmal nocturnal hemoglobinuria as a molecular disease. *Medicine 76*, 63–93.

Rusten, L.S., Jacobsen, S.E., Kaalhus, O., Veiby, O.P., Funderud, S., and Smeland, E.B. (1994). Functional differences between $CD34^-$ and DR- subfractions of $CD34^+$ bone marrow cells. *Blood 84*, 1473–1481.

Sensenbrenner, L.L., Steele, A.A., and Santos, G.W. (1997). Recovery of hematologic competence without engraftment following attempted bone marrow transplantation for aplastic anemia: Report of a case with diffusion chamber studies. *Exp. Hematol. 77*, 51–58.

Socie, G., Henry-Amar, M., Bacigalupo, A., Hows, J., Tichelli, A., Ljungman, P., McCann, S.R., Frickhofen, N., Veer-Korthof, E.V., and Gluckman, E. (1993). Malignant tumors occurring after treatment of aplastic anemia. *N. Engl. J. Med. 329*, 1152–1157.

Stahnke, K., Hecker, S., Kohne E., and Debatin, K.M. (1998). CD95 (APO-1/FAS)-mediated apoptosis in cytokine-activated hematopoietic cells. *Exp. Hematol. 26*, 844–850.

Storb, R., Blume, K.G., O'Donnell, M.R., Chauncey, T., Forman, S.J., Deeg, H.J., Hu, W.W., Appelbaum, F.R., Doney, K., Flowers, M.E., Sanders, J., and Leisenring, W. (2001). Cyclophosphamide and antithymocyte globulin to condition patients with aplastic anemia for allogeneic marrow transplantations: the experience in four centers. *Biol. Blood Marrow Transplant. 7*, 39–44.

Szklo, M., Sensenbrenner, L., Markowitz, J., Weida, S., Warm, S., and Linet, M. (1995). Incidence of aplastic anemia in metropolitan Baltimore: a population-based study. *Blood 66*, 115–119.

Thomas, E.D. and Storb, R. (1984). Acquired severe aplastic anemia: Progress and perplexity. *Blood 64*, 325–328.

Thomas, E.D., Storb, R., Fefer, A., Slichter, S.J., Bryant, J.I., Buckner, C.D., Neiman, P.E., Clift, R.A., Funk, D.D., and Lerner, K.E. (1972). Aplastic anemia treated by marrow transplantation. *Lancet 1*, 284–289.

Thomas, E.D., Storb, R., Giblett, E.R., Longpre, B., Weiden, P.L., Fefer, A., Witherspoon, R., Clift, R. A., and Buckner C.D. (1976). Recovery from aplastic anemia following attempted marrow transplantation. *Exp. Hematol. 4*, 97–102.

Tichelli, A., Gratwohl, A., Wursch, A., Nissen, C., and Speck, B. (1988). Late haematological complications in severe aplastic anaemia. *Br. J. Haematol. 69*, 413–418.

Tisdale, J.F., Dunn, D.E., Geller, N., Plante, M., Nunez, O., Dunbar, C.E., Barrett, A.J., Walsh, T.J., Rosenfeld, S.J., and Young, N.S. (2000). High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial. *Lancet 356*, 1554–1559.

Tzakis, A.G., Arditi, M., Whittington, P.F., Yanaga, K., Esquivel, C., Andrews, W.A., Makowaka, L., Malatak, J., Freese, D.K., Stock, P.G., Ascher, N.L., Johnson, F.L., Broelsch, C.E., and Starzl, T.E. (1988). Aplastic anemia complicating orthotopic liver transplantation for non-A, non-B hepatitis. *N. Engl. J. Med. 319*, 393–396.

Van Zant, G., de Hann, G., and Rich, I.N. (1997). Alternatives to stem cell renewal from a developmental viewpoint. *Exp. Hematol. 25*, 187–192.

Weitzel, J.N., Hows, J.M., Jeffreys, A.J., Min, G.L., and Goldman, J.M. (1988). Use of a hypervariable minisatellite DNA probe (33.15) for evaluating engraftment two or more years after bone marrow transplantation for aplastic anaemia. *Br. J. Haematol. 70*, 91–97.

Zeng, W., Nakao, S., Takamatsu, H., Yachi, A., Takami, A., Kondo, Y., Sugimori, N., Yamazaki, H., Miura, Y., Shiobara, S., and Matsuda, T. (1999). Characterization of T-cell repertoire of the bone marrow in immune-mediated aplastic anemia: evidence for the involvement of antigen-driven T-cell response in cyclosporine-dependent aplastic anemia. *Blood 93*, 3008–3016.

Zeng, W., Maciejewski, J.P., Chen, G., and Young, N.S. (2001). Limited heterogeneity of T-cell receptor BV usage in aplastic anemia. *J. Clin. Invest. 108*, 765–773.

Zoumbos, N.C., Gascón, P., Djeu, J.Y., Trost, S.R., and Young, N.S. (1985). Circulating activated suppressor T-lymphocytes in aplastic anemia. *N. Engl. J. Med. 312*, 257–265.

CHAPTER

45

Clotting Disorders

JEAN-MARIE R. SAINT-REMY
Center for Molecular and Vascular Biology, University of Leuven, Belgium

Disorders related to coagulation factor autoimmunity are rare but underestimated. Clinical manifestations are obviously dominated by bleeding, but these can be acute and life-threatening, or progressive with formation of hematomas in various organs. The circumstances under which an autoimmune coagulation disorder appears also vary from spontaneous occurrence in an otherwise healthy individual, as a complication in the context of a systemic autoimmune disease, to association with postpartum, surgery, drug intake or exposure to a cross-reactive antigen. The purpose of this chapter is to offer a comprehensive overview of autoimmune coagulation disorders to help clinicians and clinical investigators to establish an early diagnosis. A diagram showing the general organization of the coagulation system is provided in Figure 45.1.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

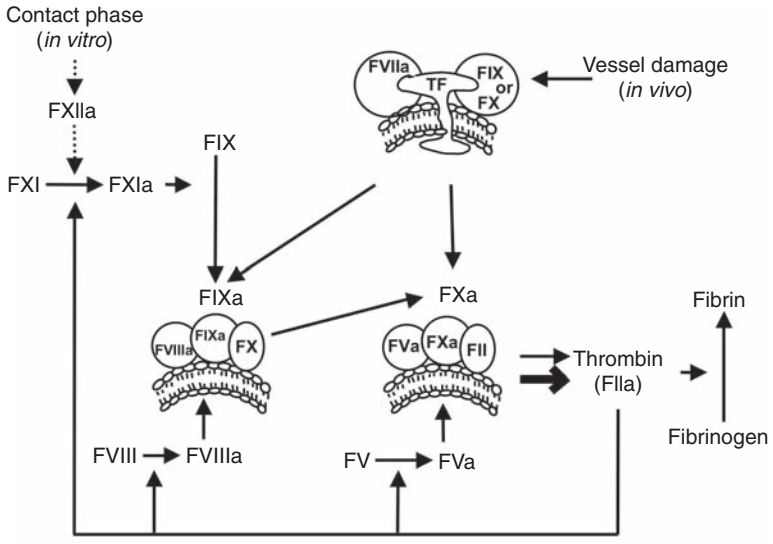


FIGURE 45.1 Overview of the coagulation pathways. 1) *In vivo*, the coagulation process is initiated when tissue factor (TF) expressed on injured vessel walls is exposed to activated Factor VII in the bloodstream. Small amounts of thrombin are generated through activation of Factor IX and Factor X. 2) Thrombin activates Factor VIII and Factor V, thereby triggering an amplification phase through the formation of the tenase complex (FVIIIa–FIXa–FX), which converts Factor X to its activated form, and the prothrombinase complex (FVa–Factor Xa–prothrombin/Factor II), which converts prothrombin into thrombin. Thrombin also activates Factor XI, which amplifies the generation of Factor IXa. 3) Thrombin converts fibrinogen into fibrin. It activates protein C, which triggers a negative feedback through inactivation of Factor VIIIa and Factor Va (not shown). Thrombin also activates the thrombin-activable fibrinolysis inhibitor (TAFI), which stabilizes the clot (not shown). 4) Direct activation of Factor XI also occurs through Factor XIIa via the contact phase system.

AUTOANTIBODIES TO FACTOR VIII

Clinical Presentation and Associated Conditions

This represents by far the most commonly encountered autoimmune reaction to a coagulation factor, although it affects only 0.2–1 individuals in a million population per year (Garvey, 1995). The precise incidence of anti-Factor VIII (FVIII) inhibitors is difficult to establish, as it can occur with only minimal symptoms (Green and Lechner, 1981). In addition, inhibitors to FVIII are also detectable in a majority of healthy donors, although in such cases inhibitors appear to be neutralized by anti-idiotypic antibodies.

The clinical picture varies from minimal signs such as easy bruising to very severe bleeding affecting the gastrointestinal tract, skin, and muscles. Epistaxis and hematuria are frequent but, in contrast to antibodies elicited by infusion of FVIII in congenital hemophilia A patients, joints are not affected. Large hematomas may form with nerve or tracheal compression. These signs can appear suddenly or very progressively. There is no gender or race preference. The vast majority of cases are observed in elderly people, yet children can be affected, too (Moraca and Ragni, 2002). The mortality rate lies between 10 and 20%.

Forty-five percent of cases are observed in individuals with no underlying disease. Associated conditions belong mainly to four groups: malignancies, systemic autoimmune diseases and/or chronic inflammation, postpartum (Hauser et al., 1995), and iatrogenic.

Malignancies include lympho- and myelo-proliferative disorders (Lin et al., 1991; Meek and Haak, 1986), and lung (Shwaiki et al., 2001) and prostate carcinomas, but this is not exclusive (Hultin, 1991). Systemic autoimmune diseases include systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren syndrome (Vignes et al., 1996), multiple sclerosis, and pemphigus vulgaris. Graft-versus-host disease (Seidler et al., 1994) and chronic inflammation, such as that of Crohn's disease, thyroiditis (Sievert et al., 1996), chronic hepatitis C (Sugishita et al., 1999), and tuberculosis (Aurousseau et al., 1996), have been described. Autoantibodies to FVIII appearing in the postpartum phase are usually of short duration. Anti-FVIII antibodies have also been shown during therapy with penicillin sulfamides (sulfonamide) (Annichino-Bizzacchi and Machado, 1991), and interferon (IFN)-α (Stricker et al., 1994), and after Bacillus Calmette–Guerin (BCG) vaccination. A few cases of inhibitors occurring after surgery have also been described (Alumkal et al., 1999).

Diagnosis

Prolonged activated prothrombin time (APTT) with normal prothrombin time (PT) and thrombin time (TT), and normal platelet count should prompt a search for an inhibitor to FVIII. Direct evaluation of FVIII, Factor IX (FIX), Factor

FXI (FXI), and Factor FXII (FXII) ensures the specificity of the antibodies. Mixing experiments in which varying volumes of normal plasma are mixed with the plasma suspected to contain an inhibitor show partial or no correction of APTT. The Bethesda assay, or better its so-called Nijmegen modification, is carried out by mixing vol/vol varying dilutions of the plasma under investigation with normal plasma. The dilution of plasma containing the inhibitor that still inhibits 50% of FVIII activity from normal plasma is taken as the number of Bethesda units. As most autoantibodies to FVIII are type II inhibitors (see below), which are time and temperature dependent, it is current practice to incubate the mixture for 2 h at 37° C.

Pathophysiology

FVIII has a mature sequence of 2332 amino acids organized in domains with the structure A1-A2-B-A3-C1-C2 (Figure 45.2). A number of functional inhibitor epitopes have been identified (arrows in Figure 45.2), corresponding to sites at which FVIII interacts with phospholipids, von Willebrand factor (vWF), FIX, and FX. As for alloantibodies to FVIII, acquired inhibitors belong primarily to the IgG4 isotypes, with some IgG1 isotypes also in a significant proportion of cases (Gilles and Saint-Remy, 1996; Matsumoto et al., 2001). A limited attempt to map the FVIII epitopes recognized by autoantibodies has indicated that, as for alloantibodies, the C2 domain is most often the main target (Di Giambattista et al., 2001), followed by the A2 domain (Scandella et al., 1989). The C2 domain contains the binding sites for phospholipids and some of the binding sites for vWF. It is therefore inferred that acquired inhibitors neutralize FVIII function by inhibiting the binding of FVIII to phospholipids, a necessary step in the formation of the tenase complex, and/or to vWF, the function of which is to protect FVIII from proteolytic inactivation and rapid clearance from the circulation. One of the mechanisms suggested by the binding to the A2 domain is related to inhibition of FVIII binding to FIX or acceleration of the release of the noncovalently bound A2 domain from A1–A3-light chain. Such an interpretation is, however, difficult to reconcile with the kinetics of FVIII inactivation usually observed with auto–anti-FVIII antibodies. Recent evidence suggests that at least some of the autoantibodies to FVIII act by inducing a conformational change in the molecule, resulting from binding to a distant site (Jacquemin et al., 2000). Recent studies have addressed the question of T-cell epitopes (Reding et al., 2003).

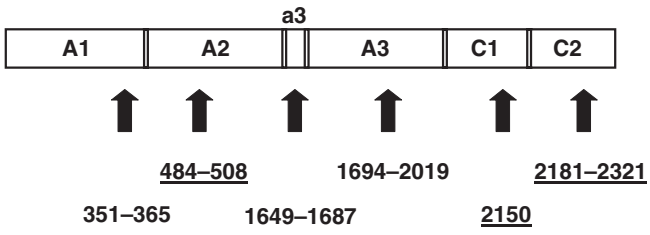


FIGURE 45.2 Major B-cell epitopes on the activated Factor VIII (FVIII) molecule. FVIII is represented in its activated form with the six identified major B-cell epitopes. For the sake of clarity, the molecule is shown as a single polypeptide chain instead of in its heterotrimeric configuration. Underlined sequences have been mapped directly with autoantibodies. Epitope mapping used to be carried out with recombinant FVIII domains, but recently a method was developed using combined transcription–translation of radiolabeled peptides made with rabbit reticulocytes. This method allows much more precision in epitope delineation. There is no current evidence to conclude that epitopes recognized by autoantibodies differ from those of alloantibodies.

According to the kinetics of FVIII inhibition, FVIII inhibitors are usually separated into two categories (Gawryl and Hoyer, 1982). Type I inhibitors inhibit FVIII function completely following second-order kinetics, while type II inhibitors inhibit FVIII function in two phases, a rapid inactivation followed by a much slower phase, and never inactivate FVIII completely. The vast majority of autoantibodies to FVIII described so far are type II inhibitors. Interestingly, there is some suggestion that idiopathic autoantibodies present type II characteristics, while antibodies appearing in the context of an autoimmune disease, or associated with a malignancy, could primarily be type I.

Treatment

Therapy includes two steps, namely the control of bleeding and the eradication of the inhibitor (Roberts, 1998). Control of bleeding is achieved by different methods. rFVIIa administration is probably the treatment of choice for acute situations that are life-threatening (Hay et al., 1997; von Depka, 2002). The success rate with rFVIIa is high, but such a treatment carries the risk, albeit limited, of thrombotic events. The therapy is nevertheless very expensive and thereby not accessible in many centers throughout the world. Low-titer inhibitors can be treated by infusion of FVIII or use of desmopressin (DDAVP) (Mudad and Kane, 1993); the latter mobilizes vWF and FVIII from storage in endothelial cells.

An alternative, though equally costly, therapy is porcine FVIII (Hoyle and Ludlam, 1986; Morrison et al., 1993). This is based on the observation that, although there exists an extensive homology between human and porcine FVIII, inhibitors elicited towards human FVIII do not cross-react with porcine FVIII. The latter is, however, immunogenic, which precludes its use over prolonged periods of time. Activated prothrombin complexes can also be used in emergency situations.

Eradication of the inhibitor is usually achieved by a therapy based on high-dose corticosteroids (Spero et al., 1981), often combined with cytostatic agents such as cyclophosphamide (Green et al., 1993; Shaffer and Phillips, 1997). Cyclosporine and calcineurin inhibitors have been used with success. Intravenous gamma-globulin (IVIG) infusion has been advocated (Crenier et al., 1996; Schwartz

et al., 1995) on the basis of the presence of anti-idiotypic antibodies that can neutralize the inhibitory activity of anti-FVIII antibodies (Sultan et al., 1984; Rossi et al., 1988). This is considered as a second-line treatment. In some instances, extracorporeal removal of immunoglobulins combined with IVIG, with or without cytostatic agents, has been recommended in refractory cases (Nilsson et al., 1984).

Recently, an antibody directed towards CD20, a B-cell surface protein, has shown efficacy in the treatment of acquired anti-FVIII inhibitors (Stasi et al., 2004). Controlled trials are underway to determine whether this constitutes a valuable alternative therapy to those listed above.

The apparent absence of consensus about treatment reflects the lack of controlled studies as well as the variety of conditions that are associated with this syndrome. Efforts are underway to validate some of these therapeutic approaches.

AUTOANTIBODIES TO FACTOR IX

The occurrence of antibodies to FIX in patients without hemophilia is very rare and only single case reports are found in the literature. Clinical presentation includes hematomas and gastrointestinal bleeding, but in some cases the discovery of an inhibitor is made by routine evaluation of a prolonged APTT. Association with pathologic conditions relies most often on single cases: postpartum, nephrotic syndrome, adenocarcinoma (Collins and Gonzalez, 1984), arteritis (Torres et al., 1980), SLE (Castro et al., 1972), and more convincingly, gastrointestinal diseases such as Crohn's and ulcerative rectocolitis (Kyriakou, 2002).

Diagnosis relies on the direct assessment of the inhibitor in a modified Bethesda assay (see above). APTT is prolonged with normal PT. FIX activity is generally <1% of normal level. Autoantibodies to FIX belong to the IgG isotype but have not been characterized in detail. It is, however, likely that they show specificity and isotype distribution similar to that of alloantibodies found in hemophilia B patients undergoing replacement therapy (Greenwood et al., 2003).

A safe and rapidly efficient treatment involves a combination of IVIG and corticosteroids (Mazzucconi et al., 1999).

AUTOANTIBODIES TO FACTOR V

Clinical Presentation and Associated Conditions

This is a rare disorder with fewer than 250 cases described, but is likely to be underdiagnosed because of the large variety of associated syndromes or triggering factors (see below) and the fact that 40% of cases are asymptomatic. The bleeding diathesis is variable with epistaxis, intestinal bleeding or occurrence of retroperitoneal or brain hematomas (Kapur et al., 1993; Schleinitz et al., 2001).

The majority of cases are discovered after cardiovascular or gastrointestinal surgery (Leclerc et al., 1995), with the suspicion that antibiotics (Thomas et al., 1989) and/or blood transfusion could play a role. The use of aminoglycosides has been associated with rare occurrences of factor V (FV) autoantibodies. As for other antibodies to coagulation factors, an association has been found with cancer, paraproteins, and benign gammopathies (van Spronsen et al., 1998), autoimmune diseases such as SLE, Hashimoto's thyroiditis (Takahashi et al., 2003), rheumatoid arthritis, and celiac diseases (Taillan et al., 1989). A special case should be made for autoantibodies occurring after the use of fibrin glues (Berruyer et al., 1993; Cruickshank et al., 1994; Muntean et al., 1994), which contain bovine thrombin contaminated with trace amounts of bovine FV (Spero, 1993), and trigger the production of cross-reactive antihuman FV antibodies. New biomaterials and use of recombinant thrombin should prevent further emergence of such cross-reacting antibodies.

Diagnosis

Laboratory investigations show prolonged APTT and PT in the presence of normal TT. Mixing experiments show lack of correction of the coagulation defect when normal plasma is added to the patient's sample.

Pathogenesis

A limited number of investigations have been carried out to characterize such autoantibodies (Nesheim et al., 1986; Kalafatis et al., 2002). Not all antibodies are inhibitors of FV function. Some appear to increase the clearance of FV. Antibodies recognize the FV light chain (Suehisa et al., 1995) and in particular the C2 domain (Ortel et al., 1998), thereby inhibiting the binding of FV to phospholipids and the formation of the prothrombinase complex. There is no obvious difference in specificity between inhibitory and noninhibitory antibodies. Interestingly, anti-FV antibodies have no effect on platelet FV, which represents ± 20% of total FV, which could explain why a significant proportion of patients with auto–anti-FV antibodies have no obvious bleeding tendency.

Treatment

There is no validated therapy for FV autoantibodies. Apart from the treatment of the underlying disease and the use of corticosteroids and/or immunosuppressants (Bayani et al., 2002), some authors advocate the use of platelets

based on the storage of FV in α granules. Infusion of IVIG is probably the treatment of choice, which, although its mechanism of action is not entirely elucidated (Fu et al., 1996), is often efficient. Prothrombin-rich complexes are used for emergency situations. Anti-CD20 antibodies (rituximab) should be considered in the context of systemic autoimmune disease.

ACQUIRED FACTOR X DEFICIENCY

Clinical Presentation and Associated Conditions

The bleeding diathesis affects the gastrointestinal tract and other mucosa (Edgin et al., 1980; Lankiewicz and Bell, 1992). Hematuria and bleeding in the oropharyngeal area are frequent, as well as cutaneous hematomas (Mulhare et al., 1991). It can present at any age as it is not necessarily associated with neoplasia or autoimmune diseases.

The presence of an autoantibody to Factor X (FX) is most often suspected from *in vitro* evaluation of FX (see below). It is likely that such antibodies are present in a majority of cases, with the exception of the classical demonstration of FX-irreversible adsorption on amyloid fibrils (Furie et al., 1981; Greipp, 1981). Infections of viral or mycoplasma origin are frequently reported. Large traumas, extensive burns (Matsunaga and Shafer, 1996), and carcinomas can be associated in some instances. Single case reports of FX autoantibodies after fungicide or paint solvent exposure and in leprosy (Ness et al., 1980) have been described, but idiopathic cases are known.

Diagnosis

A prolonged PT with a normal or moderately prolonged APTT is found. Levels of FX are very low. Mixing experiments do not show correction of PT. Activation of FX by either the FVIII/FIX or tissue factor (TF)/FVII pathways is impaired. Inhibition of direct FX activation in the Russell viper venom test can also be observed.

Pathogenesis

Antibodies that increase the clearance rate of FX and inhibitor antibodies have both been described. The mechanism by which antibodies inhibit FX function is not precisely identified, but includes inhibition of FX activation as seen in the Russell test.

Treatment

Recommended treatment combines plasma exchange and infusion of IVIG (Smith et al., 1998). The use of prothrombin-rich complexes is deemed to carry a high risk of thrombotic complications. The prognosis is dependent on the underlying cause, but in most cases the inhibitor is only transient.

AUTOANTIBODIES TO FACTOR VII

Clinical Presentation and Associated Diseases

Signs of bleeding vary between minimal and life-threatening, and appear to have no direct relationship with the residual level of Factor FVII (FVII) (Weisdorf, 1989; Okajima and Ishii, 1999; Aguiar, 2003). Bleeding affects the gastrointestinal and urinary tracts, often accompanied by ecchymoses, but the limited number of cases described also points to internal hematomas, notably retroperitoneal and intracranial (Delmer et al., 1989). Autoantibodies to FVII have been observed in the postpartum period, and in the context of SLE, carcinomas or penicillin treatment. It occurs without obvious association in 30% of cases.

Diagnosis

A prolonged or very prolonged PT with a normal APTT and platelet count point to a defect in the extrinsic pathway of coagulation. Levels of FVII are reduced to a variable extent, which is of no predictive value.

Pathogenesis

Antibodies inhibit FVIIa activity. Binding to the FVIIa light chain prevents its binding to tissue factor and/or phospholipids (Kamikubo et al., 2000).

Treatment

Prothrombin complexes and steroids are recommended (Annichino-Bizzachi et al., 1993).

AUTOANTIBODIES TO FACTOR XI OR FACTOR XII (CONTACT PHASE)

Clinical Presentation and Associated Diseases

Antibodies to FXI or FXII usually go unnoticed, as they are not associated with a bleeding diathesis. Antibodies to FXI can be associated with recurrent thrombosis or even disseminated intravascular coagulation (DIC). Associations have been described with SLE (Di Sabatino, 1979; Vercellotti and Mosher, 1982), psoriasis (Rustgi et al.,

1982), and adenovirus infection for FXI antibodies and with liver diseases such as chronic hepatitis, SLE, Waldenstrom's macroglobulinemia, and glomerulonephritis for FXII-specific antibodies (Chalkiadakis et al., 1999).

Diagnosis

The discovery of a prolonged or very prolonged APTT in patients with no apparent bleeding or a history of recurrent thrombosis is highly suggestive of either a deficiency in FXI or FXII, or for the presence of specific antibodies. This finding should be followed by evaluation of single components.

Pathogenesis

Information on anti-FXI antibodies is derived from alloantibodies generated in patients with FXI deficiency (Stern et al., 1982). Such antibodies are of the IgG isotype and bind to two distinct epitopes (De La Cadena et al., 1988) involved in the binding of FXI to high molecular weight kallikrein (Page et al., 1994; Sugi and McIntyre, 1995), or in the activation of FIX by FXI, respectively. No reports on the specificity or mechanisms of inhibition of anti-FXII antibodies have been published.

Treatment

No treatment is generally required, except for treatment of the underlying disease and for prevention of recurrent thrombotic episodes.

AUTOANTIBODIES TO FACTOR XIII

Clinical Presentation and Associated Diseases

This is a rare but often fatal bleeding diathesis, including intracranial hemorrhage, due to a lack of fibrin stabilization (Lorand et al., 2002). It has been observed in the context of systemic autoimmune diseases, such as SLE, but occurs without any apparent cause, most often in elderly patients (Lorand et al., 1999). A possible association with treatment with isoniazide has been described, with disappearance of the inhibitor upon drug withdrawal.

Diagnosis

A characteristics feature is that extensive bleeding is associated with normal APTT, PT, and bleeding time, and with normal platelet counts. This should prompt studies on fibrin stabilization and evaluation of Factor XIII (FXIII) levels.

Pathogenesis

FXIII is activated by thrombin, which induces a conformational change in the molecule so that it can cross-link fibrin I and II. Antibodies can prevent activation by thrombin or the conformational change required for full FXIIIa activity (Lorand et al., 1988). Some antibodies interfere with the activity of FXIIIa or with its binding to fibrin.

Treatment

Withdrawal of drugs such as isoniazide and cryoprecipitate to support fibrin stabilization are required. Eradication of the anti-FXIII antibody usually requires corticosteroids and cyclophosphamide (Nakamura et al., 1988).

AUTOANTIBODIES TO PROTHROMBIN

Clinical Presentation and Associated Diseases

The heterogeneity of antiprothrombin antibodies is well illustrated by association with either thrombotic events or bleeding, or sometimes as a chance discovery of routine laboratory assessment. Apart from its association with SLE (see Chapter 27) and the antiphospholipid syndrome (see Chapter 30), antibodies to prothrombin are found as a spontaneous primary event as a consequence of drug intake (phenytoin and chlorpromazine), in the context of human immunodeficiency virus (HIV) infection (37% of the cases), syphilis or even in infectious mononucleosis (3%) (Atsumi et al., 2000). Such antibodies can also be induced by exposure to bovine thrombin used in biologic glues. In such cases, IgE-mediated anaphylaxis can be observed (Wai et al., 2003).

Diagnosis

There is an increase in both PT and APTT. The level and activity of prothrombin are grossly altered. The search for antiprothrombin antibodies can be carried out with prothrombin directly insolubilized on an enzyme-linked immunosorbent assay (ELISA) plate or by interposition of a phospholipid layer. These two tests detect antibodies with different specificities and different pathologic meaning. Only antibodies binding to phospholipid–prothrombin complexes correlate with the presence of SLE (Simmelink et al., 2003).

Pathogenesis

Antibody-binding sites on prothrombin are located outside the functional domain. It is therefore thought that the

major effect of these antibodies on prothrombin results from an increase in clearance rate. At least some of the antiprothrombin antibodies cross-react with plasminogen, which could explain the thrombotic tendency by reduced fibrinolysis (Roubey, 1998).

Treatment

High-doses of prednisolone appear to reduce the clearance rate of complexes of antibodies with prothrombin. Steroids could also increase the rate of prothrombin production by the liver.

AUTOANTIBODIES TO FIBRINOGEN/FIBRIN

Clinical Presentation and Associated Diseases

When searched for, autoantibodies to fibrinogen and/or fibrin are often found in pregnancy and the peripartum period, apparently with no pathologic association (Kondera-Anasz, 1998). Such antibodies are also found in cord blood. In rare cases in which antibodies to fibrinogen and/or fibrin have been described, abnormal bleeding is found, but this is usually not severe. These antibodies can occur in the context of gammopathies or liver disease, but are most often a primary finding (Nawarawong et al., 1991). Patients developing antibodies when exposed to bovine fibrin glue represent a special case (Seifert et al., 1994; Chouhan et al., 1997; Fastenau and Mcintyre, 2000).

Diagnosis

An increased TT with normal or slightly prolonged PT and APTT is the usual finding. Levels of fibrinogen are usually within the normal range. Antibodies are thought to circulate in the form of immune complexes with fibrinogen.

Pathogenesis

Antifibrinogen antibodies either delay the release of fibrinopeptide obtained by thrombin activation of fibrinogen (Marciniak and Greenwood, 1979) or interfere with the process of fibrin polymerization (Hoots et al., 1981). Although mostly belonging to the IgG class, cases of IgM and IgA antibodies have been described.

Treatment

Corticosteroids, cyclophosphamide, and IVIG should be considered for therapy.

ACQUIRED VON WILLEBRAND SYNDROME

Clinical Presentation and Associated Diseases

Acquired von Willebrand syndrome (AvWS) is rare but most likely underdiagnosed (Veyradier et al., 2000; Budde et al., 2002; Kumar et al., 2002; 2003). It should be suspected in late-onset bleeding diathesis in patients with no personal or familial history of bleeding (Mohri, 2003). Symptoms are indistinguishable from those of congenital von Willebrand disease, except for those related to the accompanying disease. Bleeding occurs at the mucosal level: epistaxis, gingivorrhagia, menorrhagia, and often gastrointestinal, the latter being very severe. Ecchymoses are also common (Mohri et al., 1998). In a number of cases, AvWS is a fortuitous discovery. It should, however, be actively searched for in diseases commonly associated with it.

AvWS is associated with lympho- (Hunault-Berger et al., 2001) and myelo-proliferative diseases (Federici et al., 2001), multiple myeloma (Mohri et al., 1995), and benign gammopathy, or in the context of autoimmune diseases such as SLE (Hanley et al., 1994; Michiels et al., 2001; Niiya et al., 2002), and thyroiditis (Bruggers et al., 1994; Aylesworth et al., 1995). Association has also been described with uremia, Crohn's disease (Sokol et al., 2002), aortic stenosis (Vincentelli et al., 2003; Warkentin et al., 2003), Epstein–Barr virus infection (Kinoshita et al., 1991) and ciprofloxacin therapy (Castaman and Rodeghiero, 1994). AvWS can also present without any underlying association (Lazarchick et al., 1986).

Diagnosis

Apart from the evaluation of the associated condition, laboratory tests should first assess bleeding time (prolonged) and APTT (often prolonged due to the reduction in FVIII levels secondary to a decrease in vWF concentrations), in the presence of a normal platelet count and normal thrombin time. Specific tests for vWF should then include an assay for vWF antigen and an evaluation of its function in the presence of ristocetine (binding to platelets) or thrombin-activated platelets. vWF's capacity to bind to collagen is useful (van Genderen et al., 1994). Electrophoresis to evaluate for the presence of multimers is key to diagnosis, as such multimers tend to be cleared from the circulation more rapidly (see below). Finally, platelet vWF should be normal, which distinguishes AvWS from congenital forms of the diseases. If available, an evaluation of vWF propeptide level (normal in AvWS) will further establish the diagnosis.

A search for autoantibodies to vWF should be carried out (Fricke et al., 1985; Lazarchick and Green, 1986), although there is no standard method for it. Mixing experiments are recommended, by which it is possible to detect interference in normal vWF function. However, anti-vWF antibodies often exist in the form of complexes with vWF and are therefore not readily detectable with such an assay. Some recommend preparing an IgG-enriched fraction from plasma followed by dissociation of the complexes with vWF before assessing antibody activity. A negative result has little meaning as many anti-vWF antibodies recognize epitopes located outside the functional domains (see below).

Pathogenesis

Not all cases of AvWS are antibody mediated, although the proportion of cases in which antibodies play a role is not yet precisely established. It is useful to distinguish between antibody-mediated mechanisms and those that are not.

Most antibodies are thought to increase the clearance rate of vWF (Inbal et al., 1997), especially through the recognition of multimers. Clearance is mediated by the reticuloendothelial system. In a minority of cases, antibodies have been shown to bind to the sites of vWF that interact with glycoprotein (GP)Ib and/or GPIIb/IIIa receptors on platelets or collagen, thereby interfering with the normal function of vWF. Interference with the FVIII-binding site has not been described. A case of antibodies directed towards ADAMTS-13, the vWF cleaving protease, has been described and they resulted in a lack of generation of functional vWF (Scheiflinger et al., 2003).

Non-antibody-mediated mechanisms include adsorption of vWF at the surface of tumor cells with aberrant expression of GPIb and/or GPIIb/IIIa receptors or on activated platelets. Iatrogenic causes include the use of plasma expanders made from starch, which are also known to adsorb multimers of vWF (Casonato et al., 2002). vWF proteolysis by plasmin or elastase has been described in conditions with increased fibrinolysis or sepsis. Lastly, vWF can be disrupted by high shear stress, such as that occurring in aortic stenosis, together with platelet activation brought about by the same stress conditions.

Treatment

This includes the treatment of the underlying diseases (Federici et al., 1998; Federici, 2000). DDAVP is the treatment of choice as it has the capacity to mobilize vWF from storage sites (Frank et al., 2002). However, such treatment is of limited use in antibody-mediated AvWS, in which the rapid clearance compensates for increased production. Under such circumstances, infusion of IVIG is advocated (Arkel et al., 1994). Emergency situations require the use of FVIII concentrates with a high content of vWF. The prognosis is dictated by the capacity to overcome the initial bleeding episode and the underlying disease.

FUTURE PROSPECTS

Autoantibodies to coagulation factors represent unique situations that can be exploited to increase our understanding of the mechanisms leading to autoimmunity. Such autoantibodies have a narrow range of specificity, offering the possibility of evaluation at clonal level. Methods to carry out such an evaluation for both B and T cells are now accessible in many laboratories. Besides, the pathology created by autoantibodies to coagulation factors is easy to identify by routine functional assays. Further, in many circumstances, a corresponding allogeneic immune response can be found in coagulation factor-deficient patients treated with specific replacement therapy (FVIII and FIX to cite the most frequent). Likewise, an in-depth knowledge of the mechanisms leading to autoimmunity should help improve the quality of recombinant proteins for therapeutic use, from soluble coagulation factors to fibrin sealant glues.

Acknowledgment

The author wishes to express his gratitude to Dr Marc Jacquemin and Dr Claudine Mazurier for critical reading of the manuscript and constructive comments.

References

Aguiar, C., Lucia, J.F., and Hernandez, P. (2003). A case of an inhibitor autoantibody to coagulation factor VII. *Haemophilia 9*, 119–120.

Alumkal, J., Rice, L., Vempathy, H., McCarthy, J.J., and Riggs, S.A. (1999). Surgery-associated factor VIII inhibitors in patients without hemophilia. *Am. J. Med. Sci. 318*, 350–352.

Annichino-Bizzacchi, J.M. and Machado, T.F. (1991). Leprosy and acquired factor VIII inhibitor: a case report. *Lepr. Rev. 62*, 155–157.

Annichino-Bizzachi, J.M., Machado, T.F., and Arruda, V.R. (1993). Successful immunosuppressive treatment of high-titer postpartum factor VII inhibitor. *Ann. Intern. Med. 119*, 1149–1150.

Arkel, Y.S., Lynch, J., and Kamiyama, M. (1994). Treatment of acquired von Willebrand syndrome with intravenous immunoglobulin. *Thromb. Haemost. 72*, 643–644.

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.

Aurousseau, M.H., Eclache V., Fain, O., and Thomas, M. (1996). Acquired factor VIII inhibitor in a patient with tuberculosis (letter). *Haemostasis 26*, 319–320.

Aylesworth, C.A., Smallridge, R.C., Rick, M.E., and Alving, B.M. (1995). Acquired von Willebrand's disease: a rare manifestation of postpartum thyroiditis. *Am. J. Hematol. 50*, 217–219.

Bayani, N., Rugina, M., Haddad-Vergnes, L., and Lelong, F. (2002). High-titer acquired factor V inhibitor responsive to corticosteroids and cyclophosphamide in a patient with two malignant tumors. *Am. J. Hematol. 71*, 33–36.

Berruyer, M., Amiral, J., Ffrench, P., Belleville, J., Bastien, O., Clerc, J., Kassir, A., Estanove, S., and Dechavanne, M. (1993). Immunization by bovine thrombin used with fibrin glue during cardiovascular operations. Development of thrombin and factor V inhibitors. J. *Thorac. Cardiovasc. Surg. 105*, 892–897.

Bruggers, C.S., McElligott, K., and Rallison, M.L. (1994). Acquired von Willebrand disease in twins with autoimmune hypothyroidism: response to desmopressin and L-thyroxine therapy. *J. Pediatr. 125*, 911–913.

Budde, U., Bergmann, F., and Michiels, J.J. (2002). Acquired von Willebrand syndrome: experience from 2 years in a single laboratory compared with data from the literature and an international registry. *Semin. Thromb. Hemost. 28*, 227–238.

Casonato, A., Pontara, E., Doria, A., Bertomoro, A., Cattini, M.G., Gambari, P.F., and Girolami, A. (2002). Lack of multimer organization of von Willebrand factor in an acquired von Willebrand syndrome. *Br. J. Haematol. 116*, 899–904.

Castaman, G. and Rodeghiero, F. (1994). Acquired transitory von Willebrand syndrome with ciprofloxacin. *Lancet 343*, 492.

Castro, O., Farber, L.R., and Clyne, L.P. (1972). Circulating anticoagulants against factors IX and XI in systemic lupus erythematosus. *Ann. Intern. Med. 77*, 543–548.

Chalkiadakis, G., Kyriakou, D., Oekonomaki, E., Tsiaoussis, J., Alexandrakis, M., Vasilakis, S., and Kouroumalis, E. (1999). Acquired inhibitors to the coagulation factor XII associated with liver disease. *Am. J. Gastroenterol. 94*, 2551–2553.

Chouhan, V.D., De La Cadena, R.A., Nagaswami, C., Weisel, J.W., Kajani, M., and Rao, A.K. (1997). Simultaneous occurrence of human antibodies directed against fibrinogen, thrombin, and factor V following exposure to bovine thrombin: effects on blood coagulation, protein C activation and platelet function. *Thromb. Haemost. 77*, 343–349.

Collins, H.W. and Gonzalez, M.F. (1984). Acquired factor IX inhibitor in a patient with adenocarcinoma of the colon. *Acta Haematol. 71*, 49–52.

Crenier, L., Ducobu, J., desGrottes, J.M., Cerny, J., Delaunoit, C., and Capel, P. (1996). Low response to high-dose intravenous immunoglobulin in the treatment of acquired factor VIII inhibitor. *Br. J. Haematol. 95*, 750–753.

Cruickshank, M.K., Black, J., and Wall, W. (1994). Development of a factor V and thrombin inhibitor following partial hepatic resection and the use of topical thrombin concentrate. *Clin. Lab. Haematol. 16*, 187–190.

De La Cadena, R.A., Baglia, F.A., Johnson, C.A., Wenk, R.E., Amernick, R., Walsh, P.N., and Colman, R.W. (1988). Naturally occurring human antibodies against two distinct functional domains in the heavy chain of FXI/FXIa. *Blood 72*, 1748–1754.

Delmer, A., Horellou, M.H., Andreu, G., Lecompte, T., Rossi, F., Kazatchkine, M.D., Samama, M., and Zittoun, R. (1989). Life-threatening intracranial bleeding associated with the presence of an anti-factor VII autoantibody. *Blood 74*, 229–232.

Di Giambattista, M., Branckaert, T., and Laub, R. (2001). Mapping of natural anti-factor VIII antibodies in plasma pools from healthy donors: use of rationally designed synthetic peptides. *Biologicals 29*, 229–232.

Di Sabatino, C.A., Clyne, L.P., and Malawista, S.E. (1979). A circulating anticoagulant directed against factor Xia in systemic lupus erythematosus. *Arthritis Rheum. 22*, 1135–1138.

Edgin, R.A., Metz, E.N., Fromkes, J.J., and Beman, F.M. (1980). Acquired factor X deficiency with associated defects in platelet aggregation. *Am. J. Med. 69*, 137–139.

Fastenau, D.R. and McIntyre, J.A. (2000). Immunochemical analysis of polyspecific antibodies in patients exposed to bovine fibrin sealant. *Ann. Thorac. Surg. 69*, 1867–1872.

Federici, A.B. (2000). Therapeutic approaches to acquired von Willebrand syndrome. Expert Opin. Investig. *Drugs 9*, 347–354.

Federici, A.B., Stabile, F., Castaman, G., Canciani, M.T., and Mannucci, P.M. (1998). Treatment of acquired von Willebrand syndrome in patients with monoclonal gammopathy of uncertain significance: comparison of three different therapeutic approaches. *Blood 92*, 2707–2711.

Federici, A.B., Rand, J.H., and Mannucci, P.M. (2001). Acquired von Willebrand syndrome: an important bleeding complication to be considered in patients with lymphoproliferative and myeloproliferative disorders. *Hematol. J. 2*, 358–362.

Frank, R.D., Kunz, D. and Wirtz, D.C. (2002). Acquired von Willebrand disease: hemostatic management of major orthopedic surgery with high-dose immunoglobulin, desmopressin, and continuous factor concentrate infusion. *Am. J. Hematol. 70*, 64–71.

Fricke, W.A., Brinkhous, K.M., Garris, J.B., and Roberts, H.R. (1985). Comparison of inhibitory and binding characteristics of an antibody causing acquired von Willebrand syndrome: an assay for von Willebrand factor binding by antibody. *Blood 66*, 562–569.

Fu, Y.X., Kaufman, R., Rudolph, A.E., Collum, S.E., and Blinder, M.A. (1996). Multimodality therapy of an acquired factor V inhibitor. *Am. J. Hematol. 51*, 315–318.

Furie, B., Voo, L., McAdam, K.P., and Furie, B.C. (1981). Mechanism of factor X deficiency in systemic amyloidosis. *N. Engl. J. Med. 304*, 827–830.

Garvey, M.B. (1995). Incidence and management of patients with acquired factor VIII inhibitors: the practical experience of a tertiary care hospital. In C. Kessler (ed.) *Acquired Hemophilia* (pp. 91–111). Princeton: Exerpta Medica.

Gawryl, M.S. and Hoyer, L.W. (1982). Inactivation of factor VIII coagulant activity by two different types of human antibodies. *Blood 60*, 1103–1109.

Gilles, J.G. and Saint-Remy, J.M.R. (1996). Coagulation Factor VIII autoantibodies. In J. B. Peter and Y. Shoenfeld (eds) *Autoantibodies* (pp. 172–178). Amsterdam: Elsevier.

Green, D. and Lechner, K. (1981). A survey of 215 non-hemophilic patients with inhibitors to factor VIII. *Thromb. Haemost. 45*, 200–203.

Green, D., Rademaker, A.W., and Briët, E. (1993). A prospective, randomized trial of prednisone and cyclophosphamide in the treatment of patients with factor VIII autoantibodies. *Thromb. Haemost. 70*, 753–757.

Greenwood, R., Wang, B., Midkiff, K., White, G.C. II, Lin, H.F., and Frelinger, J.A. (2003). Identification of T-cell epitopes in clotting factor IX and lack of tolerance in inbred mice. *J. Thromb. Haemost. 1*, 95–102.

Greipp, P.R., Kyle, R.A., and Bowie, E.J. (1981). Factor X deficiency in amyloidosis: a critical review. *Am. J. Hematol. 11*, 443–450.

Hanley, D., Arkel, Y.S., Lynch, J., and Kamiyama, M. (1994). Acquired von Willebrand's syndrome in association with a lupus-like anticoagulant corrected by intravenous immunoglobulin. *Am. J. Hematol. 46*, 141–146.

Hauser, I., Schneider, B., and Lechner, K. (1995). Post-partum factor VIII inhibitors. A review of the literature with special reference to the value of steroid and immunosuppressive treatment. *Thromb. Haemost. 73*, 1–5.

Hay, C.R.M., Negrier, C., and Ludlam, C.A. (1997). The treatment of bleeding in acquired haemophilia with recombinant factor VIIa: a multicentre study. *Thromb. Haemost. 78*, 1463–1467.

Hoots, W.K., Carrell, N.A., Wagner, R.H., Cooper, H.A., and McDonagh, J. (1981). A naturally occurring antibody that inhibits fibrin polymerization. *N. Engl. J. Med. 304*, 857–861.

Hoyle, C. and Ludlam, C.A. (1986). Acquired factor VIII inhibitor associated with multiple sclerosis, successfully treated with porcine factor VIII (letter). *Thromb. Haemost. 57*, 233.

Hultin, M.B. (1991). Acquired inhibitors in malignant and nonmalignant disease states. *Am. J. Med. 91*(5A), 9S–13S.

Hunault-Berger, M., Rachieru, P., Ternisien, C., Jardel, H., Zandecki, M., Boasson, M., and Ifrah, N. (2001). Acquired von Willebrand disease and lymphoproliferative syndromes. *Presse Med. 30*, 209–212.

Inbal, A., Bank, I., Zivelin, A., Varon, D., Dardik, R., Shapiro, R., Rosenthal, E., Shenkman, B., Gitel, S., and Seligsohn, U. (1997). Acquired von Willebrand disease in a patient with angiodysplasia resulting from immune-mediated clearance of von Willebrand factor. *Br. J. Haematol. 96*, 179–182.

Jacquemin, M.G., Lavend'homme, R., Benhida, A., Vanzieleghem, B., d'Oiron, R., Lavergne, J.M., Brackmann, H.H., Schwaab, R., Hoylaerts, M., Gilles, J.G.G., Peerlinck, K., Vermylen, J., and Saint-Remy, J.M.R. (2000). A novel cause of mild/moderate hemophilia A: mutations scattered in the Factor VIII C1 domain reduce Factor VIII binding to von Willebrand factor. *Blood 96*, 958–965.

Jone, D.W., Nicholls, P.J., Donohoe, S., Gallimore, M.J., and Winter, M. (2002). Antibodies to factor XII are distinct from antibodies to prothrombin in patients with the anti-phospholipid syndrome. *Thromb. Haemost. 87*, 426–430.

Kalafatis, M., Simioni, P., Tormene, D., Beck, D.O., Luni, S., and Girolami, A. (2002). Isolation and characterization of an antifactor V antibody causing activated protein C resistance from a patient with severe thrombotic manifestations. *Blood 99*, 3985–3992.

Kamikubo, Y., Miyamoto, S., Iwasa, A., Ishii, M., and Okajima, K. (2000). Purification and characterization of factor VII inhibitor found in a patient with life threatening bleeding. *Thromb. Haemost. 83*, 60–64.

Kapur, A., Kelsey, P.R., and Isaacs, P.E. (1993). Factor V inhibitor in thrombosis. *Am. J. Hematol. 42*, 384–388.

Kinoshita, S., Yoshioka, K., Kasahara, M., and Takamiya, O. (1991). Acquired von Willebrand disease after Epstein–Barr virus infection. *J. Pediatr. 119*, 595–598.

Kireger, H., Leddy, J.P., and Breckenridge, R.T. (1975). Studies on a circulating anticoagulant in systemic lupus erythematosus: evidence for inhibition of the function of activated plasma thromboplastin antecedent (factor XIa). *Blood 46*, 189–197.

Kondera-Anasz, Z. (1998). Antibodies against fibrinogen in pregnant women, in post delivery women and in the newborns. *Thromb. Haemost. 79*, 963–968.

Kumar, S., Pruthi, R.K. and Nichols, W.L. (2002). Acquired von Willebrand disease. *Mayo Clin. Proc. 77*, 181–187.

Kumar, S., Pruthi, R.K., and Nichols, W.L. (2003). Acquired von Willebrand's syndrome: a single institution experience. *Am. J. Hematol. 72*, 243–247.

Kyriakou, D.S., Alexandrakis, M.G., Passam, F.H., Fondouli, K., Matalliotakis, E., Koutroubakis, I.E., Kourelis, T.V., Chalkiadakis, G., and Manoussos, O.N. (2002). Acquired inhibitors to coagulation factors in patients with gastrointestinal diseases. *Eur. J. Gastroenterol. Hepatol. 14*, 1383–1387.

Lankiewicz, M.W. and Bell, W.R. (1992). A unique circulating inhibitor with specificity for coagulation factor X. *Am. J. Med. 93*, 343–346.

Lazarchick, J. and Green, C. (1994). Acquired von Willebrand's disease following bone marrow transplantation. *Ann. Clin. Lab. Sci. 24*, 211–215.

Lazarchick, J., Pappas, A.A., Kizer, J., and Hall, S.A. (1986). Acquired von Willebrand syndrome due to an inhibitor specific for von Willebrand factor antigens. *Am. J. Hematol. 21*, 305–314.

Leclerc, J., Charre, S., Tournoys, A., Gorski, C., and Goudemand, J. (1995). Postoperative disorders of hemostasis caused by antifactor V circulating anticoagulant]. *Ann. Fr. Anesth. Reanim. 14*, 303–305.

Lin, C.K., Liang, R., Liu, H.W., Tse, P.W. and Chan, G.T. (1991). Myelodysplastic syndrome and acquired factor VIII inhibitor with severe subcutaneous haemorrhage. *Acta Haematol. 85*, 206–208.

Lorand, L., Velasco, P.T., Rinne, J.R., Amare, M., Miller, L.K., and Zucker, M.L. (1988). Autoimmune antibody (IgG Kansas) against the fibrin stabilizing factor (factor XIII) system. *Proc. Natl. Acad. Sci. USA 85*, 232–236.

Lorand, L., Velasco, P.T., Murthy, S.N., Lefebvre, P., and Green, D. (1999). Autoimmune antibody in a hemorrhagic patient interacts with thrombin-activated factor XIII in a unique manner. *Blood 93*, 909–917.

Lorand, L., Velasco, P.T., Hill, J.M., Hoffmeister, K.J., and Kaye, F.J. (2002). Intracranial hemorrhage in systemic lupus erythematosus associated with an autoantibody against factor XIII. *Thromb. Haemost. 88*, 919–923.

Marciniak, E. and Greenwood, M.F. (1979). Acquired coagulation inhibitor delaying fibrinopeptide release. *Blood 53*, 81–92.

Matsumoto, T., Shima, M., Fukuda, K., Nogami, K., Giddings, J.C., Murakami, T., Tanaka, I., and Yoshioka, A. (2001). Immunological characterization of factor VIII autoantibodies in patients with acquired hemophilia A in the presence or absence of underlying disease. *Thromb. Res. 104*, 381–388.

Matsunaga, A.T. and Shafer, F.E. (1996). An acquired inhibitor to factor X in a pediatric patient with extensive burns. *J. Pediatr. Hematol. Oncol. 18*, 223–226.

Mazzucconi, M.G., Peraino, M., Bizzoni, L., Bernasconi, S., Luciani, M., and De Rossi, G. (1999). Acquired inhibitor against factor IX in a child: successful treatment with high-dose immunoglobulin and dexamethasone. *Haemophilia 5*, 132–134.

Meek, J.C. and Haak, H.L. (1986). Acute myelomonocytic leukaemia and acquired haemophilia A with severe cutaneous haemorrhage. *Scand. J. Haematol. 37*, 168–173.

Michiels, J.J., Schroyens, W., van der Planken, M., and Berneman, Z. (2001). Acquired von Willebrand syndrome in systemic lupus erythematodes. *Clin. Appl. Thromb. Hemost. 7*, 106–112.

Mohri, H. (2003). Acquired von Willebrand syndrome: its pathophysiology, laboratory features and management. *J. Thromb. Thrombolysis. 15*, 141–149.

Mohri, H., Tanabe, J., Ohtsuka, M., Yoshida, M., Motomura, S., Nishida, S., Fujimura, Y., and Okubo, T. (1995). Acquired von Willebrand disease associated with multiple myeloma: characterization of an inhibitor to von Willebrand factor. *Blood Coagul. Fibrinolysis. 6*, 561–566.

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*, 3623–3629.

Moraca, R.J. and Ragni, M.V. (2002). Acquired anti-FVIII inhibitors in children. *Haemophilia 8*, 28–32.

Morrison, A.E., Ludlam, C.A., and Kessler, C. (1993). Use of porcine factor VIII in the treatment of patients with acquired haemophilia. *Blood 6*, 1513–1520.

Mudad, R. and Kane, W.H. (1993). DDAVP in acquired hemophilia A: case report and review of the literature. *Am. J. Hematol. 43*, 295–299.

Mulhare, P.E., Tracy, P.B., Golden, E.A., Branda, R.F., and Bovill, E.G. (1991). A case of acquired factor X deficiency with *in vivo* and *in vitro* evidence of inhibitor activity directed against factor X. *Am. J. Clin. Pathol. 96*, 196–200.

Muntean, W., Zenz, W., Finding, K., Zobel, G., and Beitzke, A. (1994). Inhibitor to factor V after exposure to fibrin sealant during cardiac surgery in a two-year-old child. *Acta Paediatr. 83*, 84–87.

Nakamura, S., Kato, A., Sakata, Y., and Aoki, N. (1988). Bleeding tendency caused by IgG inhibitor to factor XIII, treated successfully by cyclophosphamide. *Br. J. Haematol. 68*, 313–319.

Nawarawong, W., Wyshock, E., Meloni, F.J., Weitz, J., and Schmaier, A.H. (1991). The rate of fibrinopeptide B release modulates the rate of clot formation: a study with an acquired inhibitor to fibrinopeptide B release. *Br. J. Haematol. 79*, 296–301.

Nesheim, M.E., Nichols, W.L., Cole, T.L., Houston, J.G., Schenk, R.B., Mann, K.G., and Bowie, E.J. (1986). Isolation and study of an acquired inhibitor of human coagulation factor V. *J. Clin. Invest. 77*, 405–415.

Ness, P.M., Hymas, P.G., Gesme, D., and Perkins, H.A. (1980). An unusual factor X inhibitor in leprosy. *Am. J. Hematol. 8*, 397–402.

Niiya, M., Niiya, K., Takazawa, Y., Hayashi, Y., Tanio, Y., Kushiro, M., Tanimizu, M., Hasegawa, H., and Tanimoto, M. (2002). Acquired type 3-like von Willebrand syndrome preceded full-blown systemic lupus erythematosus. *Blood Coagul. Fibrinol. 13*, 361–365.

Nilsson, I.M., Sundqvist, S.B., and Freiburghaus, C. (1984). Extracorporeal protein A-sepharose and specific affinity chromatography for removal of antibodies. *Prog. Clin. Biol. Res. 150*, 225.

Okajima, K. and Ishii, M. (1999). Life-threatening bleeding in a case of autoantibody-induced factor VII deficiency. *Int. J. Hematol. 69*, 129–132.

Ortel, T.L., Moore, K.D., Quinn-Allen, M.A., Okamura, T., Sinclair, A.J., Lazarchick, J., Govindan, R., Carmagnol, F., and Kane, W.H. (1998). Inhibitory anti-Factor V antibodies bind to Factor V C2 domain and are associated with hemorrhagic manifestations. *Blood 91*, 4188–4196.

Page, J.D., DeLa Cadena, R.A., Humphries, J.E., and Colman, R.W. (1994). An autoantibody to human plasma prekallikrein blocks activation of the contact system. *Br. J. Haematol. 87*, 81–86.

Reding, M.T., Okita, D.K., Diethelm-Okita, B.M., Anderson, T.A., and Conti-Fine, B.M. (2003). Human $CD4^+$ T-cell epitope repertoire on the C2 domain of coagulation factor VIII. *J. Thromb. Haemost. 1*, 1777–1784.

Roberts, H.R. (1998). Clinical experience with activated factor VII: focus on safety aspects. *Blood Coagul. Fibrinol.* 9(Suppl 1), S115–S118.

Rossi, F., Sultan, Y., and Kazatchkine, M.D. (1988). Anti-idiotypes against autoantibodies and alloantibodies to Factor VIII:C (anti-haemophilic factor) are present in therapeutic polyspecific normal immunoglobulins. *Clin. Exp. Immunol. 74*, 311–316.

Roubey, R.A. (1998). Mechanisms of autoantibody-mediated thrombosis. Lupus 7(Suppl 2), s114-s119.

Rustgi, R.N., LaDuca, F.M., and Tourbaf, K.D. (1982). Circulating anticoagulant against factor XI in psoriasis. *J. Med. 13*, 289–301.

Scandella, D., Mattingly, M., de Graaf, S., and Fulcher C.A. (1989). Localization of epitopes for human factor VIII inhibitor antibodies by immunoblotting and antibody neutralization. *Blood 74*, 1618–1626.

Scheiflinger, F., Knobl, P., Trattner, B., Plaimauer, B., Mohr, G., Dockal, M., Dorner, F., and Rieger, M. (2003). Nonneutralizing IgM and IgG antibodies to von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura. *Blood 102*, 3241–3243.

Schleinitz, N., Veit, V., Chouquet, D., Seux, V., Arnoux, D., Mokart, D., Lelong, B., Alessi, M.C., Kaplanski, G., and Harle, J.R. (2001). Acquired factor V inhibitor: etiology, bleeding risk and therapeutic management with regard to three cases. *Rev. Med. Interne. 22*, 1119–1123.

Schwartz, R.S., Gabriel, D.A., Aledort, L.M., Green, D., and Kessler, C.M. (1995). A prospective study of treatment of acquired (autoimmune) factor VIII inhibitors with high-dose intravenous gammaglobulin. *Blood 86*, 797–804.

Schwerdtfeger, R., Hintz, G., and Huhn, D. (1991). Successful treatment of a patient with postpartum factor VIII inhibitor with recombinant human interferon α 2a. *Am. J. Hematol. 37*, 190–193.

Seidler, S.W., Mills, L.E., Flowers, M.E.D., and Sullivan, K.M. (1994). Spontaneous factor VIII inhibitor occurring in association with chronic graft-versus-host disease. *Am. J. Hematol. 45*, 240–243.

Seifert, J., Klause, N., Stobbe, J., and Egbers, H.J. (1994). Antibodies formed against fibrin glue components and their circulatory relevance. *J. Invest. Surg. 7*, 167–171.

Shaffer, L.G. and Phillips, M.D. (1997). Successful treatment of acquired hemophilia with oral immunosuppressive therapy. *Ann. Intern. Med. 127*, 206–209.

Shwaiki, A., Lara, L., Ahmed, F., Crock, R., Rutecki, G.W., and Whittier, F.C. (2001). Acquired inhibitor to factor VIII in small cell lung cancer: a case report and review of the literature. *Ann. Hematol. 80*, 124–126.

Sievert, R., Goldstein, M.L., and Surks, M.I. (1996). Graves' disease and autoimmune factor VIII deficiency. *Thyroid 6*, 247.

Simmelink, M.J., De Groot, P.G., and Derksen, R.H. (2003). A study on associations between antiprothrombin antibodies, antiplasminogen antibodies and thrombosis. *J. Thromb. Haemost. 1*, 735–739.

Smith, S.V., Liles, D.K., White, G.C. II, and Brecher, M.E. (1998). Successful treatment of transient acquired factor X deficiency by plasmapheresis with concomitant intravenous immunoglobulin and steroid therapy. *Am. J. Hematol. 57*, 245–252.

Sokol, L., Stueben, E.T., Jaikishen, J.P., and Lamarche, M.B. (2002). Turner syndrome associated with acquired von Willebrand disease, primary biliary cirrhosis, and inflammatory bowel disease. *Am. J. Hematol. 70*, 257–259.

Spero, J.A. (1993). Bovine thrombin-induced inhibitor of factor V and bleeding risk in postoperative neurosurgical patients. Report of three cases. *J. Neurosurg. 78*, 817–820.

Spero, J.A., Lewis, J.H., and Hasiba, U. (1981). Corticosteroid therapy for acquired F VIII:C inhibitors. *Br. J. Haematol. 48*, 635–642.

Stasi, R., Brunetti, M., Stipa, E., and Amadori, S. (2004). Selective B-cell depletion with rituximab for the treatment of patients with acquired hemophilia. Blood *103*, 4424–4428.

Stern, D.M., Nossel, H.L., and Owen, J. (1982). Acquired antibody to factor XI in a patient with congenital factor XI deficiency. *J. Clin. Invest. 69*, 1270–1276.

Stricker, R.B., Barlogie, B., and Kiprov, D.B. (1994). Acquired Factor VIII inhibitor associated with chronic interferon-α therapy. *J. Rheumatol. 21*, 350–352.

Suehisa, E., Toku, M., Akita, N., Fushimi, R., Takano, T., Tada, H., Iwatani, Y., and Amino, N. (1995). Study on an antibody against F1F2 fragment of human factor V in a patient with Hashimoto's disease and bullous pemphigoid. *Thromb. Res. 77*, 63–68.

Sugi, T. and McIntyre, J.A. (1995). Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen-PE complex. *Blood 86*, 3083–3089.

Sugishita, K., Nagase, H., Takahashi, T., Takenaka, K., Shiratori, Y., and Omata, M. (1999). Acquired factor VIII inhibitor in a non-hemophilic patient with chronic hepatitis C viral infection. *Intern. Med. 38*, 283–286.

Sultan, Y., Kazatchkine, M.D., Maisonneuve, P., and Nydegger U.E. (1984). Anti-idiotypic suppression of autoantibodies to Factor VIII (anti-haemophilic factor) by high-dose intravenous gammaglobulin. *Lancet ii*, 765–768.

Taillan, B., Fuzibet, J.G., Vinti, H., Pesce, A., Roul, C., Bayle, J., and Dujardin, P. (1989). Factor V inhibitor in celiac disease. *Am. J. Med. 87*, 360.

Takahashi, H., Fuse, I., Abe, T., Yoshino, N., and Aizawa, Y. (2003). Acquired factor V inhibitor complicated by Hashimoto's thyroiditis, primary biliary cirrhosis and membranous nephropathy. *Blood Coagul. Fibrinolysis. 14*, 87–93.

Thomas, F., Robert, A., Luquel, L., Dombret, H., Offenstadt, G., and Amstutz, P. (1989). Syndrome hemorragique mortel apres mithramycine. Inagregabilite plaquettaire et inhibiteur du facteur V. *Presse Med. 18*, 182.

Torres, A., Lucia, J.F., Oliveros, A., Vazquez, C., and Torres, M. (1980). Anti-factor IX circulating anticoagulant and immune thrombocytopenia in a case of Takayasu's arteritis. *Acta Haematol. 64*, 338–340.

van Genderen, P.J., Vink, T., Michiels, J.J., van 't Veer, M.B., Sixma, J.J., and van Vliet, H.H. (1994). Acquired von Willebrand disease caused by an autoantibody selectively inhibiting the binding of von Willebrand factor to collagen. *Blood 84*, 3378–3384.

van Spronsen, D.J., Oosting, J.D., Hoffmann, J.J., and Breed, W.P. (1998). Factor V inhibitor associated with cold agglutinin disease. *Ann. Hematol. 76*, 49–50.

Vercellotti, G.M. and Mosher, D.F. (1982). Acquired factor XI deficiency in systemic lupus erythematosus. *Thromb. Haemost. 27*, 250–252.

Veyradier, A., Jenkins, C.S.P., Fressinaud, E., and Meyer, D. (2000). Acquired von Willebrand syndrome: from pathophysiology to management. *Thromb. Haemost. 84*, 175–182.

Vignes, S., Le Moing, V., Meekel, P., Papo, T., Wechsler, B., and Godeau, P. (1996). Acquired hemophilia: a rare complication of Sjögren's syndrome. *Clin. Exp. Rheumatol. 14*, 559–560.

Vincentelli, A., Susen, S., Le Tourneau, T., Six, I., Fabre, O., Juthier, F., Bauters, A., Decoene, C., Goudemand, J., Prat, A., and Jude, B. (2003). Acquired von Willebrand syndrome in aortic stenosis. *N. Engl. J. Med. 349*, 343–349.

Von Depka, M. (2002). NovoSeven: mode of action and use in acquired haemophilia. *Intensive Care Med. 28*(Suppl 2), s222–s227.

Wai, Y., Tsui, V., Peng, Z., Richardson, R., Oreopoulos, D., and Tarlo, S.M. (2003). Anaphylaxis from topical thrombin (Thrombostat) during haemodialysis and evaluation of sensitization among a dialysis population. *Clin. Exp. Allergy. 33*, 1730–1734.

Warkentin, T.E., Moore, J.C., Anand, S.S., Lonn, E.M., and Morgan, D.G. (2003). Gastrointestinal bleeding, angiodysplasia, cardiovascular disease, and acquired von Willebrand syndrome. *Transfus. Med. Rev. 17*, 272–286.

Weisdorf, D., Hasegawa, D., and Fair, D.S. (1989). Acquired factor VII deficiency associated with aplastic anaemia: correction with bone marrow transplantation. *Br. J. Haematol. 71*, 409–413.

CHAPTER

46

Multiple Sclerosis

STEVEN D. BRASS, HOWARD L. WEINER AND DAVID A. HAFLER

Harvard Medical School, Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA

HISTORIC BACKGROUND

A French neurologist at the Salpetriere in Paris, Jean Martin Charcot, first gave a comprehensive account of the features of multiple sclerosis (MS) in 1868 by correlating the clinical and pathologic features of the illness in patients he examined both while alive and at autopsy. He noted both the accumulation of inflammatory cells in a perivascular distribution and demyelination accompanied by axonal sparing within the lesions or "plaques" found in the brain and spinal cord white matter of patients with intermittent episodes of neurologic dysfunction (Charcot, 1868a; 1868b; 1877). This led to the term "*sclérose en plaques disseminées,"* or multiple sclerosis. Towards the end of the 19th century, physicians throughout the Western hemisphere began to accept that MS was a specific disease. In 1868, Morris was the first physician to report an autopsy case of MS in North American, while in 1875 Moxon reported eight cases of "insular sclerosis affecting the brain and spinal cord" in a patient from England.

Over the past 100 years there have been many important historical milestones relating to MS that have advanced the field. The demonstration of an autoimmune, at times demyelinating, disease in mammals immunized with central nervous system (CNS) myelin [experimental autoimmune encephalomyelitis (EAE)] was first made by Thomas Rivers at the Rockefeller Institute in 1933 with the repeated injection of rabbit brain and spinal cord into primates (Rivers et al., 1933). This led to the generally accepted hypothesis that MS is secondary to an autoimmune response to self-antigens in a genetically-susceptible host. It should be pointed out that although the inflammation found in the CNS of patients with MS is thought to represent an autoimmune response, this is based on the inability to consistently isolate a microbial agent from the tissue of diseased patients. Nevertheless, primary viral infections in the CNS may induce an autoimmune response (Hafler, 1999), and the recurring lesson from the EAE model is that the minimal requirement for inducing inflammatory, autoimmune CNS demyelinating disease is the activation of myelin-reactive T cells in the peripheral immune system (Ben-Nun et al., 1981; Goverman et al., 1993). In 1948, Elvin Kabat described the increases in oligoclonal immunoglobulin in the cerebrospinal fluid (CSF) of patients with MS, which provided further evidence of an inflammatory nature to the disease (Kabat et al., 1948; 1950). In the past half-century, several large population-based MS twin studies have demonstrated a strong genetic basis to this clinical–pathologic entity (Mackay and

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Myrianthopoulos, 1966; Williams et al., 1980; Heltberg and Holm, 1982; Ebers et al., 1986; Kinnunen et al., 1987; French Research Group on MS, 1992; Mumford et al., 1992; Utz et al., 1993). In 1965, Schumacher et al. (1965) defined clinical diagnostic criteria based on the notion that MS is a disease that is disseminated in time and throughout the CNS. In 1981, magnetic resonance imaging (MRI) was first used to examine patients with MS (Young et al., 1981). The use of MRI in MS has since revolutionized how patients are diagnosed and further enlightened researchers on the pathophysiology of the illness.

In 1993, interferon (IFN)-β-1b was the first FDA-approved immunomodulatory drug shown to alter the clinical course of MS by reducing both disability and MRI lesion burden (Arnason, 1993). In the past 15 years, MS has evolved from a disease with no therapy to one with five approved immunomodulating treatments in the USA. These major advances have established MS as a treatable neurologic illness. Nonetheless, the development of more effective and safe treatments that can be used at the time of diagnosis for this potentially disabling illness is paramount, and predicated on a more thorough understanding of the underlying immunopathology.

Advances in immunology have provided clinicians with powerful tools to better understand the underlying causes of MS, leading to new therapeutic advances. The future calls for extending the original observations of Charcot and Kabat by defining the molecular pathology of MS at the levels of DNA haplotype structure, CNS, and peripheral mRNA and protein expression, leading to the generation of a new series of disease-related hypotheses.

CLINICAL FEATURES

The symptoms and signs of MS are variable as the disease can affect the CNS anywhere. Demyelinating lesions may develop at any site along myelinated CNS white matter tracts, and symptoms of MS therefore depend on the functions subserved by the pathways involved. In addition to demyelination, edema, inflammation, gliosis, and axonal loss all contribute to the symptomatology of a lesion. The most common symptoms and signs involve alteration or loss of sensation due to involvement of spinothalamic or posterior column fibers; visual loss from optic neuritis; limb weakness and spasticity related to disruption of corticospinal tracts; tremors and incoordination of gait or limbs, largely related to cerebellar or spinocerebellar fiber involvement; and abnormalities of cranial nerve function (such as double vision due to disturbance in conjugate eye movement) secondary to brainstem lesions. Bowel, bladder, and sexual dysfunction occur in over two-thirds of patients at some time during the course of their illness (Betts et al., 1993; Mattson et al., 1995), largely due to disruption in spinal cord pathways. Fatigue, depression, and cognitive changes are common symptoms of elusive etiology that may significantly interfere with daily functioning and are now being recognized as significant contributors to disability. A correlation with progressive brain atrophy on MRI has implicated axonal loss as the pathologic substrate of the cognitive deterioration in MS (Whitlock and Siskind, 1980; Freal et al., 1984; Rao et al., 1989; 1991; Sadovnick et al., 1996; Hohol et al., 1997).

The hallmark of MS is the great variability in the clinical presentation and course of the illness. MS may be divided into four clinical categories: clinically isolated syndrome (CIS), relapsing–remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and primary progressive multiple sclerosis (PPMS). Most patients change from a CIS to RRMS and then gradually become SPMS.

The CIS occurs when a patient presents with a first episode of demyelination in the form of optic neuritis, cerebellar or brainstem syndrome, or transverse myelitis accompanied by brain MRI lesions, making them at higher risk for further attacks and then being labeled as having "multiple sclerosis." Not all patients with CIS go on to develop MS. During an episode, neurologic symptoms develop over hours to several days, persist for days to several weeks and gradually dissipate. The resolution of symptoms appears to be due to the reduction of inflammation and edema at the site of the responsible lesion rather than to the reversal of demyelination, which may persist even in the absence of symptoms (Achiron and Barak, 2000). In a recent prospective study, patients experiencing their first episode suggestive of CNS demyelination and having MRI evidence of at least three typical lesions were followed for an average of 42 months. Within that period, a significant proportion of patients developed an additional relapse, thus qualifying for the diagnosis of clinically definite MS. If there were no MRI lesions, the probability of developing MS was substantially less. More than half of those developing MS experienced the additional relapse within 1 year of their first episode (Achiron and Barak, 2000). Thus, it seems reasonable to label the first attack of what appears to be MS as a "clinically isolated syndrome," explaining to patients that there is a high likelihood of developing MS. This indicates to the patient that there is a good understanding of the underlying problem, but the prognosis is not as yet clear, allowing patients who never have another attack to be saved from carrying a diagnosis of MS.

If a patient with a CIS develops further episodes of relapses followed by a recovery with a stable course between relapses, patients are referred to as having RRMS. At the beginning of MS, patients often make complete recovery from relapses. As the disease goes on, the recovery from a relapse diminishes, and the patient accrues disability. A relapsing–remitting onset is observed in 85–90%

of patients, with relapses often lasting 4 weeks in duration. The outcome in patients with RRMS is variable; untreated, approximately 50% of all MS patients require the use of a walking aid by 10 years after clinical onset (Svenson et al., 1994), although the consequences on prognosis of newer treatment regimens are not as yet clear. Increased attack frequency and poor recovery from attacks in the first years of clinical disease predict a more rapid deterioration. Multiple T2-weighted and/or gadolinium-enhancing lesions on the first MRI scan also predict a more severe subsequent course.

Ultimately, approximately 40–50% of relapsing–remitting patients stop having attacks and develop what may be a neurodegenerative progressive disease secondary to the chronic CNS inflammation, known as secondary progressive multiple sclerosis (SPMS) (Confavreux et al., 2000). The evolution to this secondary progressive form of the disease is associated with significantly fewer gadolinium-enhanced lesions and a decrease in brain parenchymal volume (Khoury et al., 1994; Filippi et al., 1995). Similarly, while earlier RRMS is sensitive to immunosuppression (Hohol et al., 1999), as times goes on, responsiveness to immunotherapy decreases and may disappear in later forms of SPMS. Thus, rather then conceiving of MS as first a relapsing–remitting and then a secondary progressive disease, it could be hypothesized that MS is a continuum where there are acute inflammatory events early on with secondary induction of a neurodegenerative process refractory to immunologic intervention. This hypothesis awaits experimental verification where early immunotherapy prevents the onset of secondary progressive disease. Such critical investigations require new models of investigation using natural history studies that can be performed over decades.

Primary progressive MS (PPMS) is characterized from the onset by the absence of acute attacks and instead involves a gradual clinical decline. Close to 15% of patients have a gradual clinical decline from the onset, usually in the form of a progressive myelopathy. Patients may also present with a progressive cerebellar syndrome in the form of ataxia. Clinically, this form of the disease is associated with a lack of response to any form of immunotherapy. This leads to the notion that PPMS may in fact be a very different disease from RRMS. A recent commentary points out the similarities between primary progressive MS and human T-lymphocyte virus (HTLV)-I-associated myelopathy (Hafler, 1999).

In the absence of a specific immune-based assay, the diagnosis of MS continues to be predicated on the clinical history and neurologic examination demonstrating multiple lesions disseminated in time and space in the CNS (McDonald et al., 2001). Although using McDonald's criteria, the diagnosis of MS can be made solely on the basis of a history of two relapses and objective findings on examination of two lesions disseminated in the CNS. MRI of the neuroaxis is often sought to confirm the diagnosis or to rule out other mimics of the illness. The use of MRI has had a major impact on early diagnosis by establishing newer criteria for the diagnosis of the disease, as well as providing a means to determine prognosis, and to monitor disease course and response to therapy. As part of McDonald's criteria, new T2-weighted lesions or T1-weighted gadolinium-enhancing lesions on follow-up MRI scans may serve as criteria for dissemination in space or time, thus allowing the diagnosis fulfilling the criteria of MS to be made with further confidence (McDonald, et al 2001).

MRI has allowed clinicians to further understand the pathophysiology of the lesions and ultimately the overall illness. The conventional MRI measures of disease burden include the quantification of brain hyperintense lesions on T2-weighted images, hypointense lesions on T1-weighted lesions, and the post-gadolinium-enhancing T1-weighted lesions and unconventional measures such as brain and spinal cord atrophy. Each of these markers has a neuropathologic correlate. T2-weighted cranial MRI lesions correlate well with the location on plaques in the CNS of postmortem MS patients (Newcombe et al., 1991). The lesions on MRI are often ovoid in shape and perpendicular to the ventricles, representing the perivenular location of demyelination (Bakshi et al., 2004). These lesions on T2-weighted images represent many possible histopathologic correlates, including demyelination, edema, axonal loss, and inflammation (Bakshi et al., 2004). Lesions on T2-weighted images are often clinically silent and correlate weakly with a patient's disability. This discrepancy between lesions on T2-weighted images and clinical status is labeled the clinical–imaging paradox (Bakshi et al., 2004). Hypointense lesions on T1-weighted images may be persistent or nonpersistent (Rovaris and Filippi, 1999). Persistent hypointense T1-weighted lesions represent areas of axonal loss and are labeled as "black holes," and their numbers have shown to correlate better than T2 lesion load with clinical severity of the disease (Rovaris and Filippi, 1999). Nonpersistent hypointense lesions on T1-weighted images that disappear represent reversible edema (Rovaris and Filippi, 1999). Postcontrast gadolinium enhancement of lesions on T1-weighted images represents acute disruption of the blood–brain barrier (BBB) from inflammation and lasts on average 3 weeks, but may range anywhere from 1 to 13 weeks (Bakshi et al., 2004). Brain and spinal cord atrophy may occur in MS and represents axonal loss. Brain and spinal cord atrophy has been shown to correlate with disability, and brain atrophy alone has also been correlated with cognition and depression (Bakshi et al., 2004). Other MRI techniques, such as magnetic resonance spectroscopy and magnetization transfer imaging have been used to study the brains of patients with MS; however, at present, given the lack of standardization, they are not being applied widely in the clinical setting.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

CSF findings can be used as immunologic markers that serve as adjuncts to clinical findings when considering the diagnosis of MS. The CSF of patients with MS typically shows normal glucose, a few lymphocytes (mostly T cells), normal to mildly elevated total protein, and oligoclonal immunoglobulin bands (OCBs).

When CSF from healthy controls is electrophoresed, the cathode region demonstrates a homogeneous blur of immunoglobulin. When CSF from MS patients is electrophoresed, the cathode region reveals a number of discrete bands that represent excess antibody production by one or more clones of B-cells. Often absent early in the disease, OCBs can eventually be detected in over 90% of patients with MS (McLean et al., 1990). CSF OCBs have also been described in conditions such as subacute sclerosing panencephalomyelitis (SSPE) (Mattson et al., 1980), neurosyphilis (Pedersen et al., 1982), varicella zoster virus infection (VZV) (Vartdal et al., 1982), human immunodeficiency virus (HIV) infection (Skotzek et al., 1988), and in multisystem autoimmune diseases, cerebrovascular accidents, and up to 5% of normal individuals. In diseases such as the viral encephalitides, OCBs commonly seem to bind virus determinants, in contrast to MS, where the antigen against which the majority of bands are directed has not been identified (Olek et al., 2000).

When CSF is examined for albumin, 20–30% of MS patients have an elevated level (Olek et al., 2000). Albumin is not made in the CSF, and thus when elevated implies that there is BBB disruption. Due to intrathecal synthesis from plasma cells, CSF immunoglobulin levels are also elevated in patients with MS. The immunoglobulins are mainly composed of IgG, with lesser amounts being IgM and IgA. Nearly 90% of patients demonstrate elevated levels of CSF IgG production when calculating the IgG index formula [(spinal fluid IgG/spinal fluid albumin)/(serum IgG/serum albumin)]. A spinal fluid IgG index >0.58 implies that IgG is being synthesized in the CNS.

The role of serum antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) is not clear in MS. While there are many reports of serum and CSF anti-MBP and anti-MOG autoantibodies (Berger et al., 2003), they appear to represent predominantly low-affinity, nonspecific binding. However, using sensitive solution-phase assays, high-affinity autoantibodies to MBP and MOG can be detected in the serum and CSF of patients with acute disseminated encephalomyelitis (O'Connor et al., 2003). Moreover, high-affinity anti-MOG autoantibodies can be eluted from the brain tissue of a subset of patients with MS (unpublished data).

More recently, a serum autoantibody, neuromyelitis optica immunoglobulin (NMO-IgG), was identified in patients with neuromyelitis optica, an inflammatory demyelinating disease similar to MS, affecting the optic nerves and spinal cord. The NMO-IgG was found to have a sensitivity of 73% and a specificity of 91% among patients with demyelinating conditions affecting the spinal cord and optic nerves, and was determined to be useful in differentiating this disease from MS (Lennon et al., 2004).

PATHOLOGY

Gross examination of MS brain tissue reveals multiple sharply demarcated gray plaques in the CNS white matter with a predilection to the optic nerves and white matter tracts of the periventricular regions, brain stem, and spinal cord (Figure 46.1). The gray matter contains less myelin, and thus lesions in the gray matter are less conspicuous on gross examination; however, it too is affected by MS lesions (Geurts et al., 2005). When examining the histologic features of a MS lesion there exist three major components: inflammation, demyelination, and gliosis. The inflammation in lesions is composed of lymphocytes, monocytes, and macrophages, whose proportions depend on the activity and age of the lesion. Demyelination is also an important feature of the MS lesion, as evident by reduced myelin. Although MS is described as a disease causing a loss of myelin, the notion of axonal loss is becoming more important. Trapp et al. (1998) reported that substantial axonal injuries with axonal transections are also abundant throughout active MS lesions, even in patients with early stages of the disease process. The axonal loss is evident by the presence of terminal axonal swellings on confocal microscopy, resulting from the "build up of axonally transported

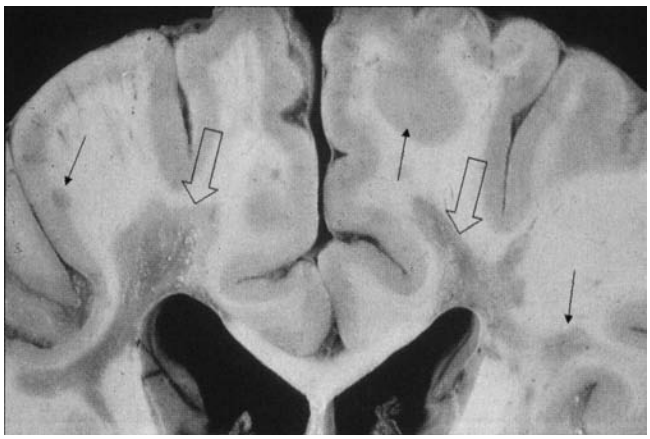


FIGURE 46.1 Coronal slice of cerebral hemispheres from a patient with multiple sclerosis. Note the extensive areas of demyelination in the periventricular regions of the lateral ventricles (open arrows) and in the subcortical white matter (small arrows). There is evidence of an increased size of the lateral ventricles as well.

Reproduced from Rudick (2004), with permission.

material collecting at the proximal end of a transected axon" (Trapp et al., 1998). The third component of a lesion is seen when reactive astrocytes and fibrillary gliosis are present in the lesion.

MS lesions can also be classified from a pathogenesis point of view into three types based on the age of the lesion: active, chronic active, and chronic inactive (Lassman, 1998). Macrophages are most prominent in the center of the *active plaques* and are seen to contain myelin debris, while oligodendrocyte counts are reduced. Lymphocytes may be found in normal appearing white matter beyond the margin of active demyelination (Prineas and Wright, 1978). The inflammatory cell profile of active lesions is also characterized by perivascular infiltration of oligoclonal T cells (Oksenberg et al., 1990; 1993; Wucherpfennig et al., 1992a), consisting of $CD4^+$ cells invading the normal appearing white matter around the lesion and $CD8^+$ T cells in the plaque margins and perivascular cuffs (Figure 46.2) (Traugott et al., 1983; Hauser et al., 1986). The inflammatory infiltrate may also include monocytes, occasional B cells, and infrequent plasma cells (Wucherpfennig et al., 1992b). In *chronic active lesions*, inflammation composed of macrophages and activated microglia are found at the border of the lesion and disappear from the center core, suggesting the presence of ongoing inflammatory activity along the lesion edge. The *chronic inactive plaque* does not have the macrophages at the border or center of the plaque. There is no ongoing demyelination and the histology demonstrates demyelinated axons with fibrillary gliosis (Lassman, 1998).

Four pathologic categories of the lesions have been described related to potentially different immunopathophysiologic disease mechanisms, although this has yet to be demonstrated at a molecular level (Lucchinetti et al., 1996): 1) T-cell- and macrophage-mediated demyelination; 2) T-cell- and macrophage-mediated, plus antibody-induced or complement-mediated, demyelination; 3) oligodendrocyte dystrophy and apoptosis with myelin protein dysregulation; and 4) primary oligodendrocyte degeneration with features similar to viral, ischemic or toxic oligodendrocyte damage. Patterns 1 and 2 are seen in acute, early active multiple sclerosis, with an intense perivenous immune reaction causing a sharply-demarcated area of demyelination and destruction of oligodendroglia, astrocytes, and axons. There is preservation of oligodendrocytes and significant remyelination (shadow plaques), and less expression of multiple myelin proteins, without pattern 1 or with pattern 2 deposition of activated complement and IgG. Pattern 3 consists of a cellular infiltrate of macrophages, microglia, and T cells, no immunoglobulin, ill-defined, nonperivenous areas of demyelination (preservation of oligodendroglia near venules, oligodendroglial destruction, loss, and apoptosis), and a marked fall in myelin-associated glycoprotein (MAG) compared to other myelin proteins. Pattern 4 consists of an inflammatory perivenous plaque with a sharp border of destruction and apoptotic loss of oligodendroglia with little remyelination. This rare pattern is exclusively seen in some patients with PPMS. It was of interest that the pattern of pathology tended to be the same in multiple lesions from any single individual with MS. These different pathologies may explain why some patients with MS have a heterogenous response to the same therapies.

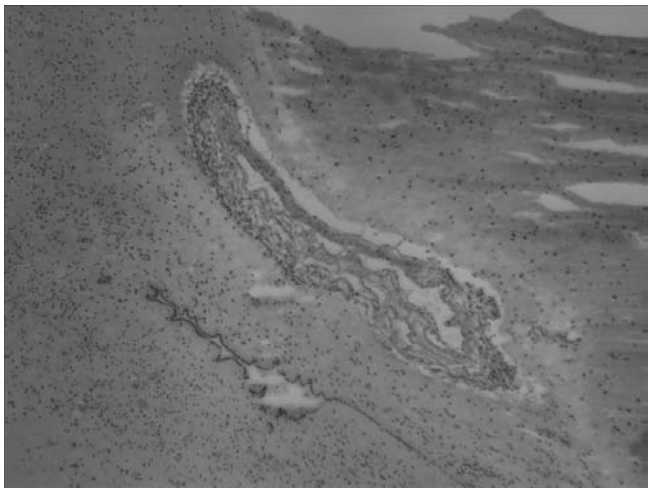


FIGURE 46.2 Inflammatory cells composed of lymphocytes and monocytes are present in the perivascular cuff of a white matter lesion (specific cell type staining not shown). Hematoxylin-eosin × 4. Histopathology provided courtesy of Gerald P. Bailey, M.D., Ph.D. and Kevin C. O'Connor, Ph.D., Harvard Medical School, Boston, MA.

EPIDEMIOLOGY

The prevalence rates of MS in North America range between 30 and 150 in 100,000. Based on a weighted mean of several studies, the average annual incidence of MS in the US is 3.2 in 100,000 per year. The median age of onset of symptoms is 23–24 years of age, with a peak age of onset for women in the early 20s, and for men in the late 30s (Schumacher et al., 1965; Studney et al., 1993; Paty et al., 1994). As in most diseases classified as autoimmune, there is a clear female predominance in MS cases (3 : 2) (Olek et al., 2000).

Evidence exists that the genetic make-up of the individual and certain unspecified environmental exposures may each play a role in making someone susceptible to MS. Studies in twins (Mackay and Myrianthopoulos, 1966; Williams et al., 1980; Heltberg and Holm., 1982; Ebers et al., 1986; Kinnunen et al. 1987; French Research group on MS, 1992; Mumford et al., 1992), which are discussed below, demonstrate shared genetic risk factors for MS. At the same time, studies of MS incidence rates in migrants (Alter et al., 1966) and apparent epidemics of MS at

geographic locations (Haines et al., 1996), also discussed below, indicate a clear role for environmental factors.

Genetic Factors

Approximately 15–20% of patients have a family history of MS, but large extended pedigrees are uncommon, with most MS families having no more than two or three affected individuals. Detailed population-based studies of familial recurrence risk (Sawcer et al., 1996) have provided estimates for familial clustering with $\lambda_{s,}$ the ratio of the risk of disease in the siblings of an affected individual compared with the general population, of approximately 15 (Margolis and Graves, 1945; Holmes et al., 1967; Shakir et al., 1983; Bias et al., 1986; Trostle et al., 1986; Sadovnik et al., 1997; Sawcer et al., 1997). In studies of twins, the monozygotic concordance rate is 30% versus the dizygotic rate of 5% (Sadovnick et al., 1993). When both parents are affected with MS, 9% of the children develop the disease (Hogancamp et al., 1997). To date, the only confirmed genetic feature to emerge from these efforts is the association and linkage of the disease with alleles and haplotypes from the major histocompatibility complex (MHC) on chromosome 6p21. It has become clear that MS represents a complex genetic disease with no clear mode of inheritance.

An alternative hypothesis emerging from the linkage studies is that MS, as a common disease, is caused by common allelic variants, each with only subtle but important variations on function. Crude theoretical modeling of human population history suggests that variants that have a high population frequency as a whole, and are likely to be responsible for complex traits (the common disease–common variant hypothesis), will generally be very old and therefore accompanied by rather little linkage dysequilibrium (Daly et al., 2001). Quantitatively, this may translate to dozens of gene regions each with risk factors of less then ×1.1–×1.4, but which in concert lead to major risk for disease development. It may be postulated that as populations emerged out of Africa between 60,000 and 100,000 years ago, exposure to new microbes resulted in what are thought to be major population bottlenecks with survival of individuals with allelic variants allowing for resistance to the novel infectious event. These combinations of different genes providing resistance to the population, when randomly coming together, result in a hyper-responsive immune system with subsequent autoimmune diseases; the price an individual may pay for the protection of the general population. Organ specificity may have emerged because each infectious agent involved with a population bottleneck would select for a single MHC-restricting element and subsequent antigen specificity. As MS is a complex genetic disease, understanding which combinations of genes provide the multitude of perhaps relatively minor risk factors, which in the population as a whole provide protection from microbial disease but together, in unfortunate random combinations, result in human autoimmune disease, is a central goal of present genetic research efforts.

Environmental Factors

Global maps of MS prevalence rates, constructed based on multiple descriptive epidemiologic studies, reveal a nonrandom geographic distribution of the disease. A diminishing North to South gradient of MS prevalence was described in the Northern hemisphere (Beebe et al., 1967; Kurtzke, 1977; Kurtzke et al., 1979; Hammond et al., 1987), with an opposite trend identified in the Southern hemisphere (Hammond et al., 1988; Hogancamp et al., 1997). This distribution likely reflects a combination of genetic and environmental influences. For example, in both Europe and the US, the prevalence of MS may reflect the degree of Scandinavian and Northern European heritage in resident populations (Hammond et al., 1988). Nonetheless, the several-fold difference in the South–North prevalence in Australia, across a genetically homogeneous population, argues for a nongenetic effect (Armon et al., 1991). The identity of such a latitude-based risk factor remains elusive (Lauer, 1995; Weinshenker, 1996; Bar-Or and Smith, 2000). Environmental deficiencies have also been known to be associated with MS, as seen by the inverse correlation of the world prevalence of MS and the environmental supply of vitamin D via sunlight exposure or dietary intake of vitamin D3 (VanAmerongen et al., 2004). At higher latitudes, the lower exposure to the sun in the winter months is insufficient to produce vitamin D given the lower exposure to the sun in winter months (VanAmerongen et al., 2004). Vitamin D is known to have some anti-inflammatory effect based on the reduction of EAE incidence and severity in animal models given the active vitamin D metabolite. Subtle defects in vitamin D metabolism by "polymorphisms in the vitamin D receptor gene could be a link in determining susceptibility to MS" (VanAmerongen et al., 2004).

Migration studies in MS have suggested that migrants from high- to low-risk areas show a decreased rate of MS (Alter et al., 1966; Kurtzke et al., 1970; Dean and Kurtzke, 1971; Gale and Martyn, 1995; Lauer, 1995). Furthermore, the age at emigration appears to influence whether the migrants acquire the risk of the new environment or retain the risk of their home country. While such comparison studies are fraught with difficulties because of differences in study design (Stazio et al., 1964; Sweeney et al., 1986; Lauer, 1995), they do suggest that some environmental exposure during a particular window of time (likely in early adolescence) may contribute to the risk of developing MS. These observations, together with studies of proposed "MS epidemics" (Lauer, 1995), have contributed to the hypothesis that MS has an infectious etiology. Despite multiple

candidate organisms, to date no infectious agent has been established as the cause of MS.

The "hygiene hypothesis" as a factor in explaining the environmental risks associated with MS has been raised by a recent study examining the correlation of exposures to infant siblings in early life and the risk of acquiring MS (Ponsonby et al., 2005). The "hygiene hypothesis" states that children who are exposed to infections early in life are less susceptible to autoimmune disorders and allergies as infections may alter T-cell receptor (TCR) diversity, B-cell maturation, and increasing IgG synthesis with re-exposures. Children growing up without exposures to siblings show increases in type 2 and type 1 helper T-cell (Th2 and Th1) immune disorders. A "mature" immune system from an early age may prevent infectious triggers in later years from causing an immunologic response that can potentially lead to an autoimmune disease such as MS. The risk of MS was reduced when sibling exposure was present in the first 6 years of life, and it was hypothesized that this related to changes in immune response and childhood infection patterns, which may lead to a reduction in MS risk (Ponsonby et al., 2005).

ANIMAL MODELS

Experimental autoimmune encephalomyelitis (EAE) is an immune-mediated disease of the CNS that is characterized by multifocal demyelinating plaques and perivascular inflammatory infiltrates, primarily comprised of T cells and monocytes, and associated with a paralytic illness. These pathologic and clinical features provide the impetus for studying EAE as a model of MS. EAE is mediated by $CD4^+$ T cells that recognize a variety of CNS autoantigens and secrete pro-inflammatory Th1-associated cytokines, such as IFN-γ and tumor necrosis factor (TNF)-α. It can be induced in several species of animals by injection of adjuvant with homogenized whole myelin or myelin components, such as MBP, proteolipid protein (PLP) or MOG. The ability to induce EAE by passive transfer of $CD4^+$ MHC class II-restricted T cells that are reactive with these antigens (Ben-Nun et al., 1981; Mokhtarian et al., 1984; Fritz et al., 1985; Zamvil et al., 1985) but not by serum from affected littermates, establishes EAE as a T-cell-mediated disease. Susceptibility to EAE and disease severity is dictated by different infectious exposures and the MHC background of the animal. The latter influences the T-cell repertoire, as well as which portions of the myelin proteins are encephalitogenic in any given strain.

Susceptibility to EAE is dependent on the strain of the animal (Baker et al., 1995; Sundvall et al., 1995; Encinas and Kuchroo, 1999). For example, mice such as the B10.Pl, SJL/J, and PL/J are susceptible, while BALB/c and C57BL/6 are relatively resistant. Disease severity and course are also influenced by the genetic background and may range broadly from a mild, uniphasic illness, through to aggressive relapsing–remitting and progressive disease patterns. Thus, there exist a variety of EAE models, each of which represents a distinct aspect of human MS. It is now recognized that the development of EAE and the pattern of EAE pathology are dictated by the coordinated expression of a number of genes that are involved in the activation and effector functions of inflammatory cells. The expression of these genes, in turn, is modulated by the animal's environment, such that genetically identical animals with different infectious exposures may manifest distinct profiles of EAE induction and phenotype. As mentioned above, an important component of susceptibility to EAE relates to the MHC background of the animal, which influences the T-cell repertoire as well as which portions of the myelin proteins are encephalitogenic in any given strain (Fritz and McFarlin, 1989). Genes that encode for costimulatory molecules, cytokines, chemokines, and adhesion molecules have been the focus of intense study. Molecular genetic techniques have enabled the dissection of the effects on disease phenotype, of under- ("knock-out") and over-expression ("transgenic") of an ever growing array of immune-related molecules.

The limitation of the EAE model is that a single inbred strain of animals may reflect single patients, and the wide range of genetic diversity and broad environmental exposure of highly outbred humans is clearly not well modeled by EAE. For example, the anti–TNF-α receptor antibody ameliorates EAE but worsens disease in patients with MS (Andersson et al., 1997).

Another use of the EAE model relates to the therapeutic application of altered peptide ligands (APLs). In the animal model, a single amino acid substitution within the peptide sequence of an encephalitogenic myelin epitope resulted in an APL that induced TCR antagonism, partial agonism, and anergy when used to stimulate T-cell clones generated against the original encephalitogenic antigen. Treatment of EAE with the APL induced a myelin-reactive T-cell repertoire that was shifted with respect to its cytokine responses from an inflammatory (Th1) towards an anti-inflammatory (Th2) profile, and was associated with improved disease (Karin et al., 1994; Nicholson et al., 1995). Based on these results, an APL of the immunodominant MBP epitope was used in a trial of MS (Nicholson et al., 1995; Windhagen et al., 1995a; Brocke et al., 1996; Ausubel et al., 1997; 1999; Bielkova et al., 2000; Kappos et al., 2000). Unfortunately, clinical deterioration and a dramatic increase in new gadolinium-enhancing lesion formation was seen in 3 of the 10 patients receiving the APL in the non-placebo-controlled trial (Bielekova et al., 2000). In contrast, Kappos et al. (2000), reporting results using the same APL in a placebo-controlled double-blinded randomized trial, found no increase in the rate of exacerbation in patients given APL

compared to those given placebo. Despite the trial ending early because of hypersensitivity reactions, Kappos et al. reported that there was evidence of improvement in lesions on MRI at the lowest 5-mg dose. They noted a regulatory Th2 cell response to altered peptide ligand that cross-reacted with the native peptide. Antagonists of the very late antigen-4 (VLA-4) were very effective in treating many inflammatory animal models, including EAE (Yednock et al., 1992; Lin and Castro, 1998). In preliminary trials the humanized anti-α4 antibody was shown to be effective at reducing new gadolinium-enhancing lesions and relapses in MS patients, but was later pulled from the market by the manufacturer after patients were diagnosed with progressive multifocal encephalopathy.

Newer experimental techniques involving EAE and the use of *antigen-specific tolerance* have emerged, including the use of active induction of tolerance by DNA vaccination. There have been mixed results in these EAE models. Animal models of EAE that were injected with DNA coding for MBP or PLP suppressed EAE but vaccination with MOG DNA worsened the EAE (Hohlfeld and Wekerle, 2004). The DNA vaccine may also be used to co-deliver genes for cytokines and the DNA of the autoantigens, which can change the clinical outcome of EAE. Garren et al. (2001) used the vaccine combination of the DNA-encoding PLP and the IL-4 gene, which helped to prevent EAE by shifting the local cytokine profile from the Th1 to the Th2 pathway. This same vaccine combination was also shown to reverse established EAE.

PATHOGENIC MECHANISMS

Current understanding of MS immunopathology is that autoreactive proinflammatory T cells are critical to the propagation of CNS tissue injury. It appears likely that when a genetically susceptible host immune system encounters a common environmental antigen (such as an infectious organism), a process of "molecular mimicry" results in the peripheral activation of cross-reactive T cells that can migrate to the CNS and mount pro-inflammatory responses to myelin epitopes. However, the mere presence of autoreactive cells in the periphery is an insufficient explanation for the development of autoimmune disease, given that myelin-reactive T cells can be found in the peripheral blood of both normal individuals and patients with MS.

Studies demonstrate that MBP-reactive T cells in MS patients are in an enhanced state of activation in the periphery of those with MS and have less stringent stimulation requirements compared to those isolated from normal individuals, suggesting a breach in peripheral tolerance. Zhang et al. (1994) found that the IL-2 receptor, a hallmark of activated T cells, was expressed on MBP-reactive T cells from MS patients but not on those obtained from normal individuals. The T-cell costimulatory pathway is represented by the engagement of CD28 on T cells by the CD80 or CD86 molecules, typically expressed on the surface of activated antigen-presenting cells (APCs). Reports have demonstrated that MBP-reactive T cells from the peripheral blood of MS patients are also less dependent upon CD80 (B7) costimulation for their activation, compared to MBP-reactive T cells from normal individuals (Williams et al., 1994; Windhagen et al., 1995b). Enhanced expression of CD80 was detected on B cells in peripheral blood, in the CSF, and in CNS plaques of MS patients compared to controls, while levels of CD86 expression were the same. The increased expression of CD80 appeared to correlate with disease activity and, in one study, treatment response to IFN-β-1b was associated with normalization of the levels of CD80 expression on the B cells of patients (Figure 46.3) (Gene et al., 1997).

Upon interaction of the T cell with the APC, the antigen-specific T cells proliferate and divide into two subsets, classified according to the cytokine profiles that they produce upon activation (Abbas et al., 1996; O'Garra, 1998). MHC class II-restricted $CD4^+$ T cells, producing IFN-γ, IL-2, lymphotoxin, and TNF-α, have been termed Th1 (inflammatory) cells and have been clearly shown to be pathogenic in most EAE models (Leonard et al., 1995; Segal et al., 1998). The Th1 cytokines activate macrophages for cellular immunity. In contrast, $CD4^+$ T cells producing IL-4, IL-5, IL-10 or IL-13 have been termed Th2 (anti-inflammatory) cells and have a protective role in EAE (Khoury et al., 1992; Owens et al., 1994; Begolka et al., 1998; Antel and Owens, 1999). TGF-β-producing cells have been termed Th3 cells and are also protective in EAE. Th2 cytokines promote humoral immune responses. Although Th2 cells are defined as anti-inflammatory cells, this is in the context of EAE.

Several lines of evidence support the hypothesis that Th1 cells may be pathogenic in MS. Th1 and Th2 cells express distinct profiles of chemokine receptors. An increased proportion of T cells from MS patients were shown to express the characteristic Th1 chemokine receptor pattern and MS plaques were found to express increased levels of the corresponding chemokine (Siveke and Hamann, 1998; Balashov et al., 1999; Sorensen et al., 1999). Analysis of cytokine mRNA in the CSF from MS patients showed a bias towards Th1 cytokines (Blain et al., 1994). Immunohistochemical studies of MS plaques *in situ* have demonstrated the presence of the proinflammatory cytokines TNF-α and IL-12 (Hofman et al., 1989; Selamj et al., 1991; Windhagen et al., 1995b). Interventions that shift or deviate the cytokine responses away from a Th1 and towards a Th2 profile have been an exploitable drug targeting route for MS.

Selective expression of adhesion molecules, chemokines and chemokine receptors, and matrix metalloproteinases (MMPs) has also been demonstrated on inflammatory cells, endothelial cells, and glial cells in samples from patients with MS. These molecules are likely to be important in

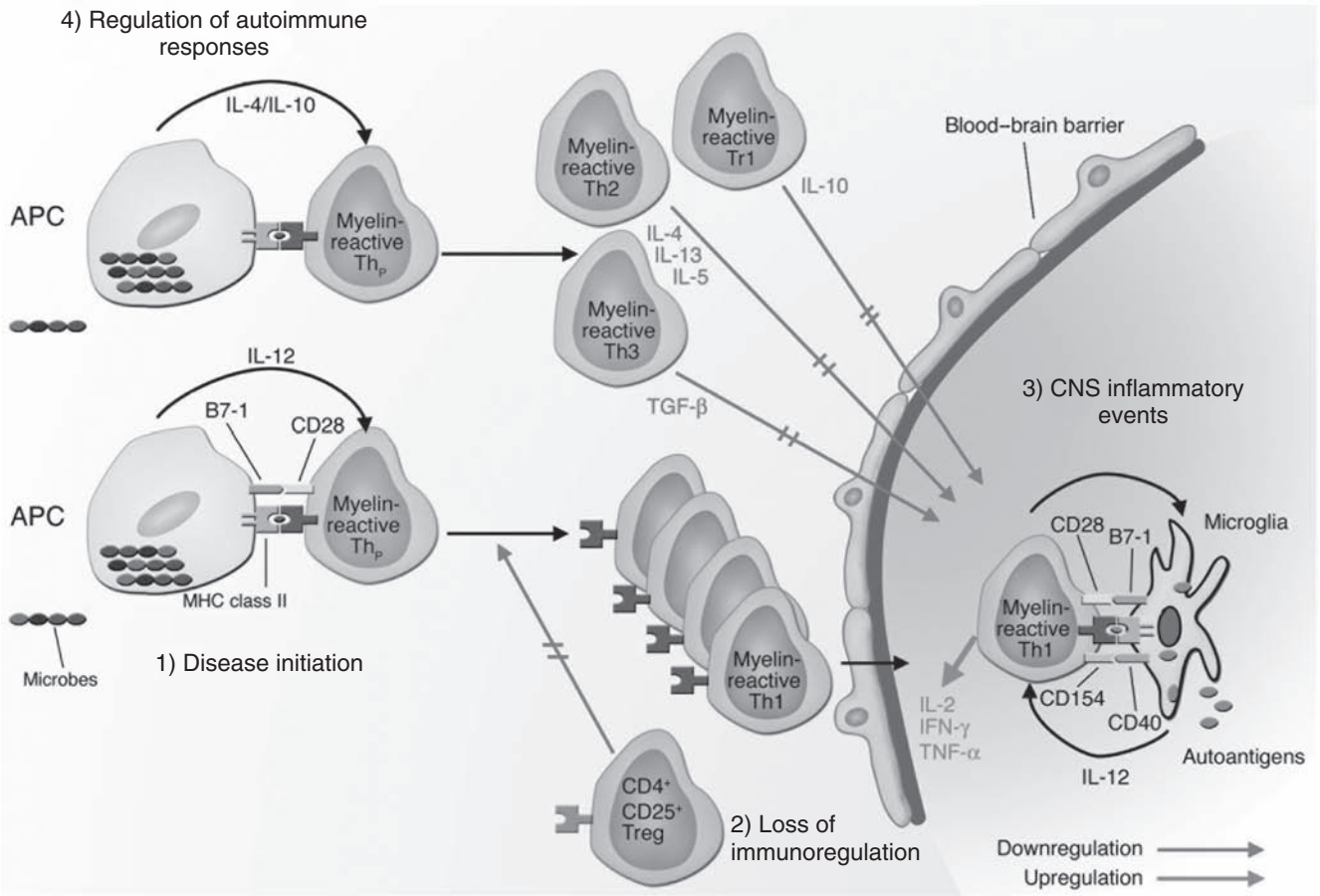


FIGURE 46.3 Presumed pathophysiology of multiple sclerosis (MS). Four main components contribute to the pathophysiology of MS. 1) *Initiation*, a presumed microbe may be taken up by an antigen-presenting cell (APC) and is presented to a myelin-reactive precursor T cell (Th_p). 2) *Loss of immunoregulation*, $CD4^+CD25^+$ regulatory T-cells (Tregs) are thought to be reduced in MS patients, which causes a decrease in the overall autoregulation of the autoimmune process. 3) *Central nervous system (CNS) inflammatory events*, in the CNS, local APCs, microglia, present antigen to myelin-reactive type 1 helper T (Th1) cells that secrete Th1 cytokines such as interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which permit the inflammation to continue. 4) *Regulation of the autoimmune responses*, three cell types enter the CNS to counterbalance the pro-inflammatory Th1 cells, which are part of the naturally-occurring regulatory mechanisms that bring the inflammatory cascade into check by counterbalancing the Th1-mediated proinflammatory response. The three cell types are the Th3 cells which secrete transforming growth factor (TGF)-β; Th2 cells which secrete Th2-mediated cytokines, including IL-4, IL-13, and IL-5; and regulatory T cells (Tr1), which secrete IL-10. See color plate section.

Reproduced from Hafler (2004), with permission conveyed through Copyright Clearence Center, Inc.

mediating the transmigration of effector cells across the BBB and into the CNS perivascular tissue. Invading autoreactive T-cells can then become reactivated upon encounter with their antigen in the CNS. The effector profile of such cells is likely to depend on several factors in the microenvironment, including the cytokine milieu and the costimulatory profile of local and infiltrating APCs. Further release of local cytokines, chemokines, and MMPs may support the recruitment of subsequent waves of infiltrating effector cells, including T cells, monocytes, and B cells. Mechanisms of myelin destruction and axonal damage are likely to be multiple and include direct effects of pro-inflammatory cytokines, oxygen radicals, and complement-fixing antibodies; antigen-specific and -nonspecific cytotoxicity; and apoptosis. Local damage results in exposure of additional myelin components that may become primary targets of subsequent waves of infiltrating cells, leading to the phenomenon of "epitope spreading." Myelin-producing oligodendrocytes scarcely produce MHC class II molecules, and thus cannot present their own autoantigens to T cells. The autoantigens must be transferred to nearby microglia or macrophages and are then taken up, processed, and presented by these APCs. Activation of resident CNS glial cells, such as microglia, may provide the basis for the generation

or maintenance of pathologic responses, even in the absence of further infiltration of exogenous inflammatory cells. In contrast, evidence that glial elements may play a role in the repair and recovery from myelin injury may lead to the development of novel therapeutic approaches in MS, and underscores the importance of elucidating the complexities of glial–immune interactions.

TREATMENT

Therapeutic approaches in MS may be broadly divided into treatments that are symptomatic and/or supportive in nature, and treatments that are directed at the underlying pathophysiology of the disorder. Effective medical management of spasticity, bladder symptoms, dysesthesias, fatigue, and depression may significantly improve daily functioning and enhance quality of life. Ambulatory aids, patient education, appropriate use of physical and occupational therapy services, and psychological and social supports should all be elements of the comprehensive management plan.

Over the past 6 years, the US FDA has approved six agents (subcutaneous IFN-β-1b, Betaferon/Betaseron®; intramuscular IFN-β-1a, Avonex®; subcutaneous IFN-β-1a, Rebif®; glatiramer acetate, Copaxone®; mitoxantrone, Novantrone®; and natalizumab, Tysabri®) as disease-modifying therapies that have been shown to alter the course of MS (Table 46.1). They are all expensive and only partially effective on average, yet their current use has marked a milestone for both patient care and the overall understanding of the pathogenesis of MS given that this disease was until recently considered to be untreatable.

The differences in study design and outcome measure definitions make direct efficacy comparisons between the approved therapies problematic.

Interferons

Compared to placebo-treated RRMS controls, treatment with alternate-day subcutaneous injections of 8 MU of IFN-β-1b (Betaseron) was shown to decrease the primary efficacy outcome measure of frequency of relapses by 34% after

TABLE 46.1 FDA-approved therapies for multiple sclerosis

Therapy	Brand name	Indications	Results	Mechanism of action
IFN-β-1a (IM, weekly)	Avonex®	Treatment of RRMS	33% reduction in relapses Reduction of new MRI T2-weighted lesions and the volume of enlarging T2-weighted lesions Reduction in the number and volume of gadolinium-enhancing lesions Slowing of brain atrophy	Acts on blood–brain barrier by interfering with T-cell adhesion to the endothelium by binding VLA-4 on T cells or by inhibiting T-cell expression of MMP Reduction in T-cell activation by interfering with HLA class II and costimulatory molecules B7/CD28 and CD40/CD40L Immune deviation of Th2 over Th1 cytokine profile
IFN-β-1a (SC, three times/week)	Rebiff®	Treatment of RRMS	Same as IFN-β-1a above	Same as IFN-β-1a above
IFN-β-1b (SC, every other day)	Betaseron®	Treatment of RRMS	Same as IFN-β-1a above	Same as IFN-β-1a above
Glatiramer acetate (SC, daily)	Copaxone®	Treatment of RRMS	33% reduction in relapses Reduction in the number and volume of gadolinium-enhancing lesions	Induces Th2 cells that enter the CNS and mediates bystander suppression at sites of inflammation
Mitoxantrone (IV infusions every 3 months)	Novantrone®	Worsening forms of RRMS SPMS	67% reduction in relapses Slowed progression in EDSS, ambulation index, and MRI disease activity	Antineoplastic that intercalates DNA Suppresses cellular and humoral immune response
Natalizumab (IV, monthly injections)	Tysabri®	Relapsing MS	Reduced rate of relapse (in up to 66%) and reduced development of new MRI lesions	Monoclonal antibody that blocks α4-integrin on surface of T cells, preventing them from crossing the blood–brain barrier

EDSS, Expanded Disability Status Scale; MMP, metalloproteinase; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

2 years (The IFNB Multiple Sclerosis Study Group, 1993). A significant decrease in the accumulation of MRI lesions was observed with treatment (The IFNB Multiple Sclerosis Study Group, 1993), although no beneficial effect on clinical disability was seen over the study period. More recent 5-year follow-up data reported that disease progression in the IFN-β-1b treated group was 35%, compared with 46% progression in the placebo group (The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group, 1995). A 30% decrease in the annual exacerbation rate in the treated group was maintained and MRI data showed significantly less accumulation of lesion burden in the IFN-β-1b-treated group (3.6%) compared to the placebo-treated patients (30.2%) over 5 years.

IFN-β-1a (Avonex, weekly intramuscular injections), a glycosylated recombinant IFN-β, was evaluated in a 2-year study of weekly intramuscular injections of 6 MU (30 μg). The primary efficacy outcome measure was timed to the onset of a sustained deterioration in disability. The proportion of patients progressing by the end of the trial was 21.9% in the treated group compared to 34.9% in the placebo group. The annual exacerbation rate was decreased by 32% in the treated group versus the placebo group. Treatment was also associated with a 40% reduction in mean MRI lesion load (Jacobs et al., 1995; 1996). The mean number of new enhancing lesions on MRI over the 2 years was 0.80 in the IFN-β-1a-treated group versus 1.65 in the placebo. In the PRISMS trial, a double-blinded placebo-controlled, phase III trial of INF-β-1a (Rebif, subcutaneous, three times weekly) in RRMS, treatment was associated with significant improvement in MRI parameters, which extended the prior evidence of benefit on relapse rate and disease progression (Li and Paty, 1999).

Common side effects of the IFN-βs include flu-like symptoms and injection site reactions (rarely necrosis with IFN-β-1b). Headache and depression are less common and, rarely, hepatotoxicity or bone marrow suppression may occur. Neutralizing antibodies (NABs) have been reported for both IFN-βs and remain a contentious issue. Some reduction in the biologic effect and clinical benefit of IFN-β in NAB^+ patients has been suggested (Cohen et al., 1999). The relative antigenicity of IFN-β-1b (alternate-day subcutaneous administration) may be greater than that of IFN-β-1a (weekly intramuscular administration); however, a prospective comparison has not been made. The effect of dose on the generation of NABs is also not established.

As a class, the IFN-βs appear to exert their effects on the pathophysiology of MS at several sites. Converging studies have suggested that they inhibit the migration of activated inflammatory cells across the BBB and into the CNS parenchyma. The IFN-βs may exert this effect at the level of adhesion molecules and/or MMPs. As noted above, the adhesion molecule VCAM-1 has been identified on both microglia and endothelial cells in chronic–active MS lesions, and its ligand, VLA-4, has been identified on the perivascular inflammatory cells of MS lesions. Treatment with IFN-β-1b was associated with decreased levels of VLA-4 on inflammatory cells in MS patients, which correlated with a decrease in the MRI lesion burden (Calabresi et al., 1997; Trojano et al., 1997). Interferon-β-1b has also been shown to suppress T-cell expression of MMPs and inhibit T-cell migration across the BBB (Stone et al., 1995; Leppert et al., 1996). The rapid effect of IFN-βs on the gadolinium-enhancing lesions of MS (Stuve et al., 1996; Stone et al., 1997) represents a promising biologic marker of treatment response and establishes the BBB as an important site of action of IFN-β therapy. A possible role for IFN-β treatment in shifting cytokines in MS from a pro-inflammatory profile to an anti-inflammatory profile is suggested by reports that treatment may decrease levels of TNF-α while enhancing levels of IL-6 (Brod et al., 1996) and of immunosuppressive cytokines (Rudick et al., 1998). Finally, by decreasing the molecules needed for antigen presentation, IFN-β leads to a decreased generation of autoreactive T cells, which limits T-cell activation. The increased levels of the costimulatory molecule CD80, reported on mononuclear cells in MS blood and CSF, are reversed in response to treatment with IFN-β-1a (Genc et al., 1997).

Glatiramer Acetate

Treatment with glatiramer acetate/copolymer 1 (Copaxone, GA), a 20 mg/day subcutaneous injectable synthetic polymer, was associated with a 2-year relapse rate reduction (primary outcome measure) of 29% compared to placebo control (Johnson et al., 1995). A subsequent MRI study revealed a beneficial effect of GA on newly forming gadolinium-enhancing lesions (Mancardi et al., 1998). GA is relatively well tolerated with typically mild injection site reactions, infrequent urticarial responses, and an occasional stereotypical vasomotor reaction. Laboratory abnormalities have not been reported and neutralizing antibodies are not an issue.

The differences in the side effect profiles of the IFN-βs and GA suggest that these treatments have distinct mechanisms of action. GA is a random sequence polypeptide of the four amino acids alanine (A), lysine (K), glutamate (E), and tyrosine (Y). In contrast to the interferons, GA has little effect on the BBB. GA treatment appears to induce GA-reactive Th2-polarized T cells that are more degenerate in their antigen recognition (Duda et al., 2000). These activated Th2 T cells may enter the CNS, where they may mediate bystander suppression at sites of inflammation, leading to decreased disease activity and the observed efficacy of GA on both clinical and MRI parameters (Yong, 2002).

Overall, the approved immunomodulating agents provide a measurable, though only partial, benefit in the treatment of RRMS. The suggestion that the mode of action of

interferons differs from that of GA raises the possibility that together these therapies may provide additive benefit, and such a combination trial is underway.

Natalizumab

Natalizumab (Tysabri) is a monoclonal antibody to the VLA-4 expressed on activated T cells and monocytes. It prevents adhesion of activated T cells to endothelial cells. The FDA approved this drug for relapsing MS patients based on data from the first year of two ongoing trials studying RRMS patients. The AFFIRM study compared natalizumab alone to placebo and the SENTINEL study looked at adding natalizumab to ongoing IFN-β-1a therapy. The initial 1-year results demonstrated that monthly intravenous infusions reduced clinical relapses by 66% when compared to the placebo arm, and a significant decrease in gadolinium-enhancing lesions was seen. However, early in 2005, the manufacturers of the drug voluntarily removed it from the market following the death of patients, who had been on combination therapy for 2 years, from progressive multifocal leukoencephalopathy; these patients had no history of HIV (National Multiple Sclerosis Society, 2005).

Other Therapies

Corticosteroids

Corticosteroids are used widely in the treatment of MS, although data supporting their use is sparse. In general, treatment of acute relapses with intravenous methylprednisolone may lead to more rapid improvement in symptoms but does not appear to change the ultimate outcome of the flare or the subsequent disease course. The mode of action of corticosteroids is not established. Relatively nonspecific suppression of T-cell responses is suspected, although differential effects on T-cell subsets have been reported. Steroids have been shown to reduce the activity of MMPs and MRI evidence of a decrease in gadolinium-enhancing lesions following treatment with steroids supports a possible therapeutic role at the BBB (Miller et al., 1992).

Cyclophosphamide

Cyclophosphamide is an alkylating agent with potent cytotoxic and immunosuppressive effects. A randomized, triple arm study demonstrated a positive effect in SPMS patients treated with a 2-week course of cyclophosphamide/ACTH as compared to patients who received ACTH alone (Hauser et al., 1983). These findings led to a randomized single-blind trial by the Northeast Cooperative Treatment Group that tested the efficacy of outpatient intravenous cyclophosphamide pulses every 2 months in 236 patients who had initially received an intravenous cyclophosphamide/ACTH induction (Weiner et al., 1993). Patients with disease for shorter durations had a more beneficial effect from cyclophosphamide treatment as compared to those not receiving the pulsed cyclophosphamide treatment.

Emerging Therapies

Daclizumab

Daclizumab is a humanized monoclonal antibody against CD25 that is expressed on activated T cells. CD25 serves as the IL-2 receptor on T cells, and when T cells are activated by IL-2, causes further T-cell expansion. Bielekova et al. (2004) reported on an open label phase II baseline to treatment trial of daclizumab in 10 RRMS patients with breakthrough disease on IFN-β therapy. Overall, there was a 78% decrease in new gadolinium-enhancing lesions on T1-weighted images and positive outcome with respect to safety and improvement in clinical outcome measures.

Statins

Statins are oral lipid-lowering agents that have immunomodulatory actions. They work by inhibiting MHC class II expression and costimulatory molecule expression and affect T-cell regulation. In a recent study of 30 patients with RRMS who received simvastatin, 80 mg/day per os for 6 months, the mean number of lesions decreased by 44% ($P = <0.0001$), and the total lesion volume decreased by 41% ($P = 0.0018$) compared to pretreatment MRI (Vollmer et al., 2004).

Bone Marrow Transplantation

Bone marrow transplantation (BMT) is currently being studied to treat severe MS. The host immune system is first ablated with chemotherapy or radiation therapy. Autologous stem cells are then infused to reconstitute a new healthy immune system to minimize the greater morbidity and mortality associated with allogenic transplantation. The objective of BMT is to stabilize patients suffering from active forms of disease (Fassas et al., 2002; Blanco et al., 2005). Autologous hematopoitic stem cell transplantation has been performed on 250 MS patients as part of open label phase I and phase II trials in which both clinical and MRI measures are followed. From a radiologic point of view, BMT causes a long-standing suppression of gadolinium-enhancing lesions on T1-weighted MRI scans up to 3 years in duration after the procedure. Only 50% of the patients had a 3-year activity-free course of MS (Yolanda et al., 2005). A preliminary summary of the results shows that BMT is most appropriate for patients with RRMS with an expanded disability status scale (EDSS) of <6 on entry, those with active disease,

and those who have failed conventional treatments (Yolanda et al., 2005). While better designed double-blind randomized trials might be indicated to examine the true effect of BMT on the progressive course of the disease, it is not clear if ultimately the benefit of this approach will ever outweigh the potentially severe side-effects associated with it.

Summary

It appears likely that immunomodulating and immunosuppressive therapies provide their greatest benefit early in the disease, during a period in which inflammatory responses are major contributors to tissue injury. As progressive disease sets in and irreversible axonal injury becomes the major process underlying clinical deterioration, the relative contribution of inflammation to ongoing injury diminishes and with it the role of anti-inflammatory therapies. This may explain why treatment of patients in the progressive phase of MS with immunomodulating therapies has been less successful.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Major strides have been made over the past years in our understanding of the immunopathogenic processes underlying the development and course of MS. Classical themes have been revisited with the application of modern techniques. The demonstration of distinct pathologic patterns in MS plaques suggests that several different mechanisms may contribute to tissue injury, and that the prevailing mechanism may not be the same across patients. In part, this may reflect the remarkable genetic diversity and broad range of environmental exposures that together define the human experience. Differences in pathogenic substrates may explain the observed heterogeneity of clinical disease course and severity, and the differential response to therapy. A more meaningful classification of MS that is based on defining the underlying process in individual patients may lead to more effective therapeutic approaches.

A variety of imaging modalities have combined with pathologic studies to highlight the substantial degree of axonal injury involved in both MS lesions and in normal appearing white matter. These abnormalities are detected early in the course of MS and may be better predictors of subsequent disability than clinical measures of disease activity. Indeed, neurologic disability in MS is likely to have at least two pathologic underpinnings: inflammatory demyelination and axonal transection. Episodic demyelination involving infiltration of inflammatory cells is the likely substrate of clinical flares. Recovery from these probably reflects resolution of local inflammation and edema in the short term. Axonal transection may remain largely silent for extended periods, due in part to the redundancy and functional compensation potential of CNS pathways. The process of axonal loss, however, is irreversible and beyond a certain threshold further degeneration translates into a slow progressive clinical decline (Bradley, 1998; Trapp et al., 1999a; 1999b). This underscores the importance of early diagnosis and intervention in MS.

While proven effective, current treatment approaches are only able to modestly impact disease progression. The development of more effective therapies will be predicated on advancing our understanding of the underlying disease process. A wealth of animal and human studies have contributed to a model of MS immunopathogenesis in which activated autoreactive T cells are likely to play an important role. By a process of molecular mimicry, exposure to an infectious agent may activate cross-reactive autoaggressive T cells that may then enter the CNS and mediate injury. Alternatively, direct insult to the CNS, such as by a neurotropic virus or trauma, may expose CNS epitopes in the setting of local inflammation, and trigger autoreactive T cells in a susceptible host. Migration of pro-inflammatory immune cells across the BBB in MS may reflect dysregulation of the complex molecular interactions involving adhesion molecules, chemokines, and MMPs. It remains to be determined whether the abnormalities in expression levels of these molecules associated with MS reflect a true pathogenic role or a mere epiphenomenon of the inflamed state. The microenvironment in which autoreactive T cells become activated, both in the periphery and in the CNS, is defined in part by the local profile of cytokines and costimulatory molecules, and influences the effector profile of the T cells, which may adopt an inflammatory or anti-inflammatory character. A pro-inflammatory environment is likely to promote "epitope spreading" and propagation of disease. In contrast, anti-inflammatory cells may mitigate the injurious responses by "bystander suppression." The roles of B cells and humoral responses, as well as the contributions of innate immunity, are receiving renewed attention. In some settings, antibodies have been implicated in augmenting or even inducing disease. In others, they may play a protective role. Studies into the neurobiology of MS, and in particular the roles of microglia and astrocytes, and the interactions of neural elements, endothelia, and inflammatory cells, have been revealing. Neural–immune interactions occur in the normal physiologic state and may represent important homeostatic mechanisms capable of promoting repair and regeneration. Indeed, these principles must be incorporated in any serious attempt to comprehensively understand MS.

Finally, ongoing studies into the mechanisms of action of the available immunomodulators are contributing to our understanding of the immunopathology and neurobiology of MS, and these insights will guide the development of the next generation of safe and effective therapies. In addition

to targeting inflammation, future approaches will undoubtedly explore neuroprotective strategies designed to prevent oligodendrocyte death and axonal transection, and complementary approaches that promote remyelination and recovery.

Acknowledgment

Dr Steven David Brass is supported by a postdoctoral fellowship from the National Multiple Sclerosis Society. We would like to thank Dr Kevin C. O'Connor for his assistance and advice with respect to the pathologic illustrations.

References

Abbas, A.K., Murphy, K.M., and Sher, A. (1996). Functional diversity of helper T lymphocytes. *Nature 383*, 787–793.

Achiron, A. and Barak, Y. (2000). Multiple sclerosis—from probable to definite diagnosis: a 7-year prospective study [see comments]. *Arch. Neurol. 57*, 974–979.

Alter, M., Leibowitz, U., and Speer, J. (1966). Risk of multiple sclerosis related to age of immigration to Israel. *Arch. Neurol. 15*, 234–237.

Andersson, P.B., Waubant, E., and Goodkin, D.E. (1997). How should we proceed with disease-modifying treatments for multiple sclerosis? [comment]. *Lancet 349*, 586–587.

Antel, J.P. and Owens, T. (1999). Immune regulation and CNS autoimmune disease. *J. Neuroimmunol. 100*, 181–189.

Armon, C., Daube, J., O'Brien, P., Kurland, L.T., and Mulder, D.W. (1991). When is an apparent excess of neurologic cases epidemiologically significant? *Neurology 41*, 1713–1718.

Arnason, B.G. (1993). Interferon beta in multiple sclerosis. *Neurology 43*, 641–643.

Ausubel, L.J., Krieger, J.I., and Hafler, D.A. (1997). Changes in cytokine secretion induced by altered peptide ligands of myelin basic protein peptide 85–99. *J. Immunol. 159*, 2502–2512.

Ausubel, L.J., Bieganowska, K.D., and Hafler, D.A. (1999). Cross-reactivity of T-cell clones specific for altered peptide ligands of myelin basic protein. *Cell. Immunol. 193*, 99–107.

Baker, D., Rosenwasser, O.A., O'Neill, J.K., and Turk, J.L. (1995). Genetic analysis of experimental allergic encephalomyelitis in mice. *J. Immunol. 155*, 4046–4051.

Bakshi, R., Hutton, G.J., Miller, J.R., and Radue, E.W. (2004). The use of magnetic resonance imaging in the diagnosis of multiple sclerosis and long term management of multiple sclerosis. *Neurology 63*(Suppl 5), s3–s11.

Balashov, K.E., Rottman, J.B., Weiner, H.L., and Hancock, W.W. (1999). CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc. Natl. Acad. Sci. USA 96*, 6873–6878.

Bar-Or, A. and Smith, D. (2000). Neuroepidemiology of multiple sclerosis. In T. Batchelor and M. Cudkowicz (eds), *Principals of Neuroepidemiology.* St Louis: Butterworth Heinemann.

Beebe, G., Kurtzke J.F., Kurland L.T., Auth, T.L., and Nagler, B. (1967). Studies on the natural history of multiple sclerosis. 3. Epidemiologic analysis of the army experience in World War II. *Neurology 17*, 1–17.

Begolka, W.S., Vanderlugt, C.L., Rahbe, S.M., and Miller, S.D. (1998). Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. *J. Immunol. 161*, 4437–4446.

Ben-Nun, A., Wekerle, H., and Cohen, I.R. (1981). The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur. J. Immunol. 11*, 195–199.

Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F., and Reindl, M. (2003). Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N. Engl. J. Med. 349*, 139–145.

Betts, C.D., D'Mellow, M.T., and Fowler, C.J. (1993). Urinary symptoms and the neurological features of bladder dysfunction in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry 56*, 245–250.

Bias, W., Reveille, J., Beaty, T., 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.

Bielekova, B., Goodwin, B., Richert, N., Cortese, I., Kondo, T., Afshar, G., Gran, B., Eaton, J., Antel, J., and Frank, J.A. (2000). Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat. Med. 6*, 1167–1175.

Bielekova, B., Richert, N., Howard, T., Blevins, G., Markovic-Plese, S., McCartin, J., Frank, J.A., Wurfel, J., Ohayen, J., Waldmann, T.A., McFarland, H.F., and Martin, R. (2004). Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon Beta. *Proc. Natl. Acad. Sci. USA 1001*, 8705–8708.

Blain, M., Nalbantoglu, J., and Antel, J. (1994). Interferon-gamma mRNA expression in immediately ex-vivo CSF T cells. *J. Neuroimmunol. 54*, 149.

Blanco, Y, Saiz, A, Carreras, E., and Graus, F. (2005). Autologous haematopoietic-stem-cell transplantation for multiple sclerosis. *Lancet Neurol. 4*, 54–63.

Bradley, W. (1998). Recent views on amyotrophic lateral sclerosis with emphasis on electrophysiological studies. *Muscle Nerve 10*, 490–502.

Brocke, S., Gijbels, K., Allegretta, M., Ferber, I., Piercy, C., Blankenstein, T., Martin, R., Utz, U., Karin, N., and Mitchell, D. (1996). Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein. *Nature 379*, 343–346.

Brod, S.A, Marshall, G.D., Jr., Henninger, E.M., Sriram, S., Khan, M., and Wolinsky, J.S. (1996). Interferon-beta 1b treatment decreases tumor necrosis factor-alpha and increases interleukin-6 production in multiple sclerosis. *Neurology 46*, 1633–1638.

Calabresi, P.A., Pelfrey, C.M., Tranquill, L.R., Maloni, H., and McFarland, H.F. (1997). VLA-4 expression on peripheral blood lymphocytes is downregulated after treatment of multiple sclerosis with interferon beta. *Neurology 49*, 1111–1116.

Charcot, J.M. (1868a). Seance du 14 mars. *Cr Soc Biol. 20*, 13–14.

Charcot, J.M. (1868b). Histologic de la sclerose en plaque. *Gazette Hopital 41*, 554–566.

Charcot, J.M. (1877). Lectures on the Diseases of the Nervous System (pp. 157–222). London: The New Sydenham Society.

Cohen, J.A., Carter, J.L,, Kinkel, R.P., and Schwid, S.R. (1999). Therapy of relapsing multiple sclerosis. Treatment approaches for nonresponders. *J. Neuroimmunol. 98*, 29–36.

Confavreux, C., Vukusic, S., Moreau, T., and Adeleine, P. (2000). Relapses and progression of disability in multiple sclerosis. *N. Engl. J. Med. 343*, 1430–1438.

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. *Nat. Genet. 29*, 229–232.

Dean, G. and Kurtzke, J.F. (1971). On the risk of multiple sclerosis according to age at immigration to South Africa. *BMJ 3*, 725–729.

Duda, P.W., Schmied, M.C., Cook, S.L., Krieger, J.L., and Hafler, D.L. (2000). Glatiramer acetate (Copaxone) induces degenerate, Th2-

polarized immune responses in patients with multiple sclerosis. *J. Clin. Invest*. *105*, 967–976.

Ebers, G.C., Bulman, D.E., Sadovnick, A.D., Paty, D.W., Warren, S., Hader, W., Murray, T.J., Seland, T.P., Duquette, P., and Grey, T. (1986). A population-based study of multiple sclerosis in twins. *N. Engl. J. Med. 315*, 1638–1642.

Ebers, G., Kukay, K., Bulman, D., Sadovnick, A. D., Rice, G., Anderson, C., Armstrong, H., Cousin, K., Bell, R.B., Hader, W., Paty, D.W., Hashimoto, S., Oger, J., Duquette, B., Warren, S., Gray, T., O'Connor, P., Nath, A., Auty, A., Metz, L., Francis, G., Paulseth, J.E., Murray, T.J., Pryse-Phillips, W., Nelson, R., Freedman, M., Brunet, D., Bouchard, J.P., Hinds, D., and Risch, N. (1996). A full genome search in multiple sclerosis. *Nat. Genet. 13*, 472–476.

Encinas, J.A. and Kuchroo, V.K. (1999) Genetics of experimental autoimmune encephalomyelitis. In A. N. Theofilopoulos (ed) *Current Directions in Autoimmunity* (pp. 247–272). Basel: Karger.

Fassas, A., Passweg, J.R., Anagnostopoulos, A., Kazis, A., Kozak, T., Havrdova, E., Carreras, E., Graus, F., Kashyap, A., Openshaw, H., Schipperus, M., Deconinck, E., Mancardi Marmont, A., Hansz, J., Rabusin, M., Zuazu Nagore, F.J., Besalduch, J., Dentamaro, T., Fouillard, L., Hertenstein, B., La Nasa, G., Musso, M., Papineschi, F., Rowe, J.M., Saccardi, R., Steck, A., Kappos, L., Gratwohl, A., Tyndall, A., Samiji, J., and Samign, J. (2002). Autoimmune Disease Working Party of the EBMT (European Group for Blood and Marrow Transplantation). *J. Neurol. 249*, 108815.

Filippi, M., Paty, D.W., Kappos, L., Barkhof, F., Compston, D.A., Thompson, A.J., Zhao, G.J., Wiles, C.M., McDonald, W.I., and Miller, D.H. (1995). Correlations between changes in disability and T2-weighted brain MRI activity in multiple sclerosis: a follow-up study. *Neurology 45*, 255–260.

Freal, J. E, Kraft, G.H., and Coryell, J.K. (1984). Symptomatic fatigue in multiple sclerosis. *Arch. Phys. Med. Rehabil. 65*, 135–138.

French Research Group on MS (1992). MS in 54 twinships: concordance rate is independent of zygosity. *Ann. Neurol. 32*, 724–727.

Fritz, R.B. and McFarlin, D.E. (1989). Encephalitogenic epitopes of myelin basic protein. *Chem. Immunol. 46*, 101–125.

Fritz, R.B., Skeen, M.J., Chou, C.H., Garcia, M., and Egorov, I.K. (1985). Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. *J. Immunol. 134*, 2328–2332.

Gale, C. and Martyn, C. (1995). Migrant studies in multiple sclerosis. *Prog. Neurobiol. 47*, 425–448.

Garren, H., Ruiz, P.J., Watkins, T.A., Fontoura, P., Nguyen, L.T., Estline, E.R., Hirschberg, D.L., and Steinman, L. (2001). Combination of gene delivery and DNA vaccination to protect from and reverse Th1 autoimmune disease via deviation to the Th2 pathway. *Immunity 15*, 15–22.

Genc, K., Dona, D.L., and Reder, A.T. (1997). Increased CD80(+) B cells in active multiple sclerosis and reversal by interferon beta-1b therapy. *J. Clin. Invest. 99*, 2664–2671.

Geurts, J.J., Bo, L., Pouwels, P.J., Castelijns, J.A., Polman, C.H., and Barkhof, F. (2005). Cortical lesions in multiple sclerosis: combined postmortem MR imaging and histopathology. *Am. J. Neuroradiol. 26*, 572–577.

Goverman, J., Woods, A., Larson, L., Weiner, L.P., Hood, L., and Zaller, D.M. (1993). Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell 72*, 551–560.

Hafler, D.A. (1999). The distinction blurs between an autoimmune versus microbial hypothesis in multiple sclerosis. *J. Clin. Invest. 104*, 527–529.

Hafler, D.A. (2004). Multiple sclerosis. *J. Clin. Invest. 113*; 790.

Haines, J., Ter-Minassian, M., Bazyk, A., Gusella, J.F., Kim, D.J., Terwedow, H., Pericak-Vance, M.A., Rimmler, J.B., Haynes, C.J., Roses, A.D., Lee, A., Shaner, B., Menfold, M., Seboun, E., Fitoussi, R.P., Gartioux, C., Reyes, C., Ribierre, F., Gyapay, G., Weissenbach, J., Hauser, J.L., Goodkin, D.E., Lincoln, R., Usuku, K., and Oksenberg, J.R. (1996). Complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. The Multiple Sclerosis Genetics Group. *Nat. Genet. 13*, 469–471.

Hammond, S., de Wytt, C., Maxwell, I., Landy, P.J., English, D., McLeod, J.G., and McCall, M.G. (1987). The epidemiology of multiple sclerosis in Queensland, Australia. *J. Neurol. Sci. 80*, 185–204.

Hammond, S.R., McLeod, J.G., Millingen, K.S., Stewart-Wynne, E.C., English, D., Holland, J.T., and McGall, M.G. (1988). The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle and Hobart. *Brain 111*, 1–25.

Hauser, S.L., Bhan, A.K., Gilles, F., Kemp, M., Kerr, C., and Weiner, H.L. (1986). Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol. 19*, 578–587.

Hauser, S.L., Dawson, D.M., Lehrich, J.R., Beal, M.F., Kevy, S.V., Propper, R.D., Mills, J.A., and Weiner, H.L. (1993). Intensive immunosuppression in progressive multiple sclerosis. A randomized, three-arm study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH. *N. Engl. J. Med. 308*, 173–180.

Heltberg, A. and Holm, N. (1982). Concordance in twins and recurrence in sibships in MS. *Lancet* 1, 1068.

Hofman, F.M., Hinton, D.R., Johnson, K., and Merrill, J.E. (1989). Tumor necrosis factor identified in multiple sclerosis brain. *J. Exp. Med. 170*, 607–612.

Hogancamp, W., Rodriguez, M., and Weinshenker, B. (1997). The epidemiology of multiple sclerosis. *Mayo Clin. Proc. 72*, 871–878.

Hohlfeld, R. and Wekerle, H. (2004). Autoimmune concepts of multiple sclerosis as a basis for selective immunotherapy: From pipe dreams to therapeutic pipelines. *Proc. Natl. Acad. Sci. USA 101*, 14599–14606.

Hohol, M.J., Guttmann, C.R., Orav, J., Mackin, G.A., Kikinis, R., Khoury, S.J., Jolesz, F.A., and Weiner, H.L. (1997). Serial neuropsychological assessment and magnetic resonance imaging analysis in multiple sclerosis. *Arch. Neurol. 54*, 1018–1025.

Hohol, M.J., Olek, M.J., Orav, E.J., Stazzone, L., Hafler, D.A., Khoury, S.J., Dawson, D.M., and Weiner, H.L. (1999). Treatment of progressive multiple sclerosis with pulse cyclophosphamide/methylprednisolone: response to therapy is linked to the duration of progressive disease. *Mult. Scler. 5*, 403–409–97.

Holmes, F.F., Stubbs, D.W., and Larsen, W.E. (1967). Systemic lupus erythematosus and multiple sclerosis in identical twins. *Arch. Intern. Med. 119*, 302–304.

The IFNB Multiple Sclerosis Study Group (1993). Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. *Neurology 43*, 655–661.

The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group (1995). Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology 45*, 1277–1285.

Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.U., and Simon, J.H. (1995). A phase III trial of intramuscular recombinant interferon beta as treatment for exacerbating-remitting multiple sclerosis: design and conduct of study and baseline characteristics of patients. Multiple Sclerosis Collaborative Research Group (MSCRG). *Mult. Scler.* 1, 118–135.

Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Hernidon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.V., Simon, J.H., Alam, J.J., Bartoszak, D.M., Bourdette, D.N., Braiman, J., Brownscheidle, C.M., Coats, M.E., Cohan, S.L., Dougherty, D.S., Kinkel, R.P., Moss, M.K., Munschauer, F.E. 3rd, Priorie, R.L., Pullicino, P.M., Scherokman, B.J., and Whitham, R.H. (1996). Intramuscular interferon beta-1a for disease progression in relapsing

multiple sclerosis. The Multiple Sclerosis Collaborative Research Group. *Ann. Neurol. 39*, 285–294.

Johnson, K.P, Brooks, B.R., Cohen, J.A., Ford, C.C., Goldstein, J., Lisak, R.P., Myers, L.W., Panitch, H.S., Rose, J.W., and Schiffer, R.B. (1995). Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology 45*, 1268–1276.

Kabat, E.A., Glusman, M., and Knaub, V. (1948). Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. *Am. J. Med. 4*, 653–662.

Kabat, E.A., Freedman, D.A., Murray, J.P., and Knaub, V. (1950). A study of the crystalline albumin, gamma globulin and the total protein in the cerebrospinal fluid of one hundred cases of multiple sclerosis and other diseases. *Am. J. Med. Sci. 219*, 55–64.

Kappos, L., Comi, G., Panitch, H., Oger, J., Antel, J., Conlon, P., and Steinman, L. (2000). Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group. *Nat. Med. 6*, 176–182.

Karin, N., Mitchell, D., Ling, N., Brocke, S., and Steinman, L. (1994). Reversal of experimental autoimmune encephalomyelitis by a soluble variant of myelin basic protein epitope: T cell receptor antagonism and reduction of Interferon-gamma and TNF production. *J. Exp. Med. 180*, 2227–2237.

Khoury, S.J., Hancock, W.W., and Weiner, H.L. (1992). Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor beta, interleukin 4, and prostaglandin E expression in the brain. *J. Exp. Med. 176*, 1355–1364.

Khoury, S.J., Guttmann, C.R., Orav, E.J., Hohol, M.J., Ahn, S.S., Hsu, L., Kikinis, R., Jolesz, F.A., and Weiner, H.L. (1994). Longitudinal MRI imaging in multiple sclerosis: correlation between disability and lesion burden. *Neurology 44*, 2120–2124.

Kinnunen, E., Koskenvuo, M., Kaprio, J., and Aho, K. (1987). Multiple sclerosis in a nationwide series of twins. *Neurology 37*, 1627–1629.

Krakowski, M. and Owens, T. (1996). Interferon-gamma confers resistance to experimental allergic encephalomyelitis. *Eur. J. Immunol. 26*, 1641.

Kurtzke, J. (1977). Geography in multiple sclerosis. *J. Neurol. 215*, 1–26.

Kurtzke, J., Dean, G., and Botha, D. (1970). A method of estimating age at immigration of white immigrants to South Africa, with an example of its importance. *S. Afr. Med. J. 44*, 663–669.

Kurtzke, J.F., Beebe, G., and Norman, J., Jr. (1979). Epidemiology of multiple sclerosis in U.S. veterans: I. Race, sex, and geographic distribution. *Neurology 29*, 1228–1235.

Lassmann, H. (1998). Neuropathology in multiple sclerosis: new concepts. *Mult. Scler. 4*, 93–98.

Lauer, L. (1995). Environmental associations with the risk of Multiple sclerosis: The contribution of ecological studies. *Acta Neurol. Scand. 161*(Suppl), 77–88.

Leonard, J.P., Waldburger, K.E., and Goldman, S.J. (1995). Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. *J. Exp. Med. 181*, 381–386.

Lennon, V.A., Wingerchuk, D.M., Kryzer, T.J., Pittock, S.J., Lucchinetti, C.F., Fujihara, K., Nakashima, I., and Weinshenker, B.G. (2004). A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet 364*, 2106–2112.

Leppert, D., Waubant, E., Burk, M.R., Oksenberg, J.R., and Hauser, S.L. (1996). Interferon beta-1b inhibits gelatinase secretion and *in vitro* migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. *Ann. Neurol. 40*, 846–852.

Li, D.K. and Paty, D.W. (1999). Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta1a in relapsing-remitting multiple sclerosis. Prevention of relapses and disability by interferon-beta1a subcutaneously in multiple sclerosis. *Ann. Neurol. 46*, 197–206.

Lin, K.C. and Castro, A.C. (1998). Very late antigen (VLA4) antagonists as anti-inflammatory agents. *Curr. Opin. Chem. Biol. 4*, 453–457.

Lucchinetti, C.F., Bruck, W., Rodriguez, M., and Lassmann, H. (1996). Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain Pathol. 6*, 259–274.

Mackay, R.P. and Myrianthopoulos, N.C. (1966). Multiple sclerosis in twins and their relatives. *Arch. Neurol. 15*, 449–462.

Mancardi, G.L., Sardanelli, F., Parodi, R.C., Melani, F., Capello, E., Inglese, M., Ferrari, A., Sormani, M.P., Ottonello, C., Levrero, F., Uccelli, A., and Bruzzi, P. (1998). Effect of copolymer-1 on serial gadolinium-enhanced MRI in relapsing remitting multiple sclerosis. *Neurology 50*, 1127–1133.

Margolis, L. and Graves, R. (1945). The occurrence of myasthenia gravis in a patient with multiple sclerosis. *North Carolina Med. J. 24*, 243–244.

Mattson, D.H., Roos, R.P., and Arnason, B.G. (1980). Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. *Nature 287*, 335–337.

Mattson, D., Petrie, M., Srivastava, D.K., and McDermott, M. (1995) Multiple sclerosis. Sexual dysfunction and its response to medications. *Arch. Neurol. 52*, 862–868.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., and Reingold, S.C. (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann. Neurol. 50*, 121–127.

McLean, B.N., Luxton, R.W., and Thompson, E.J. (1990). A study of immunoglobulin G in the cerebrospinal fluid of 1007 patients with suspected neurological disease using isoelectric focusing and the Log IgG-Index. A comparison and diagnostic applications. *Brain 113*, 1269–1289.

Miller, D.H., Thompson, A.J., Morrissey, S.P., MacManus, D.G., Moore, S.G., Kendall, B.E., Moseley, I.F., and McDonald, W.I. (1992). High dose steroids in acute relapses of multiple sclerosis: MRI evidence for a possible mechanism of therapeutic effect. *J. Neurol. Neurosurg. Psychiatry 55*, 450–453.

Mokhtarian, F., McFarlin, D.E., and Raine, C.S. (1984). Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice. *Nature 309*, 356–358.

Morris, J.C. (1868). Case of the late Dr. DW Penncock. *Am. J. Med. Sci. 56*, 138.

Moxon, W. (1875). Eight cases of insular sclerosis of the brain and spinal cord. *J. Neurol. Neurosurg. Psychiatry 20*, 437–478.

Mumford, C., Wood, N., Kellar-Wood, H., Thorpe, J.W., Miller, D.H., and Compston, D.A. (1992). The UK study of MS in twins. *J. Neurol. 239*, 62–64.

National Multiple Sclerosis Society Research/ Clinical Update (2005). www.nationalmssociety.org/Research-Tysabri.asp

Newcombe, J., Hawkins, C.P., Henderson, C.L., Patel, H.A., Woodroofe, M.N., Hayes, G.M., Cuzner, M.L., MacManus, D., du Boulay, E.P., and McDonald, W.I. (1991). Histopathology of multiple sclerosis lesions detected by magnetic resonance imaging in unfixed postmortem central nervous system tissue. *Brain 114*, 1013–1023.

Nicholson, L.B., Greer, J.M., Sobel, R.A., Lees, M.B., and Kuchroo, V.K. (1995). An altered peptide ligand mediates immune deviation and prevents autoimmune encephalomyelitis. *Immunity 3*, 397–405.

O'Connor, K.C, Chitnis, T., Griffin, D.E., Piyasirisilip, S., Bar-Or, A., Khoury, S., Wucherpfennig, K.W., and Hafler, D.A. (2003). Myelin basic protein-reactive autoantibodies in the serum and cerebrospinal

fluid of multiple sclerosis patients are characterized by low flow-affinity interactions. *J. Neuroimmunol. 136*, 140–148.

O'Garra, A. (1998). Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity 8*, 275–283.

Olek, M.J. and Dawson, D.M. (2000). Multiple sclerosis and other inflammatory demyelinating diseases of the central nervous system. In W.G. Bradley, R.B. Daroff, G.M. Fenichel, and C.D. Marsden (eds), *Neurology in Clinical Practice*, vol. II, 3rd ed. Woburn: Butterworth-Heinemann.

Oskenberg, J.R., Stuart, S., Begovich, A.B., Bell, R.B., Erlich, H.A., Steinman, L., and Bernard, C.C. (1990). Limited heterogeneity of rearranged T cell receptor transcripts in brains of multiple sclerosis patients. *Nature 345*, 344–346.

Oskenberg, J.R., Panzara, M.A., Begovich, A.B., Mitchell, D, Erlich, H.A., Murray, R.S., Shimonkevitz, R., Sherritt, M., Rothbard, J., and Bernard, C.C. (1993). Selection for T cell receptor V-D-J. gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature 362*, 68–70.

Owens, T., Renno, T., Taupin, V., and Krakowski, M. (1994). Inflammatory cytokines in the brain: does the CNS shape immune responses? *Immunol. Today 15*, 566–571.

Paty, D., Studney, D., Redekop, K., and Lublin, F. (1994). MS COSTAR: a computerized patient record adapted for clinical research purposes. *Ann. Neurol. 36*, S134–135.

Pedersen, N.S., Kam-Hansen, S., Link, H., and Mavra, M. (1982). Specificity of immunoglobulins synthesized within the central nervous system in neurosyphilis. *Acta Pathol. Microbiol. Immunol. Scand. 90*, 97–104.

Ponsonby, A., Van der Mei, I., Dwyer, T., Blizzard, L., Taylor, B., Kemp, A., Simmons, R., and Kilpatrick, K. (2005). Exposure to infant siblings during early life and risk of multiple sclerosis. *JAMA 293*, 463–469.

Prineas, J.W. and Wright, R.G. (1978). Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab. Invest. 38*, 409–421.

Rao, S.M., Leo, G.J., Haughton, V.M., St Aubin-Faubert, P., and Bernardin, L. (1989). Correlation of magnetic resonance imaging with neuropsychological testing in multiple sclerosis. *Neurology 39*, 161–166.

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.

Rivers, T.M., Sprunt, D.H., and Berry, G.P. (1933). Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. *J. Exp. Med. 58*, 39–53.

Rovaris, M. and Filippi, M. (1999). Magnetic resonance technique to monitor disease evolution and treatment trial outcomes in multiple sclerosis. *Curr. Opin. Neurol. 12*, 337–344.

Rudick, R.A. (2004). *Contemporary Diagnosis and Management of Multiple Sclerosis*. Newtown, PA: Handbook in Health Care Co.

Rudick, R.A., Ransohoff, R., Lee, J.C., Peppler, R., Yu, M., Mathisen, P.M., and Tuohy, V.K. (1988). *In vivo* effects of interferon beta-1a on immunosuppressive cytokines in multiple sclerosis. *Neurology 50*, 1294–1300 [erratum 51, 332].

Rudick, R.A. (2004) *Contemporary Diagnosis and Management of Multiple Sclerosis*. Newton, P.A. Handbook in Health Care Company Co. Color Plate page 1.

Sadovnick, A., Armstrong, H., Rice, G.P., Bulman, D., Hashimoto, L., Paty, D.W., Hashimoto, S.A., Warren, S., Hader, W., and Murray, T.J. (1993). A population based study of multiple sclerosis in twins: update. *Ann. Neurol. 33*, 281–285.

Sadovnick, A.D., Remick, R.A., Allen, J., Swartz, E., Yee, I.M., Eisen, K., Farquhar, R., Hashimoto, S.A., Hooge, J., Kastimkoff, L.F., Morrison, W., Nelson, J., Oger, J., and Paty, D.W. (1996). Depression and multiple sclerosis. *Neurology 46*, 628–632.

Sadovnick, A., Dyment, D., and Ebers, G. (1997). Genetic epidemiology of multiple sclerosis. *Epidemiol. Rev. 19*, 99–106.

Sawcer, S., Jones, H.B., Feakes, R., Gray, J., Smalden, N., Chataway, J., Robertson, N., Clayton, D., Goodfellow, P.P., and Compston, A. (1996). A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. *Nat. Genet. 13*, 464–468.

Sawcer, S., Goodfellow, P., and Compston, A. (1997). The genetic analysis of multiple sclerosis. *Trend Genet*. *13*, 234–239.

Schumacher, G.A., Beebe, G., Kibler, R.F., et al. (1965). Problems of experimental trials of therapy in multiple sclerosis: Report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Ann. N. Y. Acad. Sci*. *122*, 552–568.

Segal, B.M., Dwyer B.K., and Shevach, E.M. (1998). An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. *J. Exp. Med. 187*, 537–546.

Selmaj, K., Raine C.S., Cannella, B., and Bronsan, C.F. (1991). Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. *J. Clin Invest. 87*, 949–954.

Siveke, J.T. and Hamann, A. (1998). T helper 1 and T helper 2 cells respond differentially to chemokines. *J. Immunol. 160*, 550–554.

Shakir, R., Hussien, J., and Trontel, J.J. (1983). Myasthenia gravis and multiple sclerosis. *J. Neuroimmunol. 4*, 161–165.

Skotzek, B., Sander, T., Zimmermann, J., and Kalmel, H.W. (1998). Oligoclonal bands in serum and cerebrospinal fluid of patients with HIV infection. *J. Neuroimmunol. 20*, 151–152.

Sorensen, T.L., Tani, M., Jensen, J., Pierce, V., Lucchinetti, C., Folcik, V.A., Qin, S., Rottman, J., Sellebjerg, F., Strieter, R.M., Frederiksen, J.L., and Ransohoff, R.M. (1999). Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J. Clin. Invest. 103*, 807–815.

Stazio, A., Kurland, L., Bell, L.G., Saunders, M.G., and Rogot, E. (1964). Multiple sclerosis in Winnipeg, Manitoba. Methodological consideration of epidemiologic survey: ten-year follow-up of a community-wide study and population re-survey. *J. Chronic Dis. 17*, 415–438.

Stone, L.A., Frank, J.A., Albert, P.S., Bash, C., Smith, M.E., Maloni, H., and McFarland, H.F. (1995). The effect of interferon-beta on blood–brain barrier disruptions demonstrated by contrast-enhanced magnetic resonance imaging in relapsing-remitting multiple sclerosis. *Ann. Neurol. 37*, 611–619.

Stone, L.A., Frank, J.A., Albert, P.S., Bash, C.N., Calabresi, P.A., Maloni, H., and McFarland, H.F. (1997). Characterization of MRI response to treatment with interferon beta-1b: contrast-enhancing MRI lesion frequency as a primary outcome measure. *Neurology 49*, 862–869.

Studney, D., Lublin, F., Marcucci, L., et al. (1993). MS COSTAR: a computerized record for use in clinical research in multiple sclerosis. *J. Neurol. Rehab*. *7*, 145–152.

Stuve, O., Dooley, N.P., Uhm, J.H., Antel, J.P., Francis, G.S., Williams, G., and Yong, V.W. (1996). Interferon beta-1b decreases the migration of T lymphocytes *in vitro*: effects on matrix metalloproteinase-9. *Ann. Neurol. 40*, 853–863.

Sundvall, M., Jirholt, J., Yang, H.T., Jansson, L., Engstrom, A., Pettersson, U., and Holmdahl, R. (1995). Identification of murine loci associated with susceptibility to chronic experimental autoimmune encephalomyelitis. *Nat. Genet*. *10*, 313–317.

Svenson, L.W., Woodhead, S.E., and Platt, G.H. (1994). Regional variations in the prevalence rates of multiple sclerosis in the province of Alberta, Canada. *Neuroepidemiology 13*, 8–13.

Sweeney, V., Sadovnick, A., and Brandejs, V. (1986). Prevalence of multiple sclerosis in British Columbia. *Can. J. Neurol. Sci. 13*, 47–51.

Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S., and Bo, L. (1998) Axonal transection in the lesions of multiple sclerosis. *N. Engl. J. Med. 338*, 278–285.

Trapp, B.D., Bo, L., Mork, S., and Chang, A. (1999a). Pathogenesis of tissue injury in MS lesions. *J. Neuroimmunol. 98*, 49–56.

Trapp, B., Ransohoff, R., Fisher, E., et al. (1999b). Neurodegeneration in multiple sclerosis: relationship to neurological disability. *Neuroscientist 5*, 48–57.

Traugott, U., Reinherz, E.L., and Raine, C.S. (1983). Multiple sclerosis: distribution of T cell subsets within active chronic lesions. *Science 219*, 308–310.

Trojano, M., Avolio C., Ruggieri M., et al. (1997). Antiinflammatory effects of rIFNβ-1b in multiple sclerosis: longitudinal changes of the serum sICAM–1, TNFα and T cell subsets. *Neurology 48*, A244.

Trostle, D., Helfrich, D., and Medsger, T. (1986). Systemic sclerosis (scleroderma) and multiple sclerosis. *Arthritis Rheum. 29*, 124–127.

Utz, U., Biddison, W.E., McFarland, H.F., McFarlin, D.E., Flerlage, M., and Martin, R. (1993). Skewed T-cell receptor repertoire in genetically identical twins correlates with multiple sclerosis. *Nature 364*, 243–247.

VanAmerongen, B.M., Dijkstra, C.D., Lips, P., and Polman, C.G. (2004). Multiple sclerosis and vitamin D: an update. *Eur. J. Clin. Nutr. 58*, 1095–1109.

Vartdal, F., Vandvik, B., and Norrby, E. (1982). Intrathecal synthesis of virus-specific oligoclonal IgG, IgA and IgM antibodies in a case of varicella-zoster meningoencephalitis. *J. Neurol. Sci. 57*, 121–132.

Vollmer, T., Key, L., Durkalski, V., Tyor, W., Corboy, J., Markovic-Plese, S., Preiningerova, J., Rizzo, M., and Singh, I. (2004). Oral simvastatin treatment in relapsing-remitting multiple sclerosis. *Lancet 363*, 1607–1608.

Weiner, H.L., Mackin, G.A., Orav, E.J., Hafler, D.A., Dawson, D.M., LaPierre, Y., Hernden, R., Lehrich, J.R., Hauser, S.L., and Turel, A. (1993). Intermittent cyclophosphamide pulse therapy in progressive multiple sclerosis: final report of the Northeast Cooperative Multiple Sclerosis Treatment Group. *Neurology 43*, 910–918.

Weinshenker, B.G. (1996). Epidemiology of multiple sclerosis. *Neurol. Clin. 14*, 291–308.

Whitlock, F.A. and Siskind, M.M. (1980). Depression as a major symptom of multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry 43*, 861–865.

Williams, A., Eldridge, R., McFarland, H., Houff, S., Krebs, H., and McFarlin, D. (1980). Multiple sclerosis in twins. *Neurology 30*, 1139–1147.

Williams, K., Ulvestad, E., and Antel, J.P. (1994). B7/BB-1 antigen expression on adult human microglia studied *in vitro* and *in situ*. *Eur. J. Immunol. 24*, 3031–3037.

Windhagen, A., Scholz, C., Hollsberg, P., Fukaura, H., Sette, A., and Hafler, D.A. (1995a). Modulation of cytokine patterns of human autoreactive T cell clones by a single amino acid substitution of their peptide ligand. *Immunity 2*, 373–380.

Windhagen, A., Newcombe, J., Dangond, F., Strand, C., Woodroofe, M.N., Cuzner, M.L., and Hafler, D.A. (1995b). Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86), and interleukin 12 cytokine in multiple sclerosis lesions. *J. Exp. Med. 182*, 1985–1996.

Wucherpfennig, K.W., Newcombe, J., Li, H., Keddy, C., Cuzner, M.L., and Hafler, D.A. (1992a). Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc. Natl. Acad. Sci. USA 89*, 4588–4592.

Wucherpfennig, K.W., Newcombe, J., Li, H., Keddy, C., Cuzner, M.L., and Hafler, D.A. (1992b). T cell receptor V alpha-V beta repertoire and cytokine gene expression in active multiple sclerosis lesions. *J. Exp. Med. 175*, 993–1002.

Yednock, T.A., Cannon, C., Fritz, L.C., Sanchez-Madrid, F., Steinman, L., and Karin, N. (1992). Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha-4-1 integrin. *Nature 356*, 63–66.

Yong, V.W. (2002). Differential mechanisms of action of interferon-beta and glatiramer acetate in MS. *Neurology 59*, 802–808.

Young, I.R., Hall, A.S., Pallis, C.A., Bydder, G.M., Legg, N.J., and Steiner, R.E. (1981). Nuclear magnetic resonance imaging of the brain in multiple sclerosis. *Lancet 2*, 1063–1066.

Zamvil, S., Nelson, P., Trotter, J., Mitchell, D., Knobler, R., Fritz, R., and Steinman, L. (1985). T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature 317*, 355–358.

Zhang, J., Markovic-Plese, S., Lacet, B., Raus, J., Weiner, H.T., and Hafler, D.A. (1994). Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *J. Exp. Med. 179*, 973–984.

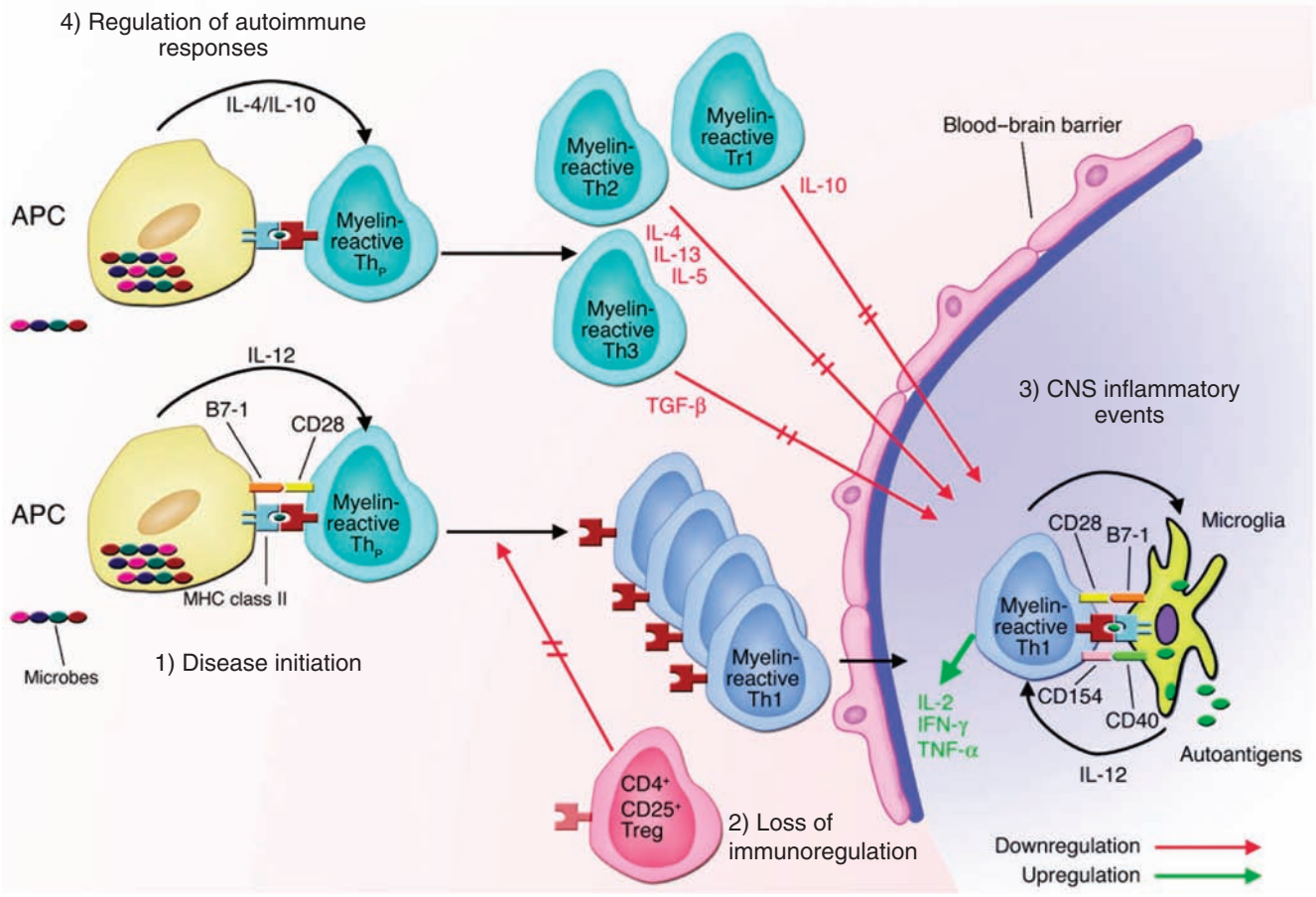


FIGURE 46.3 Presumed pathophysiology of multiple sclerosis (MS). Four main components contribute to the pathophysiology of MS. 1) *Initiation*, a presumed microbe may be taken up by an antigen- presenting cell (APC) and is presented to a myelin-reactive precursor T-cell (Th_p). 2) *Loss of immunoregulation*, $CD4^+CD25^+$ regulatory T-cells (Tregs) are thought to be reduced in MS patients, which causes a decrease in the overall autoregulation of the autoimmune process. 3) *Central nervous system (CNS) inflammatory events*, in the CNS, local APCs, microglia, present antigen to myelin-reactive type 1 helper T (Th1)-cells that secrete Th1 cytokines such as interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α, which permit the inflammation to continue. 4) *Regulation of the autoimmune responses*, three cell types enter the CNS to counterbalance the proinflammatory Th1 cells, which are part of the naturally-occurring regulatory mechanisms that bring the inflammatory cascade into check by counterbalancing the Th1-mediated proinflammatory response. The three cell types are the Th3 cells which secrete tumor growth factor (TGF)-β; Th2 cells which secrete Th2-mediated cytokines, including IL-4, IL-13, and IL-5; and regulatory T-cells (Tr1), which secrete IL-10.

Reproduced from Hafler (2004), with permission.

CHAPTER

47

Peripheral Neuropathy

MICHAEL P. T. LUNN*
*National Hospital for Neurology and Neurosurgery, Queen Square, London, UK

KAZIM A. SHEIKH†
†Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Autoimmunity is implicated in a small but important group of peripheral nerve diseases. These include the acute inflammatory neuropathies eponymously referred to as the Guillain–Barré (GBS) and Fisher syndromes, and the chronic inflammatory demyelinating polyradiculoneuropathies, both idiopathic and associated with a serum paraprotein. Substantial evidence exists for an autoimmune pathogenesis in GBS and its subtypes. Evidence is still being gathered to support similar processes in the chronic inflammatory neuropathies, including the demyelinating neuropathy associated with antibodies to myelin-associated glycoprotein (MAG). Although most current evidence supports an antibody-driven pathogenesis triggered by infection, T cell and other cellular immune components are crucial effectors of disease. Experimental allergic neuritis (EAN), an inflammatory model of neuropathy, has been instructive in the detailed study of the pathogenesis of immune-mediated neuropathies and will be discussed.

ACUTE NEUROPATHIES: THE GUILLAIN–BARRÉ SYNDROME

Historic Background

Guillain, Barré, and Strohl described a rapidly evolving flaccid paralysis with areflexia and albuminocytologic dissociation in the cerebrospinal fluid (CSF) in 1916 (Guillain et al., 1916). Early autopsy demonstrated both T-cell inflammation and demyelination in peripheral nerves (Haymaker and Kernohan 1949; Ashbury et al., 1969), leading to the notion of GBS being a single pathophysiologic entity synonymous with acute inflammatory demyelinating polyradiculoneuropathy (AIDP). AIDP is by far the most common variant of GBS in the developed world. Variants such as the Fisher syndrome (Fisher, 1956) and the axonal variants (Feasby et al., 1986), acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN) (Yuki et al., 1990; McKhann et al., 1991; 1993), are now part of a spectrum of disease with variable worldwide occurrence (Box 47.1; see below).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Box 47.1
Diversity of Guillain–Barré variants

- Acute inflammatory demyelinating polyradiculoneuropathy (AIDP); regional variants, e.g., pharyngo-cervical-brachial
- Acute motor axonal neuropathy (AMAN)
- Acute motor and sensory axonal neuropathy (AMSAN)
- (Miller) Fisher syndrome (ataxia, ophthalmoplegia, and areflexia)
- Acute panautonomic neuropathy
- Acute pure sensory neuropathy
- Acute motor conduction block neuropathy

Epidemiology

Since the near eradication of poliomyelitis, GBS has become the most common cause of acute flaccid neuromuscular paralysis in the world. The incidence of GBS is 1–1.5 in 100,000 (range 0.4–4) (Hughes and Rees, 1997; Rees et al., 1998; Emilia-Romagna Study Group, 1998). The incidence increases steadily with advancing age and males are affected more often than females by 1.25 : 1 (Hadden and Gregson, 2001). Case–control studies implicate infections as precipitating events (see below).

Severity varies from mild with full recovery in 10% of patients, to bedbound in 40%, to complete paralysis with ventilatory dependence in 20%. Death occurs in 5–8% of patients (Guillain–Barré Syndrome Study Group, 1985; Rees et al., 1998).

Clinical Features and Subtypes

The diagnosis of GBS remains clinical, but electrophysiologic studies help to subtype patients into diagnostic categories.

Acute Inflammatory Demyelinating Polyradiculoneuropathy

AIDP accounts for >95% of patients with GBS in Europe and North America. Patients present with a rapidly evolving neuropathic (sensory) motor paralysis, usually ascending, in two or more limbs over less than 4 weeks. The illness is monophasic. Most patients have numbness, tingling or pain and many complain of bladder disturbance, facial weakness or swallowing difficulty (Hughes, 1990). Autonomic disturbance is common with arrhythmia and fluctuating blood pressures. Tendon reflexes are absent or reduced. The CSF contains fewer than 50 leucocytes/μl (Asbury and Cornblath, 1990) and CSF protein is raised in 80% of cases. Antiganglioside antibodies may be detected in the serum (see below) by enzyme-linked immunosorbent assay (ELISA), often with thin layer chromatography confirmation. Assays should be performed in an experienced laboratory. Electrophysiologic studies typically show slowed motor conduction velocities, delayed F-waves and preserved compound muscle action potential (CMAP) amplitudes consistent with demyelination, but conduction failure and axonal degeneration may complicate the picture.

Multifocal perivascular T-cell infiltration with demyelination, typically patchy with involvement of proximal and terminal nerve segments, characterizes the pathology of AIDP (Asbury et al., 1969; Hall et al., 1992; Reisin et al., 1993; Massaro et al., 1998). Indications of blood–nerve barrier (BNB) breakdown and deposition of activated complement components can be seen in some but not all cases of AIDP (Hafer-Macko et al., 1996b). These observations raise the possibility that T-cell- or antibody-mediated immune injury can predominate in an individual case.

Acute Motor Axonal Neuropathy

AMAN is a pure motor variant of GBS seen most commonly in China, Japan, and Mexico (Ramos-Alvarez et al., 1969; McKhann et al., 1991; 1993; Ogawara et al., 2000), where it accounts for almost half of cases. In Europe and North America it accounts for only 5–20% of cases (Rees et al., 1995b; Visser et al., 1995), probably nearer 5%. The reasons for this are unknown. In China, AMAN occurs in seasonal epidemics, affects more children (McKhann et al., 1991), and is strongly associated with *Campylobacter jejuni* infection. Sensory impairment is minimal and autonomic involvement less common. Electrophysiologic studies are characterized by reduced CMAP amplitudes and absent F-waves, with normal distal motor latencies and conduction velocity (Kuwabara et al., 2000). Sensory involvement is absent.

The pathology of axonal GBS has largely been described from AMAN cases in northern China. The pathologic changes indicate an antibody-mediated immune attack directed preferentially against motor axons, causing primary axonal degeneration in the absence of prominent T-cell inflammation (Griffin et al., 1995; 1996a; Hafer-Macko et al., 1996a). Macrophages may be found in the periaxonal space, suggesting that the antigen of interest is on the axolemma (Figure 47.1). However, since in some patients little pathology is found (Griffin et al., 1996b) and in others recovery is too rapid for nerve fiber degeneration and regeneration (Ho et al., 1997; Kuwabara et al., 1998), axonal conduction failure and distal neuromuscular terminal failure must also make a prominent contribution to clinical weakness in AMAN.

Acute Motor and Sensory Neuropathy

AMSAN is a more severe form of AMAN, with a more severe course, sensory involvement, and delayed recovery

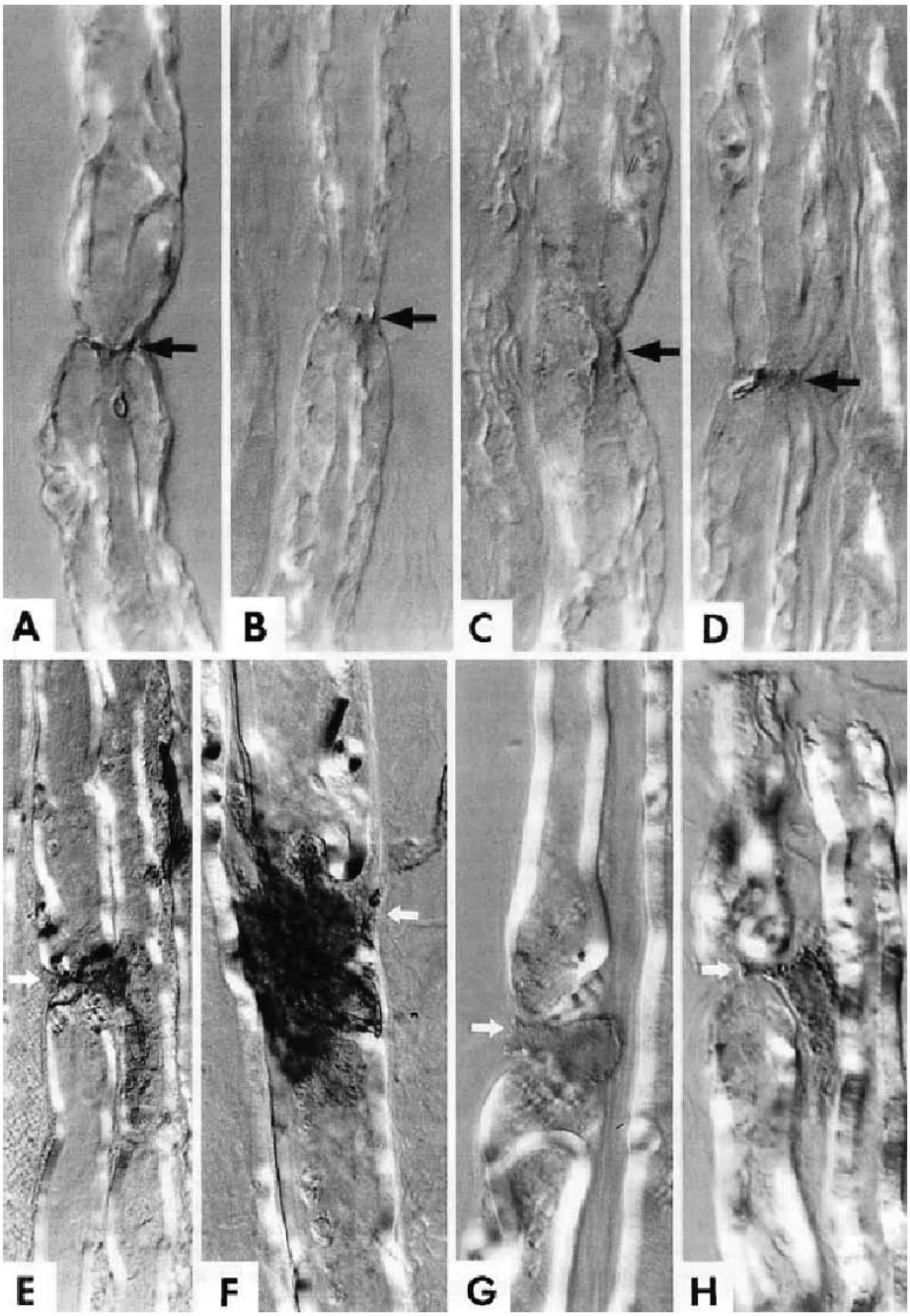


FIGURE 47.1 Immunostained teased ventral root fibers from a patient with acute motor axonal neuropathy (AMAN), 4 days after the onset of neurologic symptoms. *A–D*, The complement activation product C3d was localized discretely at the nodes of Ranvier (arrows) of the large myelinated motor fibers. The golden–brown immunoreaction product is at the nodes. *E–H*, Many motor fibers had macrophages overlying, and extending processes into, the nodes of Ranvier (arrows). [*E* and *F* were immunostained with the macrophage marker HAM-56; *G* and *H* for HLA-DR (major histocompatibility locus class II)]. See color plate section.

Reprinted from Hafer-Macko et al. (1996a).

(Feasby et al.,1986; Griffin et al., 1995). Sensory as well as motor nerve roots are involved. The pathology is similar to AMAN.

Fisher Syndrome

The Miller Fisher or Fisher syndrome (FS) (Fisher, 1956) accounts for approximately 5% of GBS cases and comprises ophthalmoplegia, ataxia, and areflexia without limb weakness. In common usage, facial and bulbar weakness have been included as part of the syndrome. Overlapping forms with AIDP are not uncommon. This syndrome is strongly associated with preceding *C. jejuni* infection and over 85% of patients have antibodies to the ganglioside GQ1b, which are almost certainly pathogenic (see below) (Willison et al., 1993; Mizoguchi, 1998; Willison and O'Hanlon, 1999; O'Hanlon et al., 2001). Anti-GQ1b antibodies are also found in GBS patients with ophthalmoplegia. The pathophysiologic and structural bases of the clinical manifestations in FS are still not completely resolved.

Acute Idiopathic Autonomic Neuropathies

These neuropathies are rare but are probably variants of GBS (Kissel et al., 2001; Freeman, 2005). They present with acute or subacute autonomic failure manifesting as presyncope and syncopal episodes with a viral prodrome in 50%. Abdominal symptoms with diarrhea, constipation, vomiting or pain, impotence, bladder dysfunction, sweating abnormalities, and pupillary symptoms and signs are variable and not uncommon. Pandysautonomia is most common but both pure sympathetic and parasympathetic failure are described. There are often few sensory or motor features other than subjective sensory disturbance and hyporeflexia. There is albuminocytologic dissociation in the CSF, and recently an association has been described with neuronal nicotinic acetylcholine receptor antibodies in the serum (Vernino et al., 2000). Recovery is often slow and incomplete.

Autoimmune Features

Molecular Mimicry

Molecular mimicry has been invoked as a mechanism in a variety of autoimmune diseases. AMAN and FS provide some of the best available evidence to support the hypothesis of molecular mimicry as a pathogenetic mechanism underlying postinfectious autoimmune disorders. AMAN and FS fulfill some of the Koch-Witebsky postulates supporting autoantibody as pathogenic (Witebsky et al., 1957; Rose and Bona, 1993). Antiganglioside antibodies can be demonstrated in patient serum. They have cognate antigens (gangliosides) enriched in peripheral nerve. It is possible to immunize animals (see below) with ganglioside antigens, either purified or as whole bacteria, to produce autoantibody and disease, albeit with difficulty. Transfer of antibody from patient/model animal to a normal subject can sometimes transfer disease. Furthermore, mechanisms of antibody action are now starting to be understood.

Antiganglioside Antibodies in Guillain–Barré Variants

Antibodies to ganglioside species are found in the serum of patients with GBS. They are polyclonal, predominantly IgG, and generally complement-fixing IgG1 and IgG3 (Willison and Veitch, 1994; Ogino et al., 1995; Yuki et al., 1995; Ho et al., 1999). This implies class-switching usually with T-cell help, both atypical of human anticarbohydrate responses (see below). Antibodies to gangliosides GM1, GM1(NeuGc), GM1b, GalNAc–GM1b, GD1a, GalNAc–GD1a, GD1b, 9-O-acetyl GD1b, GD3, GT1b, GQ1b, GQ1bα, LM1, galactocerebroside, and SGPG have been reported in more than 200 papers on inflammatory neuropathies (Willison and Yuki, 2002). Clinicoserologic correlations between GBS subtypes and serum antibodies to putative ganglioside antigens have been drawn (Rees et al., 1995a; Jacobs et al., 1996; Ho et al., 1999; Ogawara et al., 2000). Antibodies to gangliosides GM1 and GD1a, implicated as the major target antigens in AMAN (Yuki et al., 1993b; Rees et al., 1995a; Jacobs et al., 1996; Hadden et al., 1998; Ho et al., 1999; Ogawara et al., 2000), can be detected in 50–60% of AMAN patients in the Far East (Ho et al., 1999; Ogawara et al., 2000). Antibodies to GalNAc–GD1a and GM1b are found in motor predominant GBS in about 10–15% of cases (Ang et al., 1999; Yuki et al., 1999; 2000). Anti-GQ1b antibodies, frequently cross-reactive with structurally-related gangliosides, are present in 80–90% of patients with FS (Willison et al., 1993; Yuki et al., 1993a; Carpo et al., 1998). This correlation provides the strongest association between antibodies to a specific ganglioside and a clinical phenotype.

Gangliosides in Peripheral Nerve

Gangliosides are sialic acid-containing glycolipids (Figure 47.2), widely distributed in mammalian tissues, but enriched in the nervous system. GM1, GD1b, GD1a, and GT1b are the most abundant. A simple hypothesis to explain the differences between GBS variants is based on the premise that target gangliosides have differential distribution in the peripheral nervous system (PNS). In relation to FS, GQ1b is relatively enriched in the oculomotor cranial nerves (Chiba et al., 1993; 1997), although anti-GQ1b antibodies may bind elsewhere (Willison et al., 1996; Goodyear et al., 1999). Although ganglioside immunolocalization studies are technically difficult, the AMAN-associated gangliosides GM1 and GD1a, are localized at the nodes of

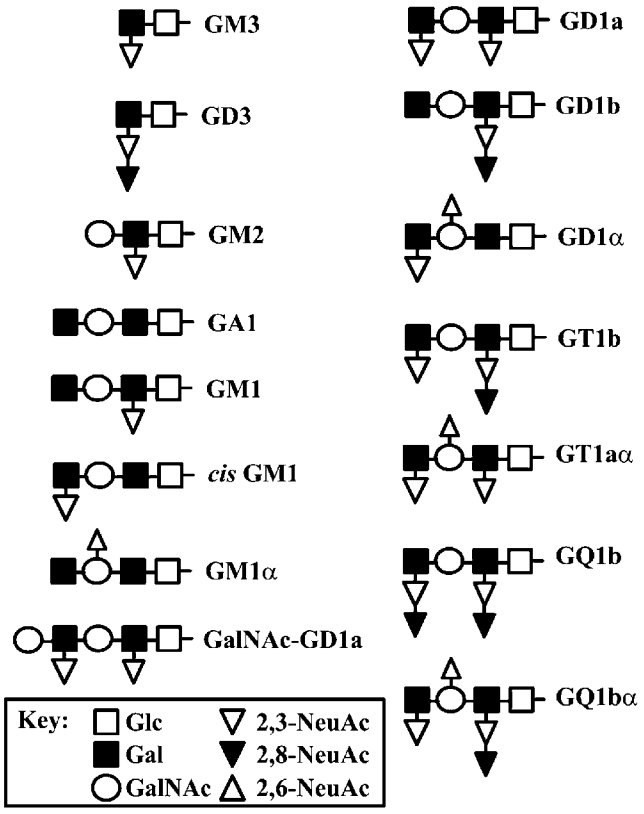


FIGURE 47.2 Ganglioside species in peripheral nerve that are potential targets for neuropathy-associated antibodies. Note the similarity in structures between species, which allows for some cross-reactivity. All have an intramembrane ceramide and display cell-surface oligosaccharides. IgG antibodies to GQ1b are associated with over 90% of cases of Fisher syndrome, but commonly demonstrate cross-reactivity with related species GT1b and GD1b. Antibodies to GM1 are associated with multifocal motor neuropathy with conduction block and a worse prognosis in Guillain–Barré syndrome. IgG antibodies to GD1a are strongly associated with acute motor axonal neuropathy. Antibodies to GM1 and GalNAc-GD1a are also less commonly found (see text). Gal, galactose, GalNAc, N-acetylgalactosamine; Glc, glucose; NeuAc, neuraminic (sialic) acid.

Ranvier and in motor nerve terminals (Sheikh et al., 1999; Gong et al., 2002). Furthermore, preferential staining of motor nerve fibers has been demonstrated with monoclonal anti-GD1a antibodies in rats (Gong et al., 2002) (Figure 47.3) and also with human anti-GD1a antibodies from a patient with AMAN (De Angelis et al., 2001). Other factors, such as variations in permeability of the BNB, density, and accessibility of target gangliosides, and relationship to functional components of the axolemma, such as ion channels, are likely to be relevant.

Functional Effects of Antibodies

Antibodies bind to nerves at nodes of Ranvier where gangliosides (especially GM1) and channels are enriched. Some studies indicate that anti-GM1 antibodies may block or alter

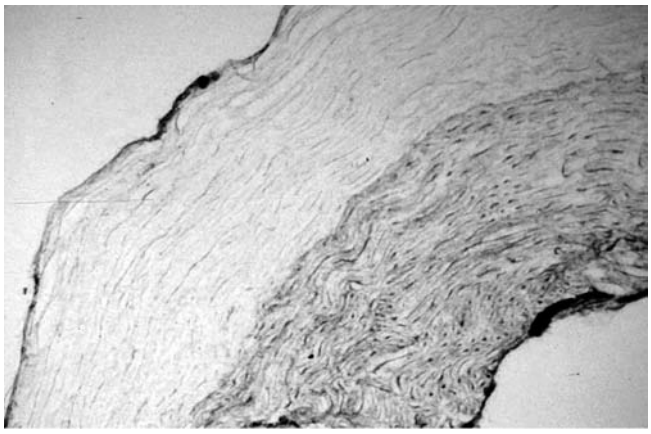


FIGURE 47.3 Unfixed fresh-frozen rat motor-sensory nerve root stained with a monospecific murine IgG anti-GD1a antibody. There is preferential strong staining of the motor axons of the ventral root (below) compared to the sensory axons of the adjacent dorsal root (above). Original magnification ×320.

channel function (Takigawa et al., 1995; 2000; Weber et al., 2000), although the exact mechanisms remain unclear (Sheikh et al., 1999). Intraneural injection of GBS patient serum, purified immunoglobulin or specific antiganglioside antibodies has produced mixed results (Saida et al., 1979; Winer et al., 1988; Sumner et al., 1992).

An alternative site of attack is at the roots and motor nerve terminals (MNTs) where the BNB is relatively deficient. MNTs degenerate in AMAN (Ho et al., 1997) and are disturbed electrophysiologically in FS (Uncini and Lugaresi, 1999). In an *ex vivo* phrenic nerve-diaphragm preparation, Willison et al. have clearly shown that anti-GQ1b antibodies bind to nerve terminals and cause complement-dependent quantal acetylcholine (ACh) release, resulting in neuromuscular blockade and a calcium-dependent disruption of the terminal bouton (Willison et al., 1996; Goodyear et al., 1999; Plomp et al., 1999; O'Hanlon et al., 2001). They have recently shown the same effect with anti-GD1a antibodies, and that this effect is dependent upon GD1a antigen density (Goodfellow et al., 2005).

In a parallel series of patch-clamp experiments, IgG GQ1b, GD1a, GD1b, and GM1 antibodies have been shown to cause reversible complement independent pre- and post-synaptic blockade, depending upon the antibody used (Buchwald et al., 1995; 1998; 2002; Buchwald and Sheikh, unpublished results).

Environmental Effects

C. jejuni is a gram-negative non-spore-forming enteropathogen that is one of the most common causes of bacterial gastroenteritis worldwide (Hughes and Rees, 1997; Friedman et al., 2000; Oberhelman and Taylor, 2000).

Infection with *C. jejuni* is found in 13–72% of patients with AMAN or GBS (Hughes and Rees, 1997; Hadden and Gregson, 2001), with an overall prevalence estimated to be around 30% (Moran et al., 2002). Only 1 in 1000 cases of *C. jejuni* infection is complicated by GBS. The exact characteristics of *C. jejuni* that determine whether GBS follows infection are still unclear. However, a relationship of GBS with a number of Penner serotypes is recognized (Prendergast and Moran, 2000). Penner serotyping distinguishes *C. jejuni* strains on the basis of strain-specific, heat-stable, extractable, capsular lipopoly- and lipooligosaccharides. Penner strains HS:19 (Fujimoto et al., 1992; Yuki et al., 1992; Kuroki et al., 1993; Rees et al., 1995b; Jacobs et al., 1997b; Sheikh et al., 1998) and HS:41 (Goddard et al., 1997) are especially over-represented in GBS and are uncommon in patients with uncomplicated gastroenteritis.

The lipopolysaccharide (LPS) and lipooligosaccharide (LOS) of *C. jejuni* carry ganglioside-like moieties. Several studies have characterized these in GBS- and diarrhea-associated *C. jejuni* strains. GM1-, GD1a-, GalNAc–GD1a-, GM1b-, GT1a-, GD2-, GD3-, and GM2-like structures have all been identified (Aspinall et al., 1993; 1994a; 1994b; Yuki et al., 1994; 1996; Sheikh et al., 1998; Nachamkin et al., 1999; 2002). Although no GQ1b-like structure exists, antibody-binding assays have shown the presence of GQ1b- and GT1a-cross-reactive moieties in *C. jejuni* LPS/LOSs (Yuki et al., 1994; Jacobs et al., 1995; 1997a). It is these structures that are likely to provide the initial stimulus to autoimmune activation.

Upper respiratory tract infection or other febrile episodes caused by cytomegalovirus (CMV) (5–22%), Epstein–Barr virus (EBV) (2–10%), *Mycoplasma pneumoniae* (5%), and *Haemophilus influenzae* have all been identified as potentially causative (Hadden and Gregson, 2001; Ju et al., 2004). *H. influenzae* carries ganglioside-like moieties (Mori et al., 2000). Swine flu and rabies vaccinations have also been causatively implicated (Kaplan et al., 1982; Hemachudha et al., 1988).

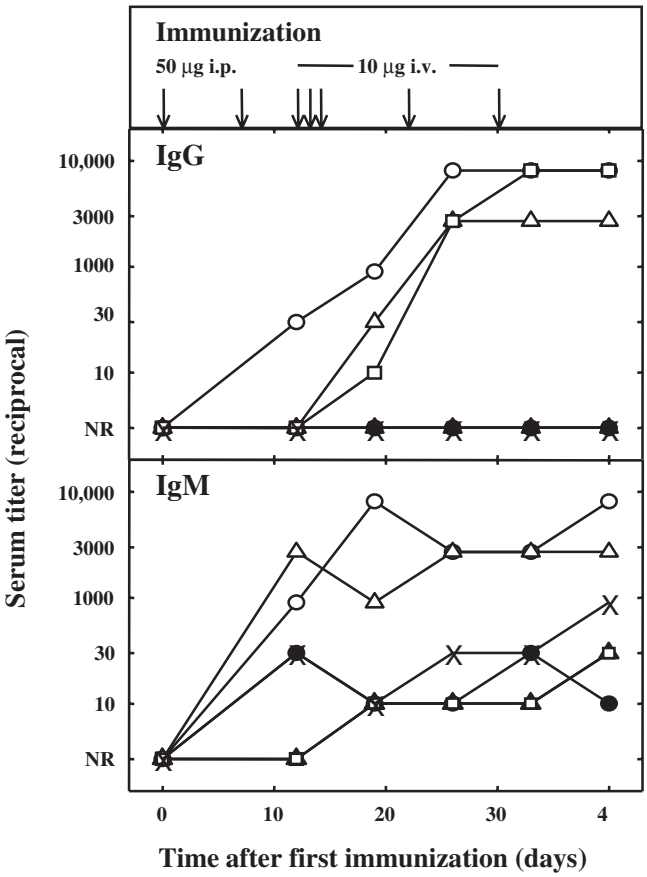


FIGURE 47.4 Mice lacking complex ganglioside species (GalNAcT$^{-/-}$) are able to switch class to complement-fixing IgG production when immunized with keyhole limpet hemocyanin (KLH)-conjugated ganglioside. Serologic responses of three individual GalNAcT$^{-/-}$ mice (open symbols) and three wild-type mice (closed symbols) immunized with GD1a-KLH, and a GalNAcT$^{-/-}$ mouse immunized with unconjugated KLH (X). Sera collected at the indicated times were tested for IgG and IgM anti-GD1a antibody titers by ELISA. Titer values are presented as the greatest dilution resulting in a signal which exceeded 3 SD above the control mean. Values were tested in triplicate. i.p., Intraperitoneal; i.v., intravenous.

Reprinted from Lunn et al. (2000), with permission.

Animal Models

Attempts to generate either IgG antiganglioside antibodies or neuropathy in animals by immunization with *C. jejuni* were for a long while either unforthcoming or not reproducible (Nagai et al., 1976; Kusunoki et al., 1996; Li et al., 1996; Prendergast and Moran, 2000). Immunization of mice with *C. jejuni* LPSs/LOSs generates mainly low-titer IgM antibodies (Wirguin et al., 1997; Goodyear et al., 1999), reflecting a high level of tolerance to self-gangliosides (Bowes et al., 2002). Tolerance to self-gangliosides can be overcome by immunization with gangliosides or *C. jejuni* LPSs conjugated to adjuvant in transgenic animals lacking complex gangliosides (Lunn et al., 2000; Bowes et al., 2002) (Figure 47.4). This model illustrates that where tolerance is circumvented, potentially pathogenic antibodies can be generated.

A sensory ataxic neuropathy with pathologic changes in the nerve and cord has been induced in rabbits by GD1b immunization and passive antibody transfer (Kusunoki et al., 1996; 1999a; 1999b). Immunization with mixed bovine brain gangliosides or GM1 alone produced an acute flaccid paralysis in rabbits reminiscent of human disease (Yuki et al., 2001; Susuki et al., 2003). The implantation of a murine IgG anti-GD1a-secreting hybridoma into mice, but not passive transfer of the same antibody, led to an axonal neuropathy (Sheikh et al., 2004). Additional cytokine factors may be required to initiate the neuropathy.

Cellular Mechanisms

Comprehensive evidence of T-cell involvement in GBS has not been so forthcoming despite the central role of T cells in the pathogenesis of EAN. Studies of animal models and predominantly human AIDP have generated a complex multistep pathogenesis for cellular involvement in the autoimmune neuropathies (Figure 47.5). Macrophages are essential effectors of damage and recovery, targeted by antibody and T cells (see below).

Multifocal lymphocytic infiltration was established as the hallmark of the pathology of GBS in early postmortem studies (Asbury et al., 1969), but it is not always seen (Cornblath et al., 1990; Honavar et al., 1991). Circulating activated T cells are found early in the course of GBS (Taylor and Hughes, 1989; Hartung and Toyka, 1990). αβ T cells with CD4 and CD8 ratios in similar proportions to peripheral blood (Cornblath et al., 1990) are the predominant cells found in nerve. Restricted usage of Vβ genes, especially Vβ15, suggests activation by a common antigen or superantigen (Khalili-Shirazi et al., 1997). Furthermore, γδ T cells have also been found in, and isolated from, GBS-affected nerves (Khalili-Shirazi et al., 1998; Winer et al., 2002). γδ T cells are capable of recognizing non-protein antigen and are thus candidates for responding to putative carbohydrate and ganglioside antigens (see above)

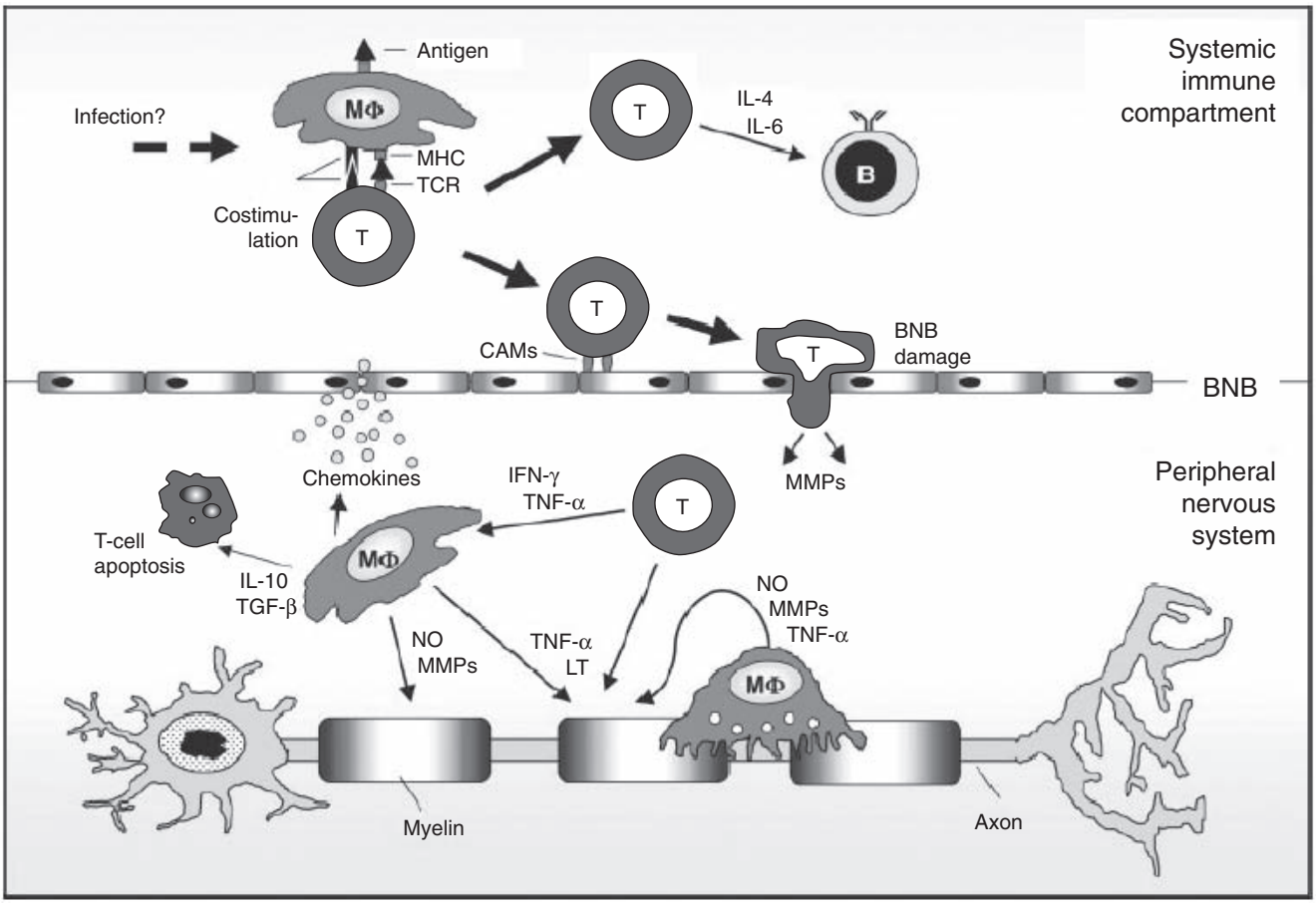


FIGURE 47.5 Schematic of the immune responses in the inflamed peripheral nervous system. Basic principles of the cellular immune responses: autoreactive T cells (T) recognize a specific autoantigen presented by major histocompatibility complex (MHC) class II molecules and the simultaneous delivery of costimulatory signals on the cell surface of antigen-presenting cells, such as macrophages (MΦ), in the systemic immune compartment. Activated T lymphocytes can cross the blood–nerve barrier (BNB) in order to enter the peripheral nervous system (PNS). Within the PNS, T cells activate macrophages that enhance phagocytic activity, production of cytokines, and the release of toxic mediators, such as nitric oxide (NO), matrix metalloproteinases (MMPs), and proinflammatory cytokines, propagating demyelination and axonal loss. The termination of the inflammatory response is mediated, in part, by macrophages by the induction of T-cell apoptosis and the release of anti-inflammatory Th2/Th3 cytokines, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). CAMs, cell adhesion molecules; LT, leukotrienes; TNF-α, tumor necrosis factor-α.

Reproduced from Kieseier et al. (2004), with permission.

(Bukowski et al., 1998). They proliferate *in vitro* in response to *C. jejuni* sonicates, but require either αβ T cells or interleukin (IL)-2/IL-15 to do so (Van Rhijn et al., 2003). Predominant use of Vγ8δ1 suggests activation of epithelially (possibly gut) resident γδ T cells (Cooper et al., 2000). αβ and γδ T cells also probably provide the necessary help to orchestrate class-switching of antiganglioside antibodies to IgG1 and IgG3.

Autoantigen in the systemic compartment is processed and presented by antigen-presenting cells (APCs). EAN can be initiated with neuritogenic epitopes of peripheral nerve proteins P0, P2, and PMP22 (Hughes et al., 1999) or by adoptive transfer (AT) of sensitized T cells. Disease severity depends upon the cell or antigen dosage (Hartung et al., 1996). Processed and unprocessed gangliosides presented in the context of CD1 molecules on APCs also stimulate T-cell activation in *in vitro* assay systems (Prigozy et al., 2001; Shamshiev et al., 2002). A limited literature is emerging demonstrating T-cell responses to glycolipids in multiple sclerosis (Shamshiev et al., 1999). The development of EAN or GBS is dependent on the presence of T cells (Holmdahl et al., 1985) and their normal function (Holmdahl et al., 1985; Hartung et al., 1987; Jung et al., 1992), and the normal function of the costimulatory partners CD80/B7.1 or CD86/B7.2 and CTLA-4/CD28 (Kiefer et al., 2000; Zhu et al. 2001a; 2001b; Zehntner et al., 2003).

Lymphocyte activation is revealed by greater numbers of circulating T cells bearing activation markers and increased concentrations of type 1 helper T-cell (Th1) cytokines, such as interferon (IFN)-γ, IL-2, IL-2 receptor, and tumor necrosis factor (TNF)-α (Taylor and Hughes, 1989; Hartung and Toyka, 1990; Hartung et al., 1991; Exley et al., 1994; Creange et al., 1996; Dahle et al., 1997). Levels of transforming growth factor (TGF)-β1 are depressed (Creange et al., 1998). In EAN, levels of IFN-γ, IL-1β, IL-6, TNF-α, TNF-β, and IL-12 are raised during development of disease (Zhu et al., 1994; 1997).

Homing and migration of activated T cells to the peripheral nerve is modulated by E-selectin and mucins binding L-selectin and sialyl Lewis antigens (Hartung et al., 2002), and then VCAM-1 and ICAM-1, which are both upregulated early in GBS and EAN progression (Enders et al., 1998; Creange et al., 2001). Blockade of VCAM-1 or its ligand VLA-4/α4β1 integrin ameliorates EAN (Enders et al., 1998). Selectin and integrin are released into the circulation and may downregulate inflammation (Hartung et al., 1988). Chemokines assist in leukocyte recruitment, localization, and trafficking (Baggiolini, 1998; Campbell et al., 1998). Recently, the chemokine receptors CCR1, CCR2, CCR4, CCR5, and CXCR3 were characterized in AIDP-affected nerves, differentially upregulated in infiltrating cell populations (Kieseier et al., 2002).

Diapedesis through the vascular endothelium and basal lamina is facilitated by matrix metalloproteinases (MMPs). MMP-2, MMP-3, MMP-7, and especially MMP-9 have been implicated in the pathogenesis of EAN and GBS (Kieseier et al., 1998b; Creange et al., 1999) and correlate with GBS severity.

Macrophages, both resident and recruited from the circulation (Hartung et al., 2002), remain the key component in perpetuating endoneural inflammatory damage through the release of specific immune mediators. Under inflammatory conditions they continue to present antigen and induce Schwann cells to do so also (Gold et al., 1995). Depletion of macrophages abrogates the development of EAN, indicating their central role in the final common pathway of nerve damage. Activated macrophages target normal-looking nerves and Schwann cells in EAN and AIDP (Prineas, 1972; 1981; Hartung et al., 1996; Hughes et al., 1999), probably by antibody-targeted cellular cytotoxicity and complement-dependent mechanisms (Hafer-Macko et al., 1996a; 1996b). Macrophage processes insinuate themselves between myelin lamellae and strip the myelin in AIDP (Hughes et al., 1999) or directly attack the axon in AMAN (see Figure 47.1) (Hafer-Macko et al., 1996a). In the endoneurium, macrophages secrete a host of inflammatory mediators, including MMPs, TNF-α, nitric oxide, eicosanoids, neutral proteases, lipases, and phospholipases, which all contribute to nerve damage (Gregson and Hall, 1973; Watson et al., 1994; Redford et al., 1997a; 1997b; Smith et al., 1999).

Macrophages may also direct the recovery. They direct T-cell apoptosis, reducing the ongoing response. During recovery, the T-cell response shifts towards Th2 with rises in IL-4 in patients (Dahle et al., 1997) and upregulation of TGF-β1, IL-10, and cytolysin in models of disease (Kiefer et al., 1996; Zhu et al., 1997). TGF-β1 favors recovery (Czarniecki et al., 1988; Miller et al., 1992; Vriesendorp et al., 1996; Zhu et al., 1997) and levels correlate with severity of GBS (Creange et al.,1998).

Synergistic Nature of Cellular and Humoral Immune Elements

Understanding of the pathomechanisms of the acute inflammatory neuropathies is incomplete. Neither antibodies nor T cells generate disease in isolation and immunization with any antigen induces responses in both cellular and humoral arms of the immune system. The absence of T cells in some biopsies is discussed above. Arguments against the early and significant involvement of antibodies include the absence of detectable antiganglioside antibodies, using current methodology, in a significant proportion of GBS (usually AIDP) cases, and the onset of AT-EAN 4 days after transfer, before antibodies could be synthesized. However, disease severity in AT-EAN is usually enhanced by cotransferring antibodies recognizing myelin or oligodendrocytes/Schwann cell epitopes (Hahn et al., 1993; Spies et al.,

1995), although this was not confirmed when pretreatment AIDP GBS sera were coadministered to rats with mild EAN (Hadden et al., 2001).

Genetic Aspects

Understanding of the role of host genetics in susceptibility to GBS is still in its infancy. No strong correlations have been established between disease susceptibility or GBS subtypes and host major histocompatiblility complex (MHC) class I or class II haplotypes in several studies (Magira et al., 2003; Geleijns et al., 2005a). The study of single nucleotide polymorphisms in genes for various components of the immune response has not identified any significant contributors (Myhr et al., 2003; Geleijns et al., 2005a; 2005b). One Dutch study identified that GBS patients homozygous for the Fcγ receptor IIa-H131 had a higher chance of developing severe disease than patients with FcγRIIa-R131, FcγRIIIa or FcγRIIIb genotypes or normal controls (van der Pol et al., 2000).

Treatment and Outcome

The effectiveness of immunotherapy for GBS is supported by good evidence (Hughes et al., 2003). Two to five sessions of plasma exchange (PEx) hastens recovery in non-ambulant patients, preferably started within 2 weeks of disease onset. Intravenous immunoglobulin (IVIG) is as effective as PEx and is still probably the intervention of choice. The concern about possible contamination of IVIG with prions means that written informed consent is essential before administration. The drive to search for new, more effective and safer therapies is stronger than ever.

A recent large randomized controlled trial (van Koningsveld et al., 2004), previous smaller trials, and a Cochrane meta-analysis, have shown steroids to be at best ineffective, and in some cases harmful. There is no indication for their use in GBS.

Complement inhibitors are already available and clinical trials in the treatment of GBS are planned. Specific, targeted immunoadsorption of pathogenic antiganglioside antibodies is being developed for possible clinical use (Willison et al., 2004).

CHRONIC NEUROPATHIES: CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired peripheral nervous system disease characterized by progressive or relapsing proximal and distal weakness with or without sensory loss. There is good evidence to indicate that CIDP is autoimmune.

Historic Background

Osler (1892) recognized a chronic relapsing or progressive form of "multiple neuritis." Austin (1958) described a slowly progressive or recurrent "steroid responsive polyneuropathy," with histology indistinguishable from that seen in GBS (Thomas et al., 1969). The term chronic inflammatory demyelinating polyradiculoneuropathy was coined in 1975 (Dyck et al., 1975).

Epidemiology

The prevalence of CIDP is 1.25–7 in 100,000 (Lunn et al., 1998; McLeod et al., 1999; Mygland and Monstad, 2001).

Clinical Features and Subtypes

The course may be progressive, relapsing–remitting, monophasic or have a GBS-like onset. Symmetrical sensory and motor deficits, both proximal and distal, reach their nadir over more than 8 weeks. The cranial nerves and diaphragm are infrequently involved. CSF examination demonstrates albuminocytologic dissociation with raised protein in over 90% of cases (Bouchard et al., 1999) and the cell count is <10 mm^3 unless complicated by human immunodeficiency virus (HIV). Electrophysiology typically demonstrates multifocal motor conduction slowing, and temporal dispersion and block, with delayed or absent F-waves. Half of patients require treatment at any one time and 13% require long-term aid to walk (Lunn et al., 1999). In a single study, 6 of 21 patients were shown to have serum antibodies to the peripheral myelin protein P0 (Yan et al., 2001), but this awaits confirmation. Morphologic examination demonstrates macrophage-associated demyelination in nerve roots, plexuses, and nerve trunks with T cells in the endoneurium. The edema, Schwann cell proliferation, and inflammatory infiltrates testify to the ongoing inflammation. Axonal degeneration occurs in severe or late cases, or in distal sensory nerve biopsies (Hadden and Hughes, 2003).

As with GBS, CIDP is heterogeneous and a number of subtypes or related conditions have emerged.

Multifocal Motor Neuropathy with Conduction Block

Multifocal motor neuropathy with conduction block (MMNCB) (Pestronk et al., 1988) is a rare but treatable condition sometimes misdiagnosed as motor neurone disease (Nobile-Orazio, 2001). Wasting and weakness begin asymmetrically in the distribution of a motor nerve, usually more distal than proximal, most commonly in the upper limb. Fasciculations and cramps occur. Males account for 70% of cases. Sensory symptoms are reported in 20% of patients.

Depending upon assay methods, between 30 and 80% of patients have anti-GM1 antibodies in the serum (Nobile-Orazio, 2001; Sander and Latov, 2003), some as a paraprotein. Rarely, anti-GD1a antibodies are described (Carpo et al., 1996). Electrophysiologic studies demonstrate multifocal conduction blocks in two or more nerves with normal sensory conduction. Many studies demonstrate evidence of more widespread slowing or axonal degeneration (Nobile-Orazio, 2001; Sander and Latov, 2003).

The etiology of MMNCB remains unresolved, as it is seldom fatal and neuropathologic material is seldom sought. Perivascular CIDP-like inflammation has been described in a motor nerve biopsy (Kaji et al., 1993), and there are minimal findings in sensory nerves (Corse et al., 1996). Inflammatory cellular infiltration and immunoglobulin deposition were described in the motor roots of an autopsy case (Oh et al., 1995). Therapeutic response to immunomodulation supports an autoimmune pathogenesis (Azulay et al., 1994; Levine and Pestronk, 1999; Federico et al., 2000; Van den Berg-Vos et al., 2000; Leger et al., 2001; Umapathi et al., 2002).

Multifocal Acquired Demyelinating Sensory and Motor Neuropathy

Lewis and Sumner described the entity now referred to as multifocal acquired demyelinating sensory and motor neuropathy (MADSAM) in 1982 (Lewis et al., 1982). Electrophysiologic examination demonstrates multifocal sensory and motor involvement with conduction block.

Multifocal Acquired Sensory and Motor Neuropathy

This group of patients is only recently described (Alaedini et al., 2003). Antiganglioside antibodies are found in the serum of 48% of multifocal acquired sensory and motor neuropathy (MASMN) cases and there is a beneficial response to immunomodulation with IVIG and steroids. The nerve conduction studies do not suggest demyelination and are more consistent with axonal degeneration. These findings require confirmation.

Paraproteinemic Demyelinating Peripheral Neuropathy

Ten percent of patients with a peripheral neuropathy will have a paraprotein in their serum. Although this should initiate a search for a malignant source, such as multiple or solitary myeloma, most are monoclonal gammopathies of undetermined significance (MGUS). A number of MGUS-neuropathy syndromes are described. The most common is a progressive sensory ataxic neuropathy with unsteadiness and tremor associated with an IgMκ paraprotein that reacts with the HNK-1 epitope of the peripheral nerve antigen myelin-associated glycoprotein (MAG). Up to 80% of patients with an IgM paraprotein and a demyelinating neuropathy have anti-MAG antibodies. Nerve conduction studies reveal motor conduction slowing, particularly distally (Kaku et al., 1994; Cocito et al., 2001). Electron microscopic examination of myelin in sural nerve biopsies reveals characteristic widening of the intraperiod line not seen in other conditions (Figure 47.6).

Neuropathies associated with IgG or IgA paraproteins present in a diverse group of patients with axonal and demyelinating forms of disease. No consistent pathogenesis has yet emerged. The demyelinating cases have a similar therapeutic response to CIDP (Suarez and Kelly, 1993; Notermans et al., 1994; Saperstein et al., 2001).

Chronic ataxic neuropathy with ophthalmoplegia, M-protein, and antidisialosyl antibodies (CANOMAD) is a rare paraproteinemic syndrome rather like a chronic FS. Its pathogenesis is not yet resolved.

The POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) is a rare condition associated with both osteosclerotic myeloma and Castleman disease. The pathogenesis does not seem to relate to the paraprotein as that is not an essential feature of the diagnosis. Cytokines and vascular growth endothelial factor (VGEF) are implicated in causation (Lagueny et al., 2004).

Autoimmune Features

Many of the features of autoimmunity displayed in the acute neuropathies are also seen in CIDP. Both cellular and

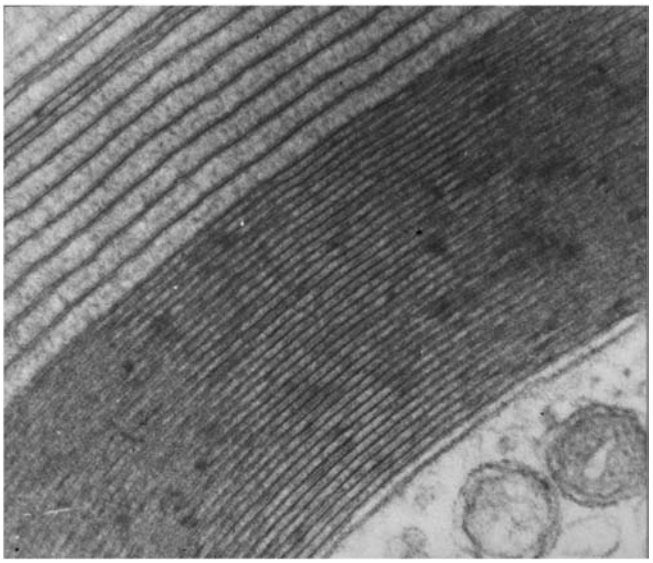


FIGURE 47.6 Widely spaced myelin. The normal myelin lamellae are tightly compacted and have a periodicity in electron microscope preparations of 12–15 nm. In the demyelinating neuropathy associated with IgM paraprotein that has activity against myelin-associated glycoprotein (MAG) (anti-MAG PDPN), the intraperiod line becomes split, giving an overall periodicity of 30–40 nm. Note the suggestion of material within the widened spaces, possibly IgM. Original magnification $\times 10^5$.

humoral responses are important to the pathogenesis. Raised circulating levels of IL-2 and TNF-α correlate with disease activity (Hartung et al., 1991; Misawa et al., 2001). T-lymphocyte migration into the endoneurium is facilitated by increased levels of chemokines, cell adhesion molecules, and MMPs (Previtali et al., 1998; 2001; Kieseier et al., 1998a; 2004). Expression of the BNB constituents claudin-5 and ZO-1 is altered in CIDP, perhaps contributing to the increased BNB permeability (Kanda et al., 2004). Within the endoneurium T cells secrete IFN-γ, IL-2, TNF-α (Mathey et al., 1999), and probably others, contributing to the inflammatory upregulation of effectors such as macrophages, continued recruitment, and direct nerve damage. Expression of downregulatory cytokines IL-4, IL-10, and TGF-β is also found, and in CIDP expression of NGF, GDNF, LIF, and their receptors may contribute to nerve regeneration (Yamamoto et al., 2002).

Unlike GBS or CIDP, in the anti-MAG paraproteinemic demyelinating peripheral neuropathy (anti-MAG PDPN) there is evidence only of antibody-mediated immunity. There is no evidence of macrophage-associated demyelination, T-cell infiltration into the endoneurium or upregulation of T-cell costimulatory molecules. There is quite strong evidence that anti-MAG antibodies may fulfill the Koch–Witebsky postulates. Anti-MAG antibodies can be found in the serum of patients with characteristic clinical presentation. MAG is expressed at the Schmidt–Lantermann incisures, the paranodes, and on the periaxonal myelin (Gabriel et al., 1998). It displays the HNK-1 carbohydrate epitope shared by several other peripheral nerve antigens. Histologic studies demonstrate IgM anti-MAG deposits on Schwann cells that colocalize with MAG (Takatsu et al., 1985; Gabriel et al., 1998). Electron-dense material seen between widened lamellae is consistent with anti-MAG IgM insinuated between the layers (Mendell et al., 1985; Ritz et al., 1999) (see Figure 47.6). Activated complement components have been described by some (Monaco et al., 1990; Ritz et al., 1999). The disruption of MAG functions and complement-mediated damage may result in alterations in the axonal neurofilament cytoskeleton (Ritz et al., 1999; Lunn et al., 2002) and these in turn may lead to slowing of nerve conduction and axonal degeneration.

Immunogenetic Features

Restricted usage of T-cell receptor (TCR) genes has been demonstrated in CIDP. The predominant TCR in CIDP is αβ but γδ T cells are found in the majority of nerve biopsy specimens (Winer et al., 2002), again implying a possible nonprotein immune response. No clonality has been demonstrated in isolated T cells (Bosboom et al., 2001). However, significant numbers of highly Th1 inflammatory natural killer T cells, identified by expression of Vα24JαQ invariant TCR chain, are found (Illes et al., 2000).

Environmental Influences

A preceding illness, infection or vaccination has been identified in 16% and 32% of patients with CIDP in the 6 weeks and 6 months preceding their illness, respectively (McCombe et al., 1987; Bouchard et al., 1999). Others have found no convincing evidence of vaccination being a trigger (Pritchard et al., 2002). No specific provoking environmental events have so far been recognized for CIDP or the paraproteinemic peripheral neuropathies.

Animal Models

The pathogenesis of CIDP is in many respects similar to that of the acute neuropathies. There is no specific animal model for CIDP. Yan et al. (2000) have demonstrated that purified IgG from patients with CIDP responsive to plasma exchange produced conduction block and demyelination by passive transfer when the BNB was breached, either by direct injection or by preceding passive T-cell transfer.

In anti-MAG PDPN, passive transfer of antibodies results in complement-mediated demyelination in cats and rabbits (Hays et al., 1987; Willison et al., 1988), although the pathologic features were not similar to those seen in human disease. Widened myelin lamellae, almost pathognomic of the condition, were demonstrated after passive transfer to chicks (Tatum, 1993). Induction of high-titer IgM antibodies cross-reactive with human MAG by immunization with the peripheral nerve glycolipid sulfated glucuronyl paraglboboside (SGPG) has been achieved in rats, rabbits, and cats (Yamawaki et al., 1996; Kohriyama et al., 1988; Kahn et al., 1989; Ilyas et al., 2002). However, no convincing neuropathy has been demonstrated as a result.

Pathogenic Mechanisms

The final common path of macrophage-mediated demyelination in CIDP is thought to be similar to that seen in GBS (see above).

Treatment and Outcome

Steroids, IVIG, plasma exchange, and immunosuppressive drugs have all been used in the treatment of CIDP. Plasma exchange and IVIG are both very potent (Hahn et al., 1996; van Doorn, 1996; Van Schaik et al., 2002; Mendell et al., 2001). There was no statistically significant difference between steroids and IVIG in a recent trial (Hughes et al., 2001), and even though steroids are cheaper in the short term, IVIG is currently the treatment of choice. Interferon-β-1a was shown to be ineffective in treatment-resistant patients (Hadden et al., 1999). There is inadequate evidence to properly assess anecdotally beneficial drugs,

such as azathioprine, cyclosporine, and mycophenolate mofetil.

In MMNCB a dramatic therapeutic response is sometimes seen to IVIG demonstrated in controlled trials, and cyclophosphamide, interferon-β-1a, and anti-CD20 antibody are also of benefit (Levine and Pestronk, 1999; Azulay et al., 1994; Federico et al., 2000; Umapathi et al., 2002; Leger et al., 2001; Van den Berg-Vos RM et al., 2000). Plasma exchange has no effect and interestingly steroids often worsen the condition by an unknown mechanism. Predictors of favorable outcome are anti-GM1 antibodies, younger age, presence of conduction block, a normal centine kinase (CK), and less severe disease at outset (Hadden and Hughes, 2003). Despite treatment, conduction blocks are dynamic and weakness probably progresses slowly over time (Taylor et al., 2000).

Anti-MAG PDPN patients only need treatment for their neuropathy if it becomes severe and disabling. Randomized controlled evidence of benefit is available for IVIG in the short term (Lunn and Nobile-Orazio, 2003). Fludarabine and anti-CD20 have been beneficial in small series of patients (Wilson et al., 1999; Renaud et al., 2003).

CONCLUDING REMARKS AND FUTURE PROSPECTS

The last decade has witnessed huge advances in the understanding of the pathogenesis of both acute and chronic inflammatory peripheral neuropathies. GBS and CIDP are heterogeneous clinical conditions in which humoral and cellular mechanisms conspire to cause disabling diseases. Although there are still innumerable advances to be made, our greater understanding of pathomechanisms is inspiring trials of novel agents for treatment. The next decade will see the emergence of further exciting discoveries that will significantly reduce death and disability from GBS and CIDP.

Acknowledgment

KAS is supported by NIH grant NS42888 and a grant from the GBS foundation.

References

Alaedini, A., Sander, H.W., Hays, A.P., and Latov N. (2003). Antiganglioside antibodies in multifocal acquired sensory and motor neuropathy. *Arch. Neurol. 60*, 42–46.

Ang, C.W., Yuki, N., Jacobs, B.C., Koga, M., van Doorn, P.A., Schmitz, P.I., and Van der Meche, F.G. (1999). Rapidly progressive, predominantly motor Guillain–Barré syndrome with anti-GalNAc-GD1a antibodies. *Neurology 53*, 2122–2127.

Asbury, A.K. and Cornblath, D.R. (1990). Current diagnostic criteria for Guillain–Barré syndrome. *Ann. Neurol. 27*, S22–S24.

Asbury, A.K., Arnason, B.G., and Adams, R.D. (1969). The inflammatory lesion in idiopathic polyneuritis. *Medicine 48*, 173–215.

Aspinall, G.O., McDonald, A.G., Raju, T.S., Pang, H., Moran, A.P., and Penner, J.L. (1993). Chemical structures of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23, and O:36 lipopolysaccharides. *Eur. J. Biochem. 213*, 1017–1027.

Aspinall, G.O., Fujimoto, S., McDonald, A.G., Pang, H., Kurjanczyk, L.A., and Penner, J.L. (1994a). Lipopolysaccharides from *Campylobacter jejuni* associated with Guillain–Barré syndrome patients mimic human gangliosides in structure. *Infect. Immun. 62*, 2122–2125.

Aspinall, G.O., McDonald, A.G., Pang, H., Kurjanczyk, L.A., and Penner, J.L. (1994b). Lipopolysaccharides of *Campylobacter jejuni* serotype O:19: structures of core oligosaccharide regions from the serostrain and two bacterial isolates from patients with the Guillain–Barré syndrome. *Biochemistry 33*, 241–249.

Austin, J.H. (1958). Recurrent polyneuropathies and their corticosteroid treatment. *Brain 81*, 157–192.

Azulay, J.P., Blin, O., Pouget, J., Boucraut, J., Bille-Turc, F., Carlos, G., and Serratrice, G. (1994). Intravenous immunoglobulin treatment in patients with motor neuron syndromes associated with anti-GM1 antibodies: a double-blind, placebo-controlled study. *Neurology 44*, 429–432.

Baggiolini, M. (1998). Chemokines and leukocyte traffic. *Nature 392*, 565–568.

Bosboom, W.M., Van den Berg, L.H., Mollee, I., Sasker, L.D., Jansen, J., Wokke, J.H., and Logtenberg, T. (2001). Sural nerve T-cell receptor Vbeta gene utilization in chronic inflammatory demyelinating polyneuropathy and vasculitic neuropathy. *Neurology 56*, 74–81.

Bouchard, C., Lacroix, C., Plante, V., Adams, D., Chedru, F., Guglielmi, J.M., and Said, G. (1999). Clinicopathologic findings and prognosis of chronic inflammatory demyelinating polyneuropathy. *Neurology 52*, 498–503.

Bowes, T., Wagner, E.R., Boffey, J., Nicholl, D., Cochrane, L., Benboubetra, M., Conner, J., Furukawa, K., Furukawa, K., and Willison, H.J. (2002). Tolerance to self gangliosides is the major factor restricting the antibody response to lipopolysaccharide core oligosaccharides in *Campylobacter jejuni* strains associated with Guillain–Barré syndrome. *Infect. Immun. 70*, 5008–5018.

Buchwald, B., Weishaupt, A., Toyka, K.V., and Dudel, J. (1995). Immunoglobulin G from a patient with Miller–Fisher syndrome rapidly and reversibly depresses evoked quantal release at the neuromuscular junction of mice. *Neurosci. Lett. 201*, 163–166.

Buchwald, B., Toyka, K.V., Zielasek, J., Weishaupt, A., Schweiger, S., and Dudel, J. (1998). Neuromuscular blockade by IgG antibodies from patients with Guillain–Barré syndrome: a macro-patch-clamp study. *Ann. Neurol. 44*, 913–922.

Buchwald, B., Ahangari, R., and Toyka, K.V. (2002). Differential blocking effects of the monoclonal anti-GQ1b IgM antibody and alpha-latrotoxin in the absence of complement at the mouse neuromuscular junction. *Neurosci. Lett. 334*, 25–28.

Bukowski, J.F., Morita, C.T., Band, H., and Brenner, M.B. (1998). Crucial role of TCR gamma chain junctional region in prenyl pyrophosphate antigen recognition by gamma delta T cells. *J. Immunol. 161*, 286–293.

Campbell, J.J., Hedrick, J., Zlotnik, A., Siani, M.A., Thompson, D.A., and Butcher, E.C. (1988). Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science 279*, 381–384.

Carpo, M., Nobile-Orazio, E., Meucci, N., Gamba, M., Barbieri, S., Allaria, S., and Scarlato, G. (1996). Anti-GD1a ganglioside antibodies in peripheral motor syndromes. *Ann. Neurol. 39*, 539–543.

Carpo, M., Pedotti, R., Lolli, F., Pitrola, A., Allaria, S., Scarlato, G., and Nobile-Orazio, E. (1998). Clinical correlate and fine specificity of anti-GQ1b antibodies in peripheral neuropathy. *J. Neurol. Sci. 155*, 186–191.

Chiba, A., Kusunoki, S., Obata, H., Machinami, R., and Kanazawa, I. (1993). Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain–Barré syndrome: clinical and immunohistochemical studies. *Neurology 43*, 1911–1917.

Chiba, A., Kusunoki, S., Obata, H., Machinami, R., Kanazawa, I. (1997). Ganglioside composition of the human cranial nerves, with special reference to pathophysiology of Miller Fisher syndrome. *Brain Res. 745*, 32–36.

Cocito, D., Isoardo, G., Ciaramitaro, P., Migliaretti, G., Pipieri, A., Barbero, P., Cucci, A., and Durelli, L. (2001). Terminal latency index in polyneuropathy with IgM paraproteinemia and anti-MAG antibody. *Muscle Nerve 24*, 1278–1282.

Cooper, J.C., Ben Smith, A., Savage, C.O., and Winer, J.B. (2000). Unusual T cell receptor phenotype V gene usage of gamma delta T cells in a line derived from the peripheral nerve of a patient with Guillain–Barré syndrome. *J. Neurol. Neurosurg. Psychiatry 69*, 522–524.

Cornblath, D.R., Griffin, D.E., Welch, D., Griffin, J.W., and McArthur, J.C. (1990). Quantitative analysis of endoneurial T-cells in human sural nerve biopsies. *J. Neuroimmunol. 26*, 113–118.

Corse, A.M., Chaudhry, V., Crawford, T.O., Cornblath, D.R., Kunci, R.W., and Griffin, J.W. (1996). Sensory nerve pathology in multifocal motor neuropathy. *Ann. Neurol. 39*, 319–325.

Creange, A., Belec, L., Clair, B., Raphael, J.C., and Gherardi, R.K. (1996). Circulating tumor necrosis factor (TNF)-alpha and soluble TNF-alpha receptors in patients with Guillain–Barré syndrome. *J. Neuroimmunol. 68*, 95–99.

Creange, A., Belec, L., Clair, B., Degos, J.D., Raphael, J.C., and Gherardi, R.K. (1998). Circulating transforming growth factor beta 1 (TGF-beta1) in Guillain–Barré syndrome: decreased concentrations in the early course and increase with motor function. *J. Neurol. Neurosurg. Psychiatry 64*, 162–165.

Creange, A., Sharshar, T., Planchenault, T., Christo, C., Poron, F., Raphael, J.C., and Cherardi, R.K. (1999). Matrix metalloproteinase-9 is increased and correlates with severity in Guillain–Barré syndrome. *Neurology 53*, 1683–1691.

Creange, A., Chazaud, B., Sharshar, T., Planquet, A., Poron, F., Eliezer, M.C., Raphael, J.C., Gherardi, R.K. (2001). Inhibition of the adhesion step of leukodiapedesis: a critical event in the recovery of Guillain–Barré syndrome associated with accumulation of proteolytically active lymphocytes in blood. *J. Neuroimmunol. 114*, 188–196.

Czarniecki, C.W., Chiu, H.H., Wong, G.H., McCabe, S.M., and Palladino, M.A. (1988). Transforming growth factor-beta 1 modulates the expression of class II histocompatibility antigens on human cells. *J. Immunol. 140*, 4217–4223.

Dahle, C., Ekerfelt, C., Vrethem, M., Samuelsson, M., and Ernerudh, J. (1997). T helper type 2 like cytokine responses to peptides from P0 and P2 myelin proteins during the recovery phase of Guillain–Barré syndrome. *J. Neurol. Sci. 153*, 54–60.

De Angelis, M.V., Di Muzio, A., Lupo, S., Gambi, D., Uncini, A., and Lugaresi, A. (2001). Anti-GD1a antibodies from an acute motor axonal neuropathy patient selectively bind to motor nerve fiber nodes of Ranvier. *J. Neuroimmunol. 121*, 79–82.

Dyck, P.J., Lais, A.C., Ohta, M., Bastron, J.A., Okazaki, H., and Groover, R.V. (1975). Chronic inflammatory polyradiculoneuropathy. *Mayo Clin. Proc. 50*, 621–637.

Emilia-Romagna Study Group (1998). Guillain–Barré syndrome variants in Emilia-Romagna, Italy, 1992–3: incidence, clinical features, and prognosis. Emilia-Romagna Study Group on Clinical and Epidemiological Problems in Neurology. *J. Neurol. Neurosurg. Psychiatry 65*, 218–224.

Enders, U., Lobb, R., Pepinsky, R.B., Hartung, H.P., Toyka, K.V., and Gold, R. (1998). The role of the very late antigen-4 and its counter-ligand vascular cell adhesion molecule-1 in the pathogenesis of experimental autoimmune neuritis of the Lewis rat. *Brain 121*, 1257–1266.

Exley, A.R., Smith, N., and Winer, J.B. (1994). Tumour necrosis factor-alpha and other cytokines in Guillain–Barré syndrome. *J. Neurol. Neurosurg. Psychiatry 57*, 1118–1120.

Feasby, T.E., Gilbert, J.J., Brown, W.F., Bolton, C.F., Hahn, A.F., Koopman, W.F., and Zochodne, D.W. (1986). An acute axonal form of Guillain–Barré polyneuropathy. *Brain 109*, 1115–1126.

Federico, P., Zochodne, D.W., Hahn, A.F., Brown, W.F., and Feasby, T.E. (2000). Multifocal motor neuropathy improved by IVIg: randomized, double-blind, placebo-controlled study. *Neurology 55*, 1256–1262.

Fisher, M. (1956). Syndrome of ophthalmoplegia, ataxia and areflexia. *N. Engl. J. Med. 255*, 57–65.

Freeman, R. (2005). Autonomic peripheral neuropathy. *Lancet 365*, 1259–1270.

Friedman, C.R., Neimann, J., Wegener, H.C., and Tauxe, R.V. (2000). Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In I. Nachamkin and M.J. Blaser (eds), *Campylobacter* (pp. 121–138). Washington, DC: American Society for Microbiology.

Fujimoto, S., Yuki, N., Itoh, T., and Amako, K. (1992). Specific serotype of *Campylobacter jejuni* associated with Guillain–Barré syndrome. *J. Infect. Dis. 165*, 183.

Gabriel, J.M., Erne, B., Bernasconi, L., Tosi, C., Probst, A., Landmann, L., and Steck, A.J. (1998). Confocal microscopic localization of anti-myelin-associated glycoprotein autoantibodies in a patient with peripheral neuropathy initially lacking a detectable IgM gammopathy. *Acta. Neuropathol. 95*, 540–546.

Geleijns, K., Laman, J.D., van Rijs, W., Tio-Gillen, A.P., Hintzen, R.Q., van Doorn, P.A., and Jacobs, B.C. (2005a). Fas polymorphisms are associated with the presence of anti-ganglioside antibodies in Guillain–Barré syndrome. *J. Neuroimmunol. 161*, 183–189.

Geleijns, K., Schreuder, G.M., Jacobs, B.C., Sintnicolaas, K., van Koningsveld, R., Meulstee, J., Lamen, J.D., and van Doorn, P.A. (2005b). HLA class II alleles are not a general susceptibility factor in Guillain–Barré syndrome. *Neurology 64*, 44–49.

Goddard, E.A., Lastovica, A.J., and Argent, A.C. (1997). Campylobacter 0:41 isolation in Guillain–Barré syndrome. *Arch. Dis. Child 76*, 526–528.

Gold, R., Toyka, K.V., and Hartung, H.P. (1995). Synergistic effect of IFN-gamma and TNF-alpha on expression of immune molecules and antigen presentation by Schwann cells. *Cell. Immunol. 165*, 65–70.

Gong, Y., Tagawa, Y., Lunn, M.P., Laroy, W., Heffer-Lauc, M., Li, C.Y., Griffin, J.W., Schnaar, R.L., and Sheikh, K.A. (2002). Localization of major gangliosides in the PNS: implications for immune neuropathies. *Brain 125*, 2491–2506.

Goodfellow, J.A., Bowes, T., Sheikh, K., Odaka, M., Halstead, S.K., Humphreys, P.D., Wagner, E.R., Yuki, N., Furukawa, K., Furukawa, K., Plomp, J.S., and Willison, H.J. (2005). Overexpression of GD1a ganglioside sensitizes motor nerve terminals to anti-GD1a antibody-mediated injury in a model of acute motor axonal neuropathy. *J. Neurosci. 25*, 1620–1628.

Goodyear, C.S., O'Hanlon, G.M., Plomp, J.J., Wagner, E.R., Morrison, I., Veitch, J., Cochrane, L., Bullens, R.W., Molenaar, P.C., Conner, J., and Willison, H.J. (1999). Monoclonal antibodies raised against Guillain–Barré syndrome-associated *Campylobacter jejuni* lipopolysaccharides react with neuronal gangliosides and paralyze muscle-nerve preparations. *J. Clin. Invest. 104*, 697–708.

Gregson, N.A. and Hall, S.M. (1973). A quantitative analysis of the effects of the intraneural injection of lysophosphatidyl choline. *J. Cell. Sci. 13*, 257–277.

Griffin, J.W., Li, C.Y., Ho, T.W., Xue, P., Macko, C., Gao, C.Y., Yang, C., Tian, M., Mishu, B., and Cornblath, D.R. (1995). Guillain–Barré

syndrome in northern China. The spectrum of neuropathological changes in clinically defined cases. *Brain 118*, 577–595.

Griffin, J.W., Li, C.Y., Ho, T.W., Tian, M., Gao, C.Y., Xue, P., Mishu, B., Cornblath, D.R., Macko, C., McKhann, G.M., and Asbury, A.K. (1996a). Pathology of the motor-sensory axonal Guillain–Barre syndrome. *Ann. Neurol. 39*, 17–28.

Griffin, J.W., Li, C.Y., Macko, C., Ho, T.W., Hsieh, S.T., Xue, P., Wang, F.A., Cornblath, D.R., McKhann, G.M., and Asbury, A.K. (1996b). Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain–Barré syndrome. *J. Neurocytol. 25*, 33–51.

Guillain, G., Barré, J.A., and Strohl, A. (1916). Sur un syndrome de radiculo-névrite avec hyperalbuminose du liquide cephalorachidien sans réaction cellulaire. Remarques sur les caracteres cliniques et graphiques des reflexes tendineux. *Bull. Soc. Méd. Hôp. Paris 40*, 1462–1470.

Guillain–Barré Syndrome Study Group (1985). Plasmapheresis and acute Guillain–Barré syndrome. The Guillain–Barré syndrome Study Group. *Neurology 35*, 1096–1104.

Hadden, R.D. and Gregson, N.A. (2001). Guillain–Barré syndrome and *Campylobacter jejuni* infection. *Symp. Ser. Soc. Appl. Microbiol.* 145S–154S.

Hadden, R.D. and Hughes, R.A. (2003). Management of inflammatory neuropathies. *J. Neurol. Neurosurg. Psychiatry 74*(Suppl 2), ii9–ii14.

Hadden, R.D., Cornblath, D.R., Hughes, R.A., Zielasek, J., Hartung, H.P., Toyka, K.V., and Swan, A.V. (1998). Electrophysiological classification of Guillain–Barré syndrome: clinical associations and outcome. Plasma Exchange/Sandoglobulin Guillain–Barré Syndrome Trial Group. *Ann. Neurol. 44*, 780–788.

Hadden, R.D., Sharrack, B., Bensa, S., Soudain, S.E., and Hughes, R.A. (1999). Randomized trial of interferon beta-1a in chronic inflammatory demyelinating polyradiculoneuropathy. *Neurology 53*, 57–61.

Hadden, R.D., Gregson, N.A., Gold, R., Willison, H.J., and Hughes, R.A. (2001). Guillain–Barré syndrome serum and anti-Campylobacter antibody do not exacerbate experimental autoimmune neuritis. *J. Neuroimmunol. 119*, 306–316.

Hafer-Macko, C., Hsieh, S.T., Li, C.Y., Ho, T.W., Sheikh, K., Cornblath, D.R., McKhann, G.M., Asbury, A.K., and Griffin, J.W. (1996a). Acute motor axonal neuropathy: an antibody-mediated attack on axolemma. *Ann. Neurol. 40*, 635–644.

Hafer-Macko, C.E., Sheikh, K.A., Li, C.Y., Ho, T.W., Cornblath, D.R., McKhann, G.M., Asbury, A.K., and Griffin, J.W. (1996b). Immune attack on the Schwann cell surface in acute inflammatory demyelinating polyneuropathy. *Ann. Neurol. 39*, 625–635.

Hahn, A.F., Feasby, T.E., Wilkie, L., and Lovgren, D. (1993). Antigalactocerebroside antibody increases demyelination in adoptive transfer experimental allergic neuritis. *Muscle Nerve 16*, 1174–1180.

Hahn, A.F., Bolton, C.F., Zochodne, D., and Feasby, T.E. (1996). Intravenous immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy. A double-blind, placebo-controlled, cross-over study. *Brain 119*, 1067–1077.

Hall, S.M., Hughes, R.A., Atkinson, P.F., McColl, I., and Gale, A. (1992). Motor nerve biopsy in severe Guillain–Barré syndrome. *Ann. Neurol. 31*, 441–444.

Hartung, H.P. and Toyka, K.V. (1990). T-cell and macrophage activation in experimental autoimmune neuritis and Guillain–Barré syndrome. *Ann. Neurol. 27*(Suppl.), S57–S63.

Hartung, H.P., Schafer, B., Fierz, W., Heininger, K., and Toyka, K.V. (1987). Ciclosporin A prevents P2 T cell line-mediated experimental autoimmune neuritis (AT-EAN) in rat. *Neurosci. Lett. 83*, 195–200.

Hartung, H.-P., Heininger, K., Schafer, B., Fierz, W., Toyka K.V. (1988). Immune mechanisms in inflammatory neuropathy. *Adv. Neuroimmunol. 540*, 122–161.

Hartung, H.P., Reiners, K., Schmidt, B., Stoll, G., and Toyka, K.V. (1991). Serum interleukin-2 concentrations in Guillain–Barré syndrome and chronic idiopathic demyelinating polyradiculoneuropathy: comparison with other neurological diseases of presumed immunopathogenesis. *Ann. Neurol. 30*, 48–53.

Hartung, H.-P., Willison, H.J., Jung, S., Pette, M., Toyka, K.V., and Giegerich, G. (1996). Autoimmune responses in peripheral nerve. *Springer Semin. Immunopathol. 18*, 97–123.

Hartung, H.P., Willison, H.J., and Kieseier, B.C. (2002). Acute immunoinflammatory neuropathy: update on Guillain–Barré syndrome. *Curr. Opin. Neurol. 15*, 571–577.

Haymaker, W. and Kernohan, J.W. (1949). The Landry–Guillain–Barré syndrome: a clinicopathologic report of fifty fatal cases and a critique of the literature. *Medicine 28*, 59–141.

Hays, A.P., Latov, N., Takatsu, M., and Sherman, W.H. (1987). Experimental demyelination of nerve induced by serum of patients with neuropathy and an anti-MAG IgM M-protein. *Neurology 37*, 242–256.

Hemachudha, T., Griffin, D.E., Chen, W.W., Johnson, R.T. (1988). Immunologic studies of rabies vaccination-induced Guillain–Barré syndrome. *Neurology 38*, 375–378.

Ho, T.W., Hsieh, S.T., Nachamkin, I., Willison, H.J., Sheikh, K., Kiehlbauch, J., Flanigan, K., McArthur, J.C., Cornblath, D.R., McKhann, G.M., and Griffin, J.W. (1997). Motor nerve terminal degeneration provides a potential mechanism for rapid recovery in acute motor axonal neuropathy after Campylobacter infection. *Neurology 48*, 717–724.

Ho, T.W., Willison, H.J., Nachamkin, I., Li, C.Y., Veitch, J., Ung, H., Wang, G.R., Liu, R.C., Cornblath, D.R., Asbury, A.K., Griffin, J.W., and McKhann, G.M. (1999). Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain–Barré syndrome. *Ann. Neurol. 45*, 168–173.

Holmdahl, R., Olsson, T., Moran, T., and Klareskog, L. (1985). *In vivo* treatment of rats with monoclonal anti-T-cell antibodies. Immunohistochemical and functional analysis in normal rats and in experimental allergic neuritis. *Scand. J. Immunol. 22*, 157–169.

Honavar, M., Tharakan, J.K., Hughes, R.A., Leibowitz, S., and Winer, J.B. (1991). A clinicopathological study of the Guillain–Barré syndrome. Nine cases and literature review. *Brain 114*, 1245–1269.

Hughes, R.A. (1990). *Guillain–Barré Syndrome*. London: Springer-Verlag.

Hughes, R.A. and Rees, J.H. (1997). Clinical and epidemiologic features of Guillain–Barré syndrome. *J. Infect. Dis. 176*(Suppl 2), S92–S98.

Hughes, R.A., Hadden, R.D., Gregson, N.A., and Smith, K.J. (1999). Pathogenesis of Guillain–Barré syndrome. *J. Neuroimmunol. 100*, 74–97.

Hughes, R., Bensa, S., Willison, H., Van den Bergh, P., Comi, G., Illa, I., Nobile-Orazio, E., van Doorn, P., Dalakas, M., Bejar, M., and Swan, A.; Inflammatory Neuropathy Cause and Treatment (INCAT) Group (2001). Randomized controlled trial of intravenous immunoglobulin versus oral prednisolone in chronic inflammatory demyelinating polyradiculoneuropathy. *Ann. Neurol. 50*, 195–201.

Hughes, R.A., Wijdicks, E.F., Barohn, R., Benson, E., Cornblath, D.R., Hahn, A.F., Meythaler, J.M., Miller, R.G., Sladky, J.T., and Stevens, J.C.; Quality Standards Subcommittee of the American Academy of Neurology (2003). Practice parameter: immunotherapy for Guillain–Barré syndrome: report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology 61*, 736–740.

Illes, Z., Kondo, T., Newcombe, J., Oka, N., Tabira, T., and Yamamura, T. (2000). Differential expression of NK T cell V alpha 24J alpha Q invariant TCR chain in the lesions of multiple sclerosis and chronic inflammatory demyelinating polyneuropathy. *J. Immunol. 164*, 4375–4381.

Ilyas, A.A., Chen, Z.W., and Prineas, J.W. (2002). Generation and characterization of antibodies to sulfated glucuronyl glycolipids in Lewis rats. *J. Neuroimmunol. 127*, 54–58.

Jacobs, B.C., Endtz, H., van der Meche, F.G., Hazenberg, M.P., Achtereekte, H.A., van Doorn, P.A. (1995). Serum anti-GQ1b IgG

antibodies recognize surface epitopes on *Campylobacter jejuni* from patients with Miller Fisher syndrome. *Ann. Neurol. 37*, 260–264.

Jacobs, B.C., van Doorn, P.A., Schmitz, P.I., Tio-Gillen, A.P., Herbrink, P., Visser, L.H., Hooijkass, H., and van der Meche, F.G. (1996). *Campylobacter jejuni* infections and anti-GM1 antibodies in Guillain–Barré syndrome. *Ann. Neurol. 40*, 181–187.

Jacobs, B.C., Endtz, H.P., van der Meche, F.G., Hazenberg, M.P., De Klerk, M.A., van Doorn, P.A. (1997a). Humoral immune response against *Campylobacter jejuni* lipopolysaccharides in Guillain–Barré and Miller Fisher syndrome. *J. Neuroimmunol. 79*, 62–68.

Jacobs, B.C., Hazenberg, M.P., van Doorn, P.A., Endtz, H.P., and van der Meche, F.G. (1997b). Cross-reactive antibodies against gangliosides and *Campylobacter jejuni* lipopolysaccharides in patients with Guillain–Barré or Miller Fisher syndrome. *J. Infect. Dis. 175*, 729–733.

Ju, Y.Y., Womersley, H., Pritchard, J., Gray, I., Hughes, R.A., and Gregson, N.A. (2004). *Haemophilus influenzae* as a possible cause of Guillain–Barré syndrome. *J. Neuroimmunol. 149,* 160–166.

Jung, S., Kramer, S., Schluesener, H.J., Hunig, T., Toyka, K., and Hartung, H.P. (1992). Prevention and therapy of experimental autoimmune neuritis by an antibody against T cell receptors-alpha/beta. *J. Immunol. 148*, 3768–3775.

Kahn, S.N., Stanton, N., Sumner, A.J., Brown, M.J., Spitalnik, S.L., and Morein, B. (1989). Analysis of the feline response to human myelin-associated glycoprotein. *J. Neurol. Sci. 89*, 141–148.

Kaji, R., Oka, N., Tsuji, T., Mezaki, T., Nishio, T., Akiguchi, I., and Kimura, J. (1993). Pathological findings at the site of conduction block in multifocal motor neuropathy. *Ann. Neurol. 33*, 152–158.

Kaku, D.A., England, J.D., and Sumner, A.J. (1994). Distal accentuation of conduction slowing in polyneuropathy associated with antibodies to myelin-associated glycoprotein and sulphated glucoronyl paraglobiside. *Brain 117*, 941–947.

Kanda, T., Numata, Y., and Mizusawa, H. (2004). Chronic inflammatory demyelinating polyneuropathy: decreased claudin-5 and relocated ZO-1. *J. Neurol. Neurosurg. Psychiatry 75*, 765–769.

Kaplan, J.E., Katona, P., Hurwitz, E.S., and Schonberger, L.B. (1982). Guillain–Barré syndrome in the United States, 1979–1980 and 1980–1981. Lack of an association with influenza vaccination. *JAMA 248*, 698–700.

Khalili-Shirazi, A., Gregson, N.A., Hall, M.A., Hughes, R.A., and Lanchbury, J.S. (1997). T cell receptor V beta gene usage in Guillain–Barré syndrome. *J. Neurol. Sci. 145*, 169–176.

Khalili-Shirazi, A., Gregson, N.A., Londei, M., Summers, L., and Hughes, R.A. (1998). The distribution of CD1 molecules in inflammatory neuropathy. *J. Neurol. Sci. 158*, 154–163.

Kiefer, R., Funa, K., Schweitzer, T., Jung, S., Bourde, O., Toyka, K.V., and Hartnung, H.P. (1996). Transforming growth factor-beta 1 in experimental autoimmune neuritis. Cellular localization and time course. *Am. J. Pathol. 148*, 211–223.

Kiefer, R., Dangond, F., Mueller, M., Toyka, K.V., Hafler, D.A., and Hartung, H.P. (2000). Enhanced B7 costimulatory molecule expression in inflammatory human sural nerve biopsies. *J. Neurol. Neurosurg. Psychiatry 69*, 362–368.

Kieseier, B.C., Clements, J.M., Pischel, H.B., Wells, G.M., Miller, K., Gearing, A.J., and Hartnung, H.P. (1998a). Matrix metalloproteinases MMP-9 and MMP-7 are expressed in experimental autoimmune neuritis and the Guillain–Barré syndrome. *Ann. Neurol. 43*, 427–434.

Kieseier, B.C., Kiefer, R., Clements, J.M., Miller, K., Wells, G.M., Schweitzer, T., Gearing, A.J., and Hartnung, H.P. (1998b). Matrix metalloproteinase-9 and -7 are regulated in experimental autoimmune encephalomyelitis. *Brain 121*, 159–166.

Kieseier, B.C., Dalakas, M.C., and Hartung, H.P. (2002). Immune mechanisms in chronic inflammatory demyelinating neuropathy. *Neurology 59*, S7–12.

Kieseier, B.C., Kiefer, R., Gold, R., Hemmer, B., Willison, H.J., and Hartung, H.P. (2004). Advances in understanding and treatment of immune-mediated disorders of the peripheral nervous system. *Muscle Nerve 30*, 131–156.

Kissel, J.T., Cornblath, D.R., and Mendell, J.R. (2001). Guillain–Barré syndrome. In J. R. Mendell, J. T. Kissel, and D. R. Cornblath (eds), *Diagnosis and Management of Peripheral Nerve Disorders* (pp. 145–172). New York: Oxford University Press.

Kohriyama, T., Ariga, T., and Yu, R.K. (1988). Preparation and characterization of antibodies against a sulfated glucuronic acid-containing glycosphingolipid. *J. Neurochem. 51*, 869–877.

Kuroki, S., Saida, T., Nukina, M., Haruta, T., Yoshioka, M., Kobayashi, Y., and Nakanishi, H. (1993). *Campylobacter jejuni* strains from patients with Guillain–Barré syndrome belong mostly to Penner serogroup 19 and contain beta-N-acetylglucosamine residues. *Ann. Neurol. 33*, 243–247.

Kusunoki, S., Shimizu, J., Chiba, A., Ugawa, Y., Hitoshi, S., and Kanazawa, I. (1996). Experimental sensory neuropathy induced by sensitization with ganglioside GD1b. *Ann. Neurol. 39*, 424–431.

Kusunoki, S., Hitoshi, S., Kaida, K., Arita, M., and Kanazawa, I. (1999a). Monospecific anti-GD1b IgG is required to induce rabbit ataxic neuropathy. *Ann. Neurol. 45*, 400–403.

Kusunoki, S., Hitoshi, S., Kaida, K., Murayama, S., and Kanazawa, I. (1999b). Degeneration of rabbit sensory neurons induced by passive transfer of anti-GD1b antiserum. *Neurosci. Lett. 273*, 33–36.

Kuwabara, S., Yuki, N., Koga, M., Hattori, T., Matsuura, P., Miyake, M., and Noda, M. (1998). IgG anti-GM1 antibody is associated with reversible conduction failure and axonal degeneration in Guillain–Barré syndrome. *Ann. Neurol. 44*, 202–208.

Kuwabara, S., Ogawara, K., Mizobuchi, K., Koga, M., Mori, M., Hattori, T., and Yuki, N. (2000). Isolated absence of F waves and proximal axonal dysfunction in Guillain–Barré syndrome with antiganglioside antibodies. *J. Neurol. Neurosurg. Psychiatry 68*, 191–195.

Lagueny, A., Bouillot, S., Vital, C., Ferrer, X., Larrieu, J.M., Vital, A. (2004). [POEMS syndrome (or Crow-Fukase syndrome)]. *Rev. Neurol (Paris) 160*, 285–295.

Leger, J.M., Chassande, B., Musset, L., Meininger, V., Bouche, P., and Baumann, N. (2001). Intravenous immunoglobulin therapy in multifocal motor neuropathy: a double-blind, placebo-controlled study. *Brain 124*, 145–153.

Levine, T.D. and Pestronk, A. (1999). IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab. *Neurology 52*, 1701–1704.

Lewis, R.A., Sumner, A.J., Brown, M.J., and Asbury, A.K. (1982). Multifocal demyelinating neuropathy with persistent conduction block. *Neurology 32*, 958–964.

Li, C.Y., Xue, P., Tian, W.Q., Liu, R.C., and Yang, C. (1996). Experimental *Campylobacter jejuni* infection in the chicken: an animal model of axonal Guillain–Barré syndrome. *J. Neurol. Neurosurg. Psychiatry 61*, 279–284.

Lunn, M.P., Johnson, L.A., Fromholt, S.E., Itonori, S., Huang, J., Vyas, A.A., Hildreth, J.E., Griffin, J.W., Schnaer, R.I., and Sheikh, K.A. (2000). High-affinity anti-ganglioside IgG antibodies raised in complex ganglioside knockout mice: reexamination of GD1a immunolocalization. *J. Neurochem. 75*, 404–412.

Lunn, M.P. and Nobile-Orazio, E. (2003). Immunotherapy for IgM anti-myelin-associated glycoprotein paraprotein-associated peripheral neuropathies. *Cochrane Database Syst. Rev.*, CD002827.

Lunn, M.P.T., Manji, H., Choudhary, P.P., Hughes, R.A.C., and Thomas, P.K. (1998). Chronic inflammatory demyelinating polyradiculoneuropathy: A prevalence study in South East England. *J. Neurol. Neurosurg. Psychiatry 66*, 677–680.

Lunn, M.P., Manji, H., Choudhary, P.P., Hughes, R.A., and Thomas, P.K. (1999). Chronic inflammatory demyelinating polyradiculoneu-

ropathy: a prevalence study in south east England. *J. Neurol. Neurosurg. Psychiatry 66*, 677–680.

Lunn, M.P., Crawford, T.O., Hughes, R.A., Griffin, J.W., and Sheikh, K.A. (2002). Anti-myelin-associated glycoprotein antibodies alter neurofilament spacing. *Brain 125*, 904–911.

Magira, E.E., Papaioakim, M., Nachamkin, I., Ashbury, A.K., Li, C.Y., Ho, T.W., Griffin, J.W., McKhann, G.M., and Monos, D.S. (2003). Differential distribution of HLA-DQ beta/DR beta epitopes in the two forms of Guillain–Barré syndrome, acute motor axonal neuropathy and acute inflammatory demyelinating polyneuropathy (AIDP): identification of DQ beta epitopes associated with susceptibility to and protection from AIDP. *J. Immunol. 170*, 3074–3080.

Massaro, M.E., Rodriguez, E.C., Pociecha, J., Arroyo, H.A., Sacolitti, M., Taratuto, A.L., Fejerman, N., and Reisin, R.C. (1988). Nerve biopsy in children with severe Guillain–Barré syndrome and inexcitable motor nerves. *Neurology 51*, 394–398.

Mathey, E.K., Pollard, J.D., and Armati, P.J. (1999). TNF alpha, IFN gamma and IL-2 mRNA expression in CIDP sural nerve biopsies. *J. Neurol. Sci. 163*, 47–52.

McCombe, P.A., Pollard, J.D., and McLeod, J.G. (1987). Chronic inflammatory demyelinating polyradiculoneuropathy. A clinical and electrophysiological study of 92 cases. *Brain 110*, 1617–1630.

McKhann, G.M., Cornblath, D.R., Ho, T., Li, C.Y., Bai, A.Y., Wu, H.S., Yei, Q.F., Zhang, W.C., Zhaori, Z., and Jiang, Z. (1991). Clinical and electrophysiological aspects of acute paralytic disease of children and young adults in northern China. *Lancet 338*, 593–597.

McKhann, G.M., Cornblath, D.R., Griffin, J.W., Ho, T.W., Li, C.Y., Jiang, Z., Wu, H.S., Zhaori, G., Liu, Y., and Jou, L.P. (1993). Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. *Ann. Neurol. 33*, 333–342.

McLeod, J.G., Pollard, J.D., Macaskill, P., Mohamed, A., Spring, P., and Khurana, V. (1999). Prevalence of chronic inflammatory demyelinating polyneuropathy in New South Wales, Australia. *Ann. Neurol. 46*, 910–913.

Mendell, J.R., Sahenk, Z., Whitaker, J.N., Trapp, B.D., Yates, A.J., Griggs, R.C., and Quarles, R.H. (1985). Polyneuropathy and IgM monoclonal gammopathy: Studies on the pathogenic role of the myelin-associated glycoprotein antibody. *Ann. Neurol. 17*, 243–254.

Mendell, J.R., Barohn, R.J., Freimer, M.L., Kissel, J.T., King, W., Nagaraja, H.N., Rice, R., Campbell, W.W., Donofrio, P.D., Jackson, C.E., Lewis, R.A., Shy, M., Simpson, D.M., Parry, G.J., Rivner, M.H., Thornton, C.A., Bromberg, M.B., Tanden, R., Harati, Y., and Giuliani, M.J.; Working Group on Peripheral Neuropathy (2001). Randomized controlled trial of IVIg in untreated chronic inflammatory demyelinating polyradiculoneuropathy. *Neurology 56*, 445–449.

Miller, A., Lider, O., Roberts, A.B., Sporn, M.B., and Weiner, H.L. (1992). Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor beta after antigen-specific triggering. *Proc. Natl. Acad. Sci. USA 89*, 421–425.

Misawa, S., Kuwabara, S., Mori, M., Kawaguchi, N., Yoshiyama, Y., and Hattori, T. (2001). Serum levels of tumor necrosis factor-alpha in chronic inflammatory demyelinating polyneuropathy. *Neurology 56*, 666–669.

Mizoguchi, K. (1998). Anti-GQ1b IgG antibody activities related to the severity of Miller Fisher syndrome. *Neurol. Res. 20*, 617–624.

Monaco, S., Bonetti, B., Ferrari, S., Moretto, G., Nardelli, E., Tedesco, F., Mollners, T.E., Nobile-Orazio, E., Monfredini, E., and Bonazzi, L. (1990). Complement-mediated demyelination in patients with IgM monoclonal gammopathy and polyneuropathy. *N. Engl. J. Med. 322*, 649–652.

Moran, A.P., Prendergast, M.M., Hogan, E.L. (2002). Sialosyl-galactose: a common denominator of Guillain–Barré and related disorders? *J. Neurol. Sci. 196*, 1–7.

Mori, M., Kuwabara, S., Miyake, M., Noda, M., Kuroki, H., Kanno, H., Ogawara, K., and Hattori, T. (2000). *Haemophilus influenzae* infection and Guillain–Barré syndrome. *Brain 123*, 2171–2178.

Mygland, A. and Monstad, P. (2001). Chronic polyneuropathies in Vest-Agder, Norway. *Eur. J. Neurol. 8*, 157–165.

Myhr, K.M., Vagnes, K.S., Maroy, T.H., Aarseth, J.H., Nyland, H.I., and Vedeler, C.A. (2003). Interleukin-10 promoter polymorphisms in patients with Guillain–Barré syndrome. *J. Neuroimmunol. 139*, 81–83.

Nachamkin, I., Ung, H., Moran, A.P., Yoo, D., Prendergast, M.M., Nicholson, M.A., Sheikh, K., Ho, T., Ashbury, A.K., McKhann, G.M., and Griffin, J.W. (1999). Ganglioside GM1 mimicry in Campylobacter strains from sporadic infections in the United States. *J. Infect. Dis. 179*, 1183–1189.

Nachamkin, I., Liu, J., Li, M., Ung, H., Moran, A.P., Prendergast, M.M., and Sheikh, K. (2002). *Campylobacter jejuni* from patients with Guillain–Barré syndrome preferentially expresses a GD(1a)-like epitope. *Infect. Immun. 70*, 5299–5303.

Nagai, Y., Momoi, T., Saito, M., Mitsuzawa, E., and Ohtani, S. (1976). Ganglioside syndrome, a new autoimmune neurologic disorder, experimentally induced with brain gangliosides. *Neurosci. Lett. 2*, 107–111.

Nobile-Orazio, E. (2001). Multifocal motor neuropathy. *J. Neuroimmunol. 115*, 4–18.

Notermans, N.C., Wokke, J.H.J., Lokhorst, H.M., Franssen, H., Van Der Graaf, Y., and Jennekens, F.G.I. (1994). Polyneuropathy associated with monoclonal gammopathy of undertermined significance: a prospective study of the prospective value of clinical and laboratory abnormalities. *Brain 117*, 1385–1393.

O'Hanlon, G.M., Plomp, J.J., Chakrabarti, M., Morrison, I., Wagner, E.R., Goodyear, C.S., Yin, X., Trapp, B.D., Conner, J., Molenaar, P.C., Stewart, J., Rowan, E.G., and Willison, H.J. (2001). Anti-GQ1b ganglioside antibodies mediate complement-dependent destruction of the motor nerve terminal. *Brain 124*, 893–906.

Oberhelman, R.A. and Taylor D.N. (2000). Campylobacter infections in developing countries. In I. Nachamkin and M. J. Blaser (eds), *Campylobacter* (pp. 139–153). Washington DC: American Society for Microbiology.

Ogawara, K., Kuwabara, S., Mori, M., Hattori, T., Koga, M., and Yuki, N. (2000). Axonal Guillain–Barré syndrome: relation to anti-ganglioside antibodies and *Campylobacter jejuni* infection in Japan. *Ann. Neurol. 48*, 624–631.

Ogino, M., Orazio, N., and Latov, N. (1995). IgG anti-GM1 antibodies from patients with acute motor neuropathy are predominantly of the IgG1 and IgG3 subclasses. *J. Neuroimmunol. 58*, 77–80.

Oh, S.J., Claussen, G.C., Odabasi, Z., and Palmer, C.P. (1995). Multifocal demyelinating motor neuropathy: pathologic evidence of 'inflammatory demyelinating polyradiculoneuropathy'. *Neurology 45*, 1828–1832.

Osler, W. (1892). Acute ascending (Landry's) paralysis. In J. Young (ed.) *Principles and Practice of Medicine*. London: Pentland.

Pestronk, A., Cornblath, D.R., Ilyas, A.A., Baba, H., Quarles, R.H., Griffin, J.W., Alderson, K., and Adams, R.N. (1988). A treatable multifocal motor neuropathy with antibodies to GM1 ganglioside. *Ann. Neurol. 24*, 73–78.

Plomp, J.J., Molenaar, P.C., O'Hanlon, G.M., Jacobs, B.C., Veitch, J., Daha, M.R., van Doorn, P.A., van der Meche, F.G., Vincent, A., Morgan, B.P., and Willison, H.J. (1999). Fisher anti-GQ1b antibodies: alpha-latrotoxin-like effects on motor end plates. *Ann. Neurol. 45*, 189–199.

Prendergast, M.M. and Moran, A.P. (2000). Lipopolysaccharides in the development of the Guillain–Barré syndrome and Miller Fisher syndrome forms of acute inflammatory peripheral neuropathies. *J. Endotoxin Res. 6*, 341–359.

Previtali, S.C., Archelos, J.J., and Hartung, H.P. (1998). Expression of integrins in experimental autoimmune neuritis and Guillain–Barré syndrome. *Ann. Neurol. 44*, 611–621.

Previtali, S.C., Feltri, M.L., Archelos, J.J., Quattrini, A., Wrabetz, L., and Hartung, H. (2001). Role of integrins in the peripheral nervous system. *Prog. Neurobiol. 64*, 35–49.

Prigozy, T.I., Naidenko, O., Qasba, P., Elewaut, D., Brossay, L., Khurana, A., Natori, T., Koezuka, Y., Kulkarni, A., and Kronenberg, M. (2001). Glycolipid antigen processing for presentation by CD1d molecules. *Science 291*, 664–667.

Prineas, J.W. (1972). Acute idiopathic polyneuritis. An electron microscope study. *Lab. Invest. 26*, 133–147.

Prineas, J.W. (1981). Pathology of the Guillain–Barré syndrome. *Ann. Neurol. 9*(Suppl), 6–19.

Pritchard, J., Mukherjee, R., and Hughes, R.A. (2002). Risk of relapse of Guillain–Barré syndrome or chronic inflammatory demyelinating polyradiculoneuropathy following immunisation. *J. Neurol. Neurosurg. Psychiatry 73*, 348–349.

Ramos-Alvarez, M., Bessudo, L., and Sabin, A.B. (1969). Paralytic syndromes associated with noninflammatory cytoplasmic or nuclear neuronopathy. Acute paralytic disease in Mexican children, neuropathologically distinguishable from Landry–Guillain–Barré syndrome. *JAMA 207*, 1481–1492.

Redford, E.J., Kapoor, R., and Smith, K.J. (1997a). Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. *Brain 120*, 2149–2157.

Redford, E.J., Smith, K.J., Gregson, N.A., Davies, M., Hughes, P., Gearing, A.J., Miller, K., and Hughes, R.A. (1997b). A combined inhibitor of matrix metalloproteinase activity and tumour necrosis factor-alpha processing attenuates experimental autoimmune neuritis. *Brain 120*, 1895–1905.

Rees, J.H., Gregson, N.A., and Hughes, R.A. (1995a). Anti-ganglioside GM1 antibodies in Guillain–Barré syndrome and their relationship to *Campylobacter jejuni* infection. *Ann. Neurol. 38*, 809–816.

Rees, J.H., Soudain, S.E., Gregson, N.A., Hughes, R.A. (1995b). *Campylobacter jejuni* infection and Guillain–Barré syndrome. *N. Engl. J. Med. 333*, 1374–1379.

Rees, J.H., Thompson, R.D., Smeeton, N.C., and Hughes, R.A. (1998). Epidemiological study of Guillain–Barré syndrome in south east England. *J. Neurol. Neurosurg. Psychiatry 64*, 74–77.

Reisin, R.C., Cersosimo, R., Garcia, A.M., Massaro, M., and Fejerman, N. (1993). Acute "axonal" Guillain–Barré syndrome in childhood. *Muscle Nerve 16*, 1310–1316.

Renaud, S., Gregor, M., Fuhr, P., Lorenz, D., Deuschl, G., Gratwohl, A., and Steck, A.J. (2003). Rituximab in the treatment of polyneuropathy associated with anti-MAG antibodies. *Muscle Nerve 27*, 611–615.

Ritz, M.F., Erne, B., Ferracin, F., Vital, A., Vital, C., and Steck, A.J. (1999). Anti-MAG IgM penetration into myelinated fibers correlates with the extent of myelin widening. *Muscle Nerve 22*, 1030–1037.

Rose, N.R. and Bona, C. (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today 14*, 426–430.

Saida, K., Saida, T., Brown, M.J., and Silberberg, D.H. (1979). *In vivo* demyelination induced by intraneural injection of anti-galactocerebroside serum: a morphologic study. *Am. J. Pathol. 95*, 99–116.

Sander, H.W. and Latov, N. (2003). Research criteria for defining patients with CIDP. *Neurology 60*, S8–15.

Saperstein, D.S., Katz, J.S., Amato, A.A., and Barohn, R.J. (2001). Clinical spectrum of chronic acquired demyelinating polyneuropathies. *Muscle Nerve 24*, 311–324.

Shamshiev, A., Donda, A., Carena, I., Mori, L., Kappos, L., and De Libero, G. (1999). Self glycolipids as T-cell autoantigens. *Eur. J. Immunol. 29*, 1667–1675.

Shamshiev, A., Gober, H.J., Donda, A., Mazorra, Z., Mori, L., and De Libero, G. (2002). Presentation of the same glycolipid by different CD1 molecules. *J. Exp. Med. 195*, 1013–1021.

Sheikh, K.A., Nachamkin, I., Ho, T.W., Willison, H.J., Veitch, J., Ung, H., Nicholson, M., Li, C.Y., Wu, H.S., Shen, B.Q., Cornblath, D.R., Ashbury, A.K., McKhann, G.M., and Griffin, J.W. (1998). *Campylobacter jejuni* lipopolysaccharides in Guillain–Barré syndrome: molecular mimicry and host susceptibility. *Neurology 51*, 371–378.

Sheikh, K.A., Deerinck, T.J., Ellisman, M.H., and Griffin, J.W. (1999). The distribution of ganglioside-like moieties in peripheral nerves. *Brain 122*, 449–460.

Sheikh, K.A., Zhang, G., Gong, Y., Schnaar, R.L., and Griffin, J.W. (2004). An anti-ganglioside antibody-secreting hybridoma induces neuropathy in mice. *Ann. Neurol. 56*, 228–239.

Smith, K.J., Kapoor, R., and Felts, P.A. (1999). Demyelination: the role of reactive oxygen and nitrogen species. *Brain Pathol. 9*, 69–92.

Spies, J.M., Pollard, J.D., Bonner, J.G., Westland, K.W., and McLeod, J.G. (1995). Synergy between antibody and P2-reactive T cells in experimental allergic neuritis. *J. Neuroimmunol. 57*, 77–84.

Suarez, G.A. and Kelly, J.J., Jr. (1993). Polyneuropathy associated with monoclonal gammopathy of undetermined significance: further evidence that IgM MGUS neuropathies are different than IgG-MGUS. *Neurology 43*, 1304–1308.

Sumner, A.J., Said, G., Idy, I., and Metral, S. (1992). Demyelinative conduction block produced by intraneural injection of human Guillain–Barré syndrome serum into rat sciatic nerve. *Neurology 32*, A106.

Susuki, K., Nishimoto, Y., Yamada, M., Baba, M., Veda, S., Hirata, K., and Yuki, N. (2003). Acute motor axonal neuropathy rabbit model: immune attack on nerve root axons. *Ann. Neurol. 54*, 383–388.

Takatsu, M., Hays, A.P., Latov, N., Abrams, G.M., Nemni, R., Sherman, W.H., Nobile-Orazio, E., Saito, T., and Freddo, L. Immunofluorescence study of patients with neuropathy and IgM M proteins. *Ann. Neurol. 18*, 173–181.

Takigawa, T., Yasuda, H., Kikkawa, R., Shigeta, Y., Saida, T., and Kitasato, H. (1995). Antibodies against GM1 ganglioside affect K^+ and Na^+ currents in isolated rat myelinated nerve fibers. *Ann. Neurol. 37*, 436–442.

Takigawa, T., Yasuda, H., Terada, M., Haneda, M., Kashiwagi, A., Saito, T., Saida, T., Kitasato, H., and Kikkawa, R. (2000). The sera from GM1 ganglioside antibody positive patients with Guillain–Barré syndrome or chronic inflammatory demyelinating polyneuropathy blocks Na^+ currents in rat single myelinated nerve fibers. *Intern. Med. 39*, 123–127.

Tatum, A.H. (1993). Experimental paraprotein neuropathy, demyelination by passive transfer of human IgM anti-myelin-associated glycoprotein. *Ann. Neurol. 33*, 502–506.

Taylor, W.A. and Hughes, R.A. (1989). T lymphocyte activation antigens in Guillain–Barré syndrome and chronic idiopathic demyelinating polyradiculoneuropathy. *J. Neuroimmunol. 24*, 33–39.

Taylor, B.V., Wright, R.A., Harper, C.M., and Dyck, P.J. (2000). Natural history of 46 patients with multifocal motor neuropathy with conduction block. *Muscle Nerve 23*, 900–908.

Thomas, P.K., Lascelles, R.G., Hallpike, J.F., and Hewer, R.L. (1969). Recurrent and chronic relapsing Guillain–Barré polyneuritis. *Brain 92*, 589–606.

Umapathi, T., Hughes, R.A., Nobile-Orazio, E., and Leger, J.M. (2002). Immunosuppressive treatment for multifocal motor neuropathy. *Cochrane Database Syst. Rev.*, CD003217.

Uncini, A. and Lugaresi, A. (1999). Fisher syndrome with tetraparesis and antibody to GQ1b: evidence for motor nerve terminal block. *Muscle Nerve 22*, 640–644.

Van den Berg-Vos, R.M., Van den Berg, L.H., Franssen, H., van Doorn, P.A., Merkies, I.S, and Wokke, J.H. (2000). Treatment of multifocal motor neuropathy with interferon-beta1A. *Neurology 54*, 1518–1521.

van der Pol, W.L., Van den Berg, L.H., Scheepers R.H., et al. (2000). IgG receptor IIa alleles determine susceptibility and severity of Guillain–Barré syndrome. *Neurology 54*, 1661–1665.

van Doorn, P.A. (1996). Treatment of patients with chronic inflammatory demyelinating polyneuropathy. *Revue Neurologique 152*, 383–386.

van Koningsveld, R., Schmitz, P.I., Meche, F.G., Visser, L.H., Meulstee, J., van Doorn, P.A. (2004). Effect of methylprednisolone when added to standard treatment with intravenous immunoglobulin for Guillain–Barré syndrome: randomised trial. *Lancet 363*, 192–196.

Van Rhijn, I., Van den Berg, L.H., Ang, C.W., Admiraal, J., and Logtenberg, T. (2003). Expansion of human gammadelta T cells after *in vitro* stimulation with *Campylobacter jejuni*. *Int. Immunol. 15*, 373–382.

Van Schaik, I.N., Winer, J.B., de Haan, R., and Vermeulen, M. (2002). Intravenous immunoglobulin for chronic inflammatory demyelinating polyradiculoneuropathy. *Cochrane Database Syst. Rev.*, CD001797.

Vernino, S., Low, P.A., Fealey, R.D., Stewart, J.D., Farrugia, G., Lennon, V.A. (2000). Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N. Engl. J. Med. 343*, 847–855.

Visser, L.H., van der Meche, F.G., van Doorn P.A., Meulstee, J., Jacobs, B.C., Oomers, P.G., Kleyweg, R.P., and Meulstee, J. (1995). Guillain–Barré syndrome without sensory loss (acute motor neuropathy). A subgroup with specific clinical, electrodiagnostic and laboratory features. Dutch Guillain–Barré Study Group. *Brain 118*, 841–847.

Vriesendorp, F.J., Flynn, R.E., Khan, M., Pappolla, M.A., and Brod, S.A. (1996). Oral administration of type I interferon modulates the course of experimental allergic neuritis. *Autoimmunity 24*, 157–165.

Watson, S.L., Westland, K., and Pollard, J.D. (1994). An electrophysiological and histological study of trypsin induced demyelination. *J. Neurol. Sci. 126*, 116–125.

Weber, F., Rudel, R., Aulkemeyer, P., and Brinkmeier, H. (2000). Anti-GM1 antibodies can block neuronal voltage-gated sodium channels. *Muscle Nerve 23*, 1414–1420.

Willison, H.J. and O'Hanlon, G.M. (1999). The immunopathogenesis of Miller Fisher syndrome. J. Neuroimmunol. *100*, 3–12.

Willison, H.J. and Veitch, J. (1994). Immunoglobulin subclass distribution and binding characteristics of anti-GQ1b antibodies in Miller Fisher syndrome. *J. Neuroimmunol. 50*, 159–165.

Willison, H.J. and Yuki, N. (2002). Peripheral neuropathies and anti-glycolipid antibodies. *Brain 125*, 2591–2625.

Willison, H.J., Trapp, B.D., Bacher, J.D., Dalakas, M.C., Griffin, J.W., and Quarles, R.H. (1988). Demyelination induced by intraneural injection of human antimyelin-associated glycoprotein antibodies. *Muscle Nerve 11*, 1169–1176.

Willison, H.J., Veitch, J., Paterson, G., and Kennedy, P.G. (1993). Miller Fisher syndrome is associated with serum antibodies to GQ1b ganglioside. *J. Neurol. Neurosurg. Psychiatry 56*, 204–206.

Willison, H.J., O'Hanlon, G.M., Paterson, G., Veitch, J., Wilson, G., Roberts, M., Tang, T., and Vincent, A. (1996). A somatically mutated human antiganglioside IgM antibody that induces experimental neuropathy in mice is encoded by the variable region heavy chain gene, V1-18. *J. Clin. Invest. 97*, 1155–1164.

Willison, H.J., Townson, K., Veitch, J., Boffey, J., Isaacs, N., Andersen, S.M., Zhang, P., Ling, C.C., and Bundle, D.R. (2004). Synthetic disialylgalactose immunoadsorbents deplete anti-GQ1b antibodies from autoimmune neuropathy sera. *Brain 127*, 680–691.

Wilson, H.C., Lunn, M.P., Schey, S., and Hughes, R.A. (1999). Successful treatment of IgM paraproteinaemic neuropathy with fludarabine. *J. Neurol. Neurosurg. Psychiatry 66*, 575–580.

Winer, J.B., Gray, I.A., Gregson, N.A., Hughes, R.A., Leibowitz, S., Shepherd, P., Taylor, W.A., and Yewdall, V. (1988). A prospective study of acute idiopathic neuropathy. III. Immunological studies. *J. Neurol. Neurosurg. Psychiatry 51*, 619–625.

Winer, J., Hughes, S., Cooper, J., Ben Smith, A., and Savage, C. (2002). Gamma delta T cells infiltrating sensory nerve biopsies from patients with inflammatory neuropathy. *J. Neurol. 249*, 616–621.

Wirguin, I., Briani, C., Suturkova-Milosevic, L., Fisher, T., Della-Lotta, P., Chaliff, P., and Latov, N. (1997). Induction of GM1 ganglioside antibodies by *Campylobacter jejuni* lipopolysaccharides. *J. Neuroimmunol. 78*, 138–142.

Witebsky, E., Rose, N.R., Terplan, K., Paine, J.R., and Egan, R.W. (1957). Chronic thyroiditis and autoimmunization. *JAMA 164*, 1439–1447.

Yamamoto, M., Ito, Y., Mitsuma, N., Li, M., Hattori, N., and Sobue, G. (2002). Parallel expression of neurotrophic factors and their receptors in chronic inflammatory demyelinating polyneuropathy. *Muscle Nerve 25*, 601–604.

Yamawaki, M., Vasquez, A., Ben Younes, A., Yoshino, H., Kanda, T., Ariga, T., Baumann, N., and Yu, R.K. (1996). Sensitization of Lewis rats with sulfoglucuronosyl paragloboside: electrophysiological and immunological studies of an animal model of peripheral neuropathy. *J. Neurosci. Res. 44*, 58–65.

Yan, W.X., Taylor, J., Andrias-Kauba, S., and Pollard, J.D. (2000). Passive transfer of demyelination by serum or IgG from chronic inflammatory demyelinating polyneuropathy patients. *Ann. Neurol. 47*, 765–775.

Yan, W.X., Archelos, J.J., Hartung, H.P., and Pollard J.D. (2001). P0 protein is a target antigen in chronic inflammatory demyelinating polyradiculoneuropathy. *Ann. Neurol. 50*, 286–292.

Yuki, N., Yoshino, H., Sato, S., and Miyatake, T. (1990). Acute axonal polyneuropathy associated with anti-GM1 antibodies following Campylobacter enteritis. *Neurology 40*, 1900–1902.

Yuki, N., Sato, S., Fujimoto, S., Yamada, S., Tsujino, Y., Kinoshita, A., and Itoh, T. (1992). Serotype of *Campylobacter jejuni*, HLA, and the Guillain–Barré syndrome. *Muscle Nerve 15*, 968–969.

Yuki, N., Sato, S., Tsuji, S., Ohsawa, T., and Miyatake, T. (1993a). Frequent presence of anti-GQ1b antibody in Fisher's syndrome. *Neurology 43*, 414–417.

Yuki, N., Yamada, M., Sato, S., Ohama, E., Kawase, Y., Ikuta, F., and Miyatake, T. (1993b). Association of IgG anti-GD1a antibody with severe Guillain-Barre syndrome. *Muscle Nerve 16*, 642–647.

Yuki, N., Taki, T., Takahashi, M., Saito, K., Yoshino, H., Tai, T., Handa, S., and Miyatake, T. (1994). Molecular mimicry between GQ1b ganglioside and lipopolysaccharides of *Campylobacter jejuni* isolated from patients with Fisher's syndrome. *Ann. Neurol. 36*, 791–793.

Yuki, N., Ichihashi, Y., and Taki, T. (1995). Subclass of IgG antibody to GM1 epitope-bearing lipopolysaccharide of *Campylobacter jejuni* in patients with Guillain–Barré syndrome. *J. Neuroimmunol. 60*, 161–164.

Yuki, N., Taki, T., and Handa, S. (1996). Antibody to GalNAc-GD1a and GalNAc-GM1b in Guillain–Barré syndrome subsequent to *Campylobacter jejuni* enteritis. *J. Neuroimmunol. 71*, 155–161.

Yuki, N., Ho, T.W., Tagawa, Y., Koga, M., Li, C. Y., Hirata, K., and Griffin, J.W. (1999). Autoantibodies to GM1b and GalNAc-GD1a: relationship to *Campylobacter jejuni* infection and acute motor axonal neuropathy in China. J *Neurol. Sci. 164*, 134–138.

Yuki, N., Ang, C.W., Koga, M., Jacobs, B.C., van Doorn, P.A., Hirata, K., and van der Meche, F.G. (2000). Clinical features and response to treatment in Guillain–Barré syndrome associated with antibodies to GM1b ganglioside. *Ann. Neurol. 47*, 314–321.

Yuki, N., Yamada, M., Koga, M., Odaki, M., Susuki, K., Tagawa, Y., Ueda, S., Kasama, T., Ohnishi, A., Hayashi, S., Takahashi, H., Kamijo, M., and Hirata, K. (2001). Animal model of axonal Guillain–Barré syndrome induced by sensitization with GM1 ganglioside. *Ann. Neurol. 49*, 712–720.

Zehntner, S.P., Brisebois, M., Tran, E., Owens, T., and Fournier, S. (2003). Constitutive expression of a costimulatory ligand on antigen-presenting cells in the nervous system drives demyelinating disease. *FASEB J. 17*, 1910–1912.

Zhu, J., Mix, E., Olsson, T., and Link, H. (1994). Cellular mRNA expression of interferon-gamma, IL-4 and transforming growth factor-beta (TGF-beta) by rat mononuclear cells stimulated with peripheral nerve myelin antigens in experimental allergic neuritis. *Clin. Exp. Immunol. 98*, 306–312.

Zhu, J., Bai, X. F., Mix, E., and Link, H. (1997). Experimental allergic neuritis: cytolysin mRNA expression is upregulated in lymph node cells during convalescence. *J. Neuroimmunol. 78*, 108–116.

Zhu, J., Zou, L., Zhu, S., Mix, E., Shi, F., Wang, H., Volkmann, I., Winblad, B., Schalling, M., and Ljunggren, H. (2001a). Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade enhances incidence and severity of experimental autoimmune neuritis in resistant mice. *J. Neuroimmunol. 115*, 111–117.

Zhu, Y., Ljunggren, H., Mix, E., Li, H.L., van der Meide, P., Elhassan, A.M., Winblad, B., and Zhu, J. (2001b). CD28-B7 costimulation: a critical role for initiation and development of experimental autoimmune neuritis in C57BL/6 mice. *J. Neuroimmunol. 114*, 114–121.

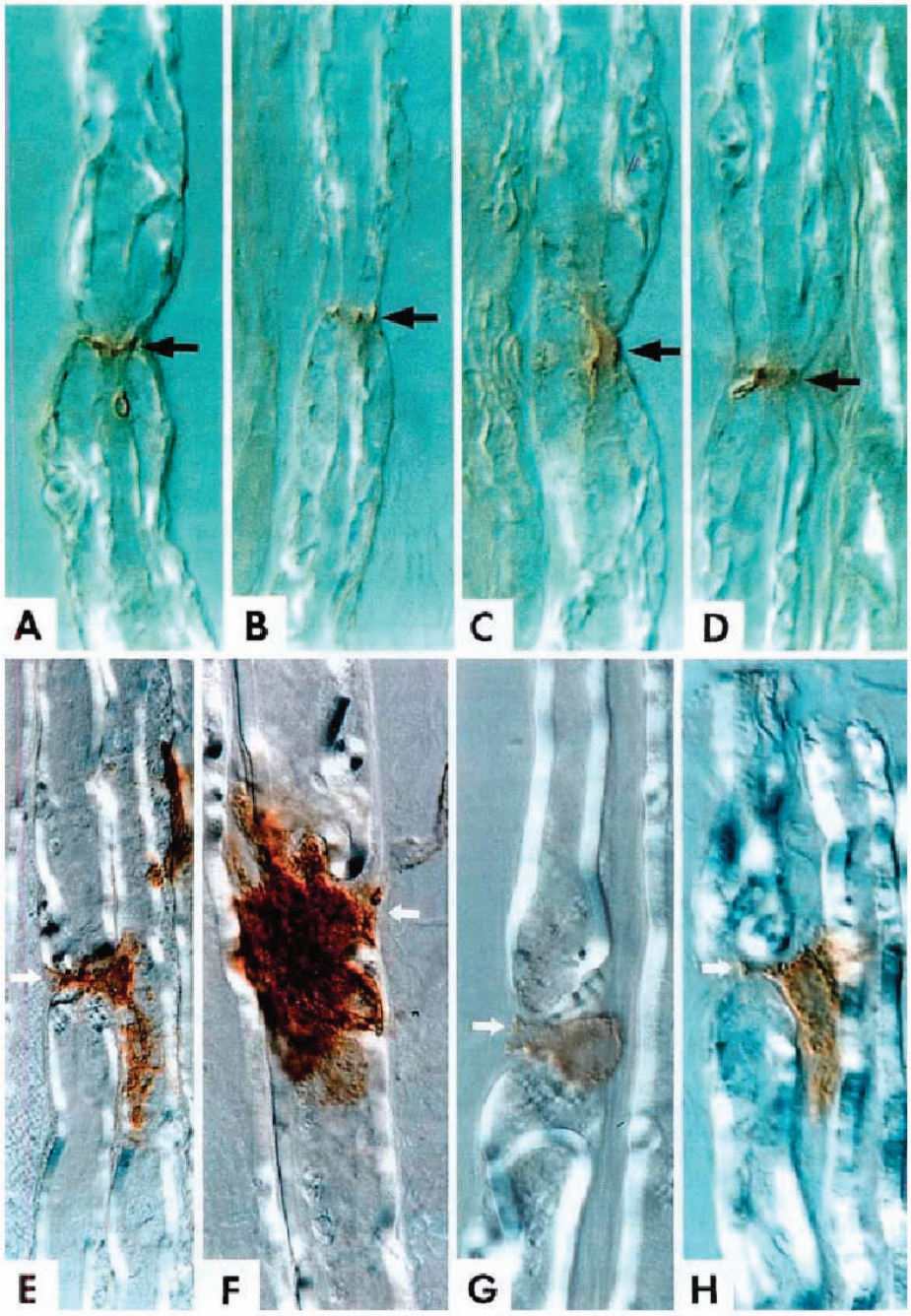


FIGURE 47.1 Immunostained teased ventral root fibers from a patient with acute motor axonal neuropathy (AMAN), 4 days after the onset of neurologic symptoms. *A–D*, The complement activation product C3d was localized discretely at the nodes of Ranvier (arrows) of the large myelinated motor fibers. The golden–brown immunoreaction product is at the nodes. *E–H*, Many motor fibers had macrophages overlying, and extending processes into, the nodes of Ranvier (arrows). [*E* and *F* were immunostained with the macrophage marker HAM-56; *G* and *H* for HLA-DR (major histocompatibility locus class II)].

Reprinted from Hafer-Macko et al. (1996a).

CHAPTER

48

Myasthenia Gravis and other Antibody-Associated Neurological Diseases

ANGELA VINCENT AND CAMILLA BUCKLEY

Neurosciences Group, Department of Clinical Neurology, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK

THE NEUROMUSCULAR JUNCTION AS A MODEL SYNAPSE

The neuromuscular junction (NMJ) is a relatively simple synapse that consists of a motor nerve terminal and postsynaptic motor "endplate." The presynaptic nerve terminal contains mitochondria and many synaptic vesicles that store the neurotransmitter, acetylcholine (ACh). There are specialized active zones, which are the site of ACh release and where voltage-gated calcium channels (VGCC) are concentrated. Voltage-gated potassium channels (VGKC) are also found in the presynaptic motor nerve membrane. The postsynaptic membrane is highly specialized and thrown into a series of postsynaptic or junctional folds. The peaks of the postsynaptic folds are electron dense, corresponding to the distribution of the highly concentrated (10,000/μm^2) ACh receptors (AChRs). The AChRs are anchored by a cytoplasmic protein called RAPsyn. During development, a soluble protein, agrin, is released from the motor nerve terminal, interacts with muscle-specific kinase (MuSK) on the postsynaptic membrane and leads to clustering of RAPsyn and the AChRs.

Neuromuscular transmission depends on the arrival of a nerve impulse which depolarizes the motor nerve terminal (Figure 48.1A). This leads to opening of the VGCCs, influx of calcium, the calcium-dependent release of many packets, or quanta, of ACh from the motor nerve terminal, and the binding of ACh to the postsynaptic AChRs. The AChRs open and allow positively charged ions to enter the muscle fiber, leading to a depolarization of the motor endplate. This results in the generation of an action potential that initiates contraction of the muscle fiber. The release of ACh is stopped when the VGKCs open and the motor nerve repolarizes. Meanwhile, ACh is hydrolyzed by acetylcholine esterase (AChE), which is present in the basal lamina between the motor nerve and the postsynaptic membrane and in the secondary clefts (which are particularly long in humans).

Neuromuscular transmission defects can be caused in two main ways: a reduction in the amount of ACh released or a reduction in the number of AChRs. In both cases, increasing the duration of action of ACh by inhibition of AChE can be a helpful symptomatic treatment. In addition, a reduction in the number of VGKCs leads to an increase in ACh release and muscle hyperactivity. Each of these situations can arise as the result of the presence of autoantibodies directed towards the AChRs or voltage-gated ion channels.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

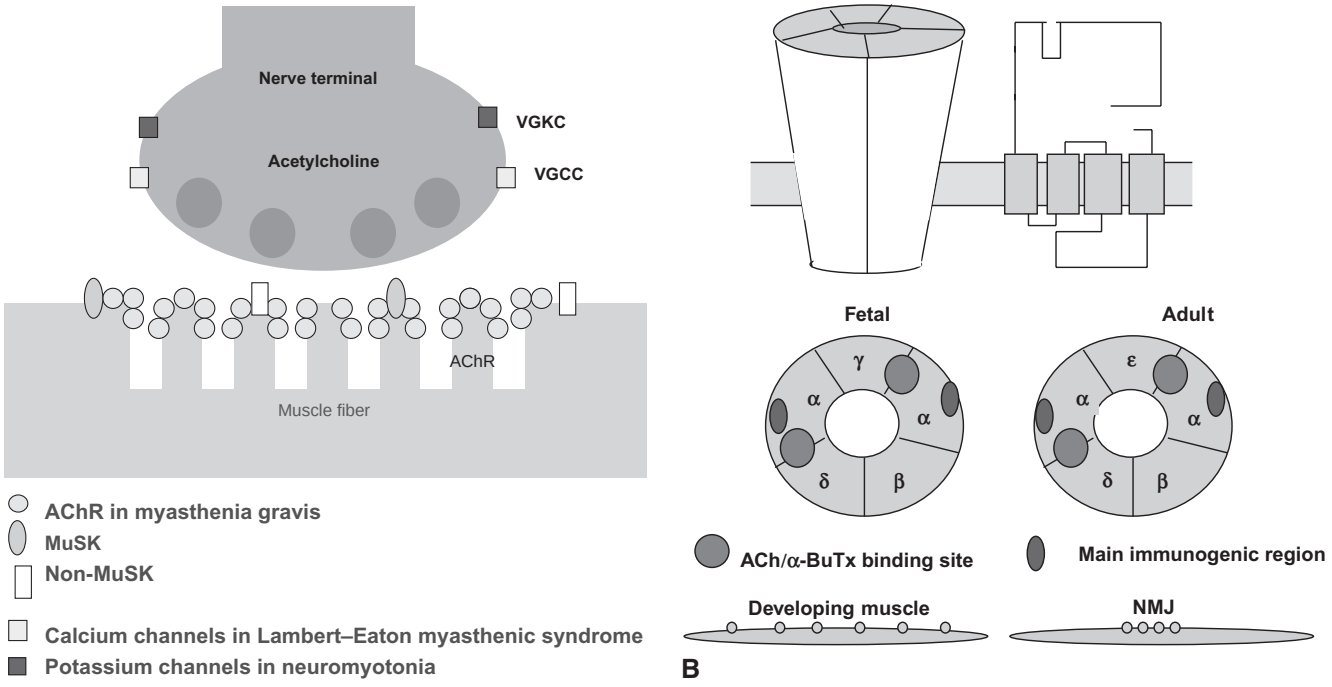


FIGURE 48.1 Ion channel targets for autoantibodies at the neuromuscular junction (NMJ). *A*, Neuromuscular transmission depends on the calcium-dependent release of vesicles of acetylcholine (ACh). ACh binds to the ACh receptors (AChRs) on the postsynaptic membrane, resulting in a depolarization. If depolarization reaches a critical threshold, an action potential is initiated in the muscle, leading to contraction. ACh is immediately destroyed by acetylcholinesterase. AChRs, voltage-gated calcium channels (VGCCs), and voltage-gated potassium channels (VGKCs) are all targets for antibody-mediated neurologic diseases. Recently, the receptor muscle-specific kinase (MuSK) has been found to be a target for antibodies in a proportion of patients with myasthenia gravis (MG) without AChR antibodies. *B*, The AChR is a transmembrane protein with $(\alpha)_2,\beta,\gamma,\delta$ subunits in the fetal form and $(\alpha)_2,\beta,\delta,\varepsilon$ subunits in the adult form. A high proportion of antibodies in MG bind to the main immunogenic regions that are on both the α subunits. In addition, many patients' antibodies bind to the fetal-specific γ subunit. In some cases, antibodies that inhibit the function of the fetal form, selectively cross the placenta, causing fetal muscle paralysis with severe and often fatal deformities. α-BuTx, α-bungarotoxin.

In this chapter, myasthenia gravis will be discussed as the model antibody-mediated disease, and the newer neurologic diseases will be reviewed only briefly.

PERIPHERAL DISORDERS ASSOCIATED WITH ANTIBODIES

Myasthenia Gravis

Historic Background

Table 48.1 lists some of the landmarks of myasthenia gravis (MG) research, which are also reviewed by Vincent and Drachman (2002) and Vincent (2002). Early studies on MG indicated that the muscle weakness was associated with a defect in neuromuscular transmission (Jolly, 1895) and that the symptoms of MG were partially reversed by AChE inhibitors (Walker, 1934). The special involvement of the thymus in MG was demonstrated by the improvement that followed removal of the gland during thyroid surgery by Ernst Sauerbruch in 1913 (Sauerbruch et al., 1913) and the surgical treatment of a patient with thymoma (Blalock et al., 1939).

In 1905, Buzzard reported collections of lymphocytes—"lymphorrhages"—in MG patients' muscles and suggested that MG might be due to "autotoxicity." However, the most dramatic advances in understanding the pathophysiology of MG were not made until the 1960s and 1970s. Nastuk et al. (1960) and Strauss et al. (1960) proposed an autoimmune basis for the disease and examined the role of complement. The occurrence of MG in young females, its association with other autoimmune diseases, the presence of thymic pathology, and the transfer of MG from mothers to their neonates, leading to a reversible "neonatal MG," led Simpson (1960) to propose that MG was an autoimmune disease with antibodies directed against an "endplate" protein. Subsequent studies by Elmqvist et al. (1964) showed that the miniature endplate potentials (MEPPs) were substantially reduced at the endplates from MG muscle biopsies, compared with control biopsies. (MEPPs are the small postsynaptic depo-

TABLE 48.1 History of myasthenia gravis (MG) research

Date	Key observation	Reference
1672	Thomas Willis publishes what is arguably the first clinical description of MG	Willis (1672)
1895	Jolly shows that defect is at the neuromuscular junction	Jolly (1895)
1913; 1939	Thymectomy appears to produce clinical improvement in patients with thymoma or nonthymomatous MG	Sauerbruch et al. (1913); Blalock et al. (1939)
1934	Mary Walker demonstrates the effectiveness of cholinesterase inhibitors as treatment	Walker et al. (1934)
1960	Simpson proposes that MG is caused by antibodies to an "endplate" protein	Simpson (1960)
1962	The snake toxin, α-bungarotoxin, can be used as a label for acetylcholine receptors at the neuromuscular junction	Chang and Lee (1962)
1964	Elmqvist et al. show that the miniature endplate potentials are reduced in MG	Elmqvist et al. (1964)
1971	Several groups begin to purify acetylcholine receptors (AChRs) from electric organs of electric rays using affinity chromatography on neurotoxin columns	
1973	Immunization against purified electric ray AChRs leads to an experimental autoimmune MG (EAMG) in rabbits	Patrick and Lindstrom (1973)
1973	AChRs are reduced in number at neuromuscular junctions, as determined by ^{125}I-α-bungarotoxin binding	Fambrough et al. (1973)
1975	MG can be passively transferred to mice by injection of patients' IgG	Toyka et al. (1975)
1976	MG patients have AChR antibodies as shown by radioimmunoprecipitation of ^{125}I-α-bungarotoxin-labeled AChRs	Lindstrom et al. (1976a)
1976–1978	Plasma exchange produces striking clinical improvement in MG, which correlates inversely with AChR antibody levels	Pinching et al. (1977), Newsom-Davis et al. (1978)
1977	IgG and complement are present at the neuromuscular junctions in MG patients and in mice with EAMG	Engel et al. (1977)
1980	MG can present with different HLA, thymic pathology, age at onset, and muscle antibodies	E.g.; Compston et al. (1980)
1981	The MG thymus contains plasma cells making AChR antibody	Scadding et al. (1981)
1977–present	Experimental autoimmune model used to determine pathogenic and immunologic mechanisms	
1984–present	Study of T cells from MG patients and their responses to AChR epitopes	

larizations that occur each time a packet of ACh is released spontaneously.) In theory, the cause of this reduced MEPP could be either presynaptic (less ACh released) or postsynaptic (fewer AChRs). However, at that time, there was no way of measuring directly the concentration of AChRs, and it was several years before a method was recognized. In 1973, Fambrough et al., using the snake toxin α-bungarotoxin (α-BuTx) that binds specifically to AChRs (Chang and Lee, 1962), showed that the number of AChRs was reduced in MG. The same year, Patrick and Lindstrom (1973) showed that immunization against affinity-purified AChRs induced weakness and paralysis in rabbits that, like MG, was responsive to AChE inhibitors. It was then relatively simple to show that AChR antibodies were present in MG patients (Lindstrom et al., 1976b), and that transfer of MG immunoglobulins to mice produced signs and electrophysiologic evidence of the disease (Toyka et al., 1975).

Electron microscopy of the NMJ shows an essentially normal nerve terminal but the postsynaptic membrane lacks junctional folds, there are reduced areas of contact between the presynaptic nerve terminal and the muscle membrane, and there is debris within the synaptic cleft (Engel et al., 1977). These changes are consistent with an immune-mediated attack on the postsynaptic membrane; there are only very infrequent reports of cellular infiltration and the mechanisms are now thought to be entirely antibody driven. The major pathology in MG is in the thymus gland (see below).

Clinical and Pathophysiologic Features

Myasthenia gravis (MG) is an acquired autoimmune disorder in which the postsynaptic AChRs are reduced in number or function, leading to muscle fatigue and weakness. The weakness often starts in the eye muscles, resulting in double vision and ptosis (drooping eyelids), but may involve any muscle group. MG is frequently associated with thymic abnormalities, so-called thymitis, in around 60% and thymoma in around 10% of patients. The clinical features are reviewed in more detail by Vincent and Drachman (2002) and Vincent et al. (2001).

Pathogenic Role and Mechanisms of Action of Acetylcholine Receptor Antibodies

The AChR antibodies were first measured by immunoprecipitation of ^{125}I α-BuTx-labeled AChR in detergent extracts of human muscle (Lindstrom et al, 1976a), but most current assays employ AChRs extracted from muscle-like cell lines that express a mixture of fetal and adult AChRs (Figure. 48.1B) (Beeson et al., 1996). Enzyme-linked immunosorbent assay (ELISA) and other alternative assays

have not proved successful. AChR antibody titers are highly variable among MG patients, ranging from 0 to >1000 nm/L, and between individuals titers do not correlate well with clinical severity, although the level of antibody within an individual correlates well with clinical scores after plasma exchange (Newsom Davis et al., 1978), thymectomy (Vincent et al., 1983; Kuks et al., 1991), or immunosuppressive treatment. The presence of anti-AChR antibody is diagnostic for MG and the levels reflect the severity of the disease within an individual; however, by itself this does not prove that the antibodies cause the disease. Nevertheless, MG fulfills the criteria of Witbesky: the presence of antibodies to a defined antigen, the induction of disease experimentally by immunization with the purified antigen (Patrick and Lindstrom, 1973), and transfer of such disease to naïve litter mates by injection of serum (Lindstrom et al, 1976a); transfer by patient's serum (Toyka et al., 1975), and the demonstration that these antibodies are involved in the pathology of the human disease (Rose and Bona, 1993). The beneficial effects of plasma exchange and immunosuppression provide further confirmation.

Information concerning the mechanisms of action of the antibodies came from Engel et al. (1977; Engel and Arahata, 1987) who showed that IgG and complement components are present at the NMJ, both on the postsynaptic membrane and in the synaptic debris; the distribution of IgG corresponds well with the distribution of AChRs, as shown by peroxidase α-BuTx binding; and the amount of membrane attack complex is inversely related to the number and density of AChRs.

In addition, the divalent antibodies can cross-link the AChRs, leading to their internalization and degradation. Drachman et al. (1978) and Stanley and Drachman (1978) showed that the rate of AChR degradation in cultured cell lines was increased several fold. It was particularly striking that *in vivo* the half life for AChRs at the NMJ was reduced from about 10 days to less than 5 days. However, there may be a compensatory increase in AChR synthesis, as shown in experimental studies of AChR turnover (Wilson et al., 1983) and in raised mRNA levels for AChR subunits in MG (Guyon et al., 1994) and in experimental autoimmune myasthenia gravis (EAMG) (Asher et al., 1990).

Only a few investigators have described direct functional effects of serum or an IgG preparation on the amplitudes of MEPPs *in vitro* (Burges et al., 1990), although MG sera can acutely reduce AChR function in cultured human cells (Lang et al., 1988). Antibodies specifically inhibiting fetal AChRs were found in two mothers with a history of recurrent fetal arthrogryposis (see below). Potential mechanisms of functional antibodies are discussed in Chapter 17.

The Acetylcholine Receptor and Antibodies

The AChR is an oligomeric membrane protein with five subunits: (α^2, β, γ, and δ in embryonic or denervated muscle, and α^2, β, δ, and ε at the adult endplate (Figure 48.1B)). The AChR has not been crystallized for structural studies (this is very difficult for multimeric membrane proteins), but studies by Unwin (2002) have demonstrated many of the features of the molecule.

AChR antibodies are heterogeneous in their characteristics both within and between individual MG patients. The antibodies have variable light chain and IgG subclass, although IgG1 predominates in most patients (Vincent et al., 1987). In many patients, a high proportion of the anti-AChR antibody competes with monoclonal antibodies directed toward the main immunogenic region (MIR) on the α subunits, which is the epitope to which a majority of experimentally-induced antibodies bind (Tzartos et al., 1980; 1982; 1998). However, some antibodies bind to other sites (Whiting et al., 1986), which appear to be on other AChR subunits (Jacobson et al., 1999a).

The antibodies are highly specific for the intact human autoantigen and bind badly or not at all to denatured AChR subunits or recombinant polypeptides. Their high affinity (around 10^{10} M) and specificity for the human AChR suggest that the antibodies are induced by some form of the native AChR molecule (Vincent et al., 1987; 1998a).

There have been few reports of monoclonal antibodies derived from immortalized AChR-specific MG cells, owing to the low precursor frequencies and to the difficulty in obtaining stable IgG-secreting, rather than IgM-secreting, hybrids or Epstein–Barr virus (EBV)-transformed lines. On the other hand, phage-display techniques have produced high-affinity Fabs that bind to human AChR and compete with monoclonal AChR antibodies (Graus et al., 1997; Farrar et al., 1997; Matthews et al., 2002).

Clinical Heterogeneity

Myasthenia is not a single disease. In the majority of MG patients, generalized weakness with AChR antibodies develops after puberty, without obvious precipitating factors. Patients are divided in terms of their age at onset into early and late onset, after separating out those patients who have a thymic tumor (thymoma). This division exposes the different thymic pathology and HLA associations in early- and late-onset patients (Table 48.2) (Compston et al., 1980), and it seems likely that these two subgroups reflect different precipitating factors, rather than an intrinsic difference in the patients' immune responses to a single precipitating factor. In addition, many non-HLA immunogenetic associations have been reported in MG, suggesting that polymorphisms in other immune mediators are important (Hjelmstrom et al., 1997; 1998), but few of these studies have divided the patients into the subgroups described in Table 48.2, and the associations are often weak and not always reproducible.

TABLE 48.2 Myasthenia gravis patients divided on the basis of age at onset, thymic pathology, HLA association, and associated autoantibodies

Subtype*	Age at onset	Sex M : F	Typical thymic pathology	HLA association	Associated autoantibodies
Early onset	<41 years	1 : 3	Thymitis	B8, DR3	May have other tissue antibodies, e.g., thyroid,
Thymoma associated	Mainly 40–60 years	1 : 1	Epithelial tumor containing many lymphocytes	No clear association	Titin and ryanodine receptor antibodies very common. Also cytokine antibodies
Late onset	>40 years	1.5 : 1	Normal or atrophied	B7, DR2 in males	Titin and ryanodine receptor antibodies common, particularly after age 60 years
AChR antibody negative MuSK antibody positive	2–70 years	1 : 3	Normal or atrophied in most	Not known	Not clear
AChR antibody negative MuSK antibody negative	1–80 years	2 : 3	Mild thymitis in some	Not known	Not clear

*These subtypes are not appropriate in patients with purely ocular MG and in other ethnic populations.

There are also patients without detectable AChR antibodies, some of whom have antibodies to MuSK. In addition, there are patients with purely ocular myasthenia; whether these have a distinct form of disease is not altogether clear. The patients with AChR antibodies and generalized MG will be considered first, divided into the three main subgroups shown in Table 48.2.

Early-Onset Acetylcholine Receptor-Positive Myasthenia Gravis

These patients present before 40 years of age and frequently have thymitis. In Northern Europeans, there is a strong association with the HLA A1 B8 DRB1*0301 DRB*0101 DQB1*0201 DQA1*0501 ancestral haplotype (Carlsson et al., 1990; Viera et al., 1993).

Involvement with a particular AChR α subunit microsatellite, i.e., a noncoding region of the gene, has also been reported (Garchon et al., 1994) and this may be associated with the DRB1*0301 (Djabiri et al., 1997). This raises the possibility that polymorphisms in gene regulatory sequences that increase AChR expression lead to presentation of AChR by DRB1*0301 to a susceptible immune system. Alternatively or in addition, AChR coding sequence polymorphisms or heteroallelic mutations might result in abnormal antigen processing. For instance, a single case report of MG arising in a woman who had a recessive inherited form of myasthenia, with heteroallelic AChR mutations, suggested that clinically-silent AChR mutations on a single allele might predispose to development of the autoimmune disease (Croxen et al., 2002). However, preliminary investigations in a large number of early-onset MG patients have not proved fruitful (Bonifati et al., 2004).

Although the pathogenic autoantibodies depend on class II-restricted helper T (Th) cells, the associations are stronger with B8 than with DR3 (Compston et al., 1980), and the hot spot appears to be between the class II and class I region. Various possibilities, such as genes for tumor necrosis factor (TNF), heat-shock proteins, and transporter proteins associated with antigen processing (TAP) have been considered (Janer et al., 1999), but it is still not clear which gene is responsible for conferring susceptibility.

In early-onset MG the thymus gland often contains T-cell areas in the medulla, some containing lymphoid follicles (Levine and Rosai, 1978; Hohlfeld and Wekerle, 1994). Lymphocytes cultured from the thymus gland synthesize anti-AChR antibody (Scadding et al., 1981), and serum anti-AChR levels frequently decline slowly after thymectomy (Vincent et al., 1983). Germinal centers of the MG thymus contain polyclonal activated B cells expressing a range of V_H and V_K genes, similar to those in the peripheral blood lymphocytes (Guigou et al., 1991) and somatic hypermutation of B-cells occurs in thymic germinal centers (Sims et al., 2001). These findings suggest that B cells somatically mutate and mature in these germinal centers, but that similar B cells are present in the periphery. It is not clear, therefore, whether or not the disease starts in the thymus. In favor of this possibility, the thymus contains the antigen, AChR, on muscle-like "myoid" cells in the thymic medulla (Kao and Drachman, 1977; Wekerle and Ketelsen, 1977; Schluep et al., 1987), and sometimes in the germinal centers themselves. Careful examination of the pathology suggests that the myoid cells are targets for autoantibody attack and that this provokes germinal center formation (Roxanis et al., 2002). Moreover, some of the germinal centers can be shown to bind AChR, suggesting that they contain many AChR-specific B cells. But whether or not germinal center formation is a primary event in the disease, or occurs secondary to the autoimmune response, is still not clear.

In fact, molecular studies show that all five AChR subunits, including a differentially spliced isoform, are expressed in MG thymuses (Andreetta et al., 1997), and AChR may be expressed not only in the myoid cells but also in thymic epithelia (Salmon et al., 1998). The presence of AChR in normal as well as MG thymus suggests that it may be involved in inducing T-cell tolerance in normal individuals or in breaking tolerance in MG, but why the latter occurs in MG is still unknown.

It is widely believed that the thymus in MG generates AChR-specific T-cells that direct antibody synthesis by B cells both in the germinal centers in the thymus and elsewhere. Since the antibody response in MG is thought to be directed towards the native AChRs, the T cells should be able to recognize AChR taken up and presented by AChR-specific B cells. Many studies have looked at T-cell responses, lines and clones, generated from peripheral blood lymphocytes or thymic suspensions and stimulated either with AChR peptides or with recombinant or purified AChR (Hawke et al., 1996; Conti-Fine et al., 1998). The rare T cells generated against recombinant polypeptides frequently respond to very small amounts of purified AChR antigen, processed and presented by appropriate antigen-presenting cell (APC), as well as to the relevant smaller peptides, suggesting that they could recognize the autoantigen *in vivo*. Conversely, the much more plentiful T cells that can be generated against synthetic AChR peptides do not appear to respond strongly to purified AChR. These results question the relevance to the disease of the T-cell responses generated against antigenic peptides rather than against native antigen or larger recombinant fragments.

Muscle does not normally express MHC class II, but there is evidence that MHC class II can be induced by viral infections *in vivo* (Bao et al., 1992) or by interferon (IFN)-γ *in vitro* (Hohlfeld and Engel, 1990). The possibility that the T-cell response is initiated or perpetuated by presentation of AChR peptides by class II on nonprofessional presenting cells has been addressed. T-cell clones specific for AChR α144–163 recognized AChR endogenously presented by the TE671 muscle cell line after it was transfected with DNA for the α and β chains of DR4.Dw4.2, or by human myotube cultures stimulated with IFN-γ (Baggi et al., 1993; Curnow et al., 2001). The responding T cells showed only modest proliferation, but bound to the presenting cells, secreted IFN-γ, and killed the presenting cells.

Late-Onset Acetylcholine Receptor Antibody Positive Myasthenia Gravis

Recent surveys of AChR antibodies in sera sent for routine diagnosis have shown a striking increase in incidence after the age of about 60 years, peaking in the late 70s (Vincent et al., 2003a). Even when the declining numbers of the population are taken into account, there is a fall off in diagnosis of MG after the age of 75 years, which suggests that MG is being underdiagnosed in the elderly. In patients who present with symptoms of MG after the age of 40 years, the thymus is mainly atrophic or involuted, and there are weak biases toward males and HLA B7, DR2 (Compston et al., 1980) or DR4, DQw8 (Carlsson et al., 1990). These patients do not have a thymoma (by definition), but nevertheless are often positive for antibodies to titin, ryanodine receptor, and cytokines (Aarli, 2001; Buckley et al., 2001b; Somnier and Engel, 2002). The cause of the disease is entirely unknown, and given its increasing incidence, more efforts should be directed towards defining immunogenetic and environmental factors that might predispose to the development of this condition.

Thymoma-Associated Myasthenia Gravis

Thymoma, which occurs in about 10% of patients in reported studies (a figure that may need to be revised with the increasing number of older patients) can arise at any age, but most patients with MG and thymoma present between the ages of 30 and 60 years. The thymus gland is usually removed, but the myasthenia rarely improves (Palmisani et al., 1994; Somnier, 1994), anti-AChR levels seldom fall (Kuks et al., 1991), and immunosuppressive therapy is often required. HLA associations have been reported in single studies but no consensus has emerged. Thymomas associated with MG are epithelial in origin, corresponding mainly to the World Health Organization type B1 and B2 (Muller-Hermelink and Marx, 2000).

The thymus adjacent to a thymoma often shows changes of thymitis, and a few B-cells are present in thymoma tissue. However, anti-AChR synthesis by thymoma cells is rare (see above), contrasting with that which occurs in early-onset thymuses and suggesting that the thymoma tissue does not initiate and/or sustain the antibody response to this antigen. However, >90% of patients with thymoma have antibodies to antigens of striated and cardiac muscle (Aarli, 2001). These include myosin, actin, α-actinin, ryanodine receptor, and a particular epitope on the giant structural protein titin (Gautel et al., 1993). Both titin and ryanodine receptor epitopes are expressed in cortical thymomas (Mygland et al., 1995; Marx et al., 1996; Romi et al., 2002), although there is some question as to their relevance (Kusner et al., 1998). In addition, and surprisingly, antibodies to the cytokines, IFN-α and interleukin (IL)-12, are also present in both thymoma-related MG patients and older MG patients (Meager et al., 1997), and in the former may predict the recurrence of a thymoma (Buckley et al., 2001b). Both cytokines are expressed by immune cells (probably macrophages; Y. Kadota and N. Willcox, personal communication) in thymoma. Moreover, B cells from MG thymoma tissue synthesize IFN-α antibodies in culture, and IFN-α-specific IgG Fabs can be cloned and expressed by combinatorial library techniques from B cells present in thymoma tissue (Shiono et al., 2003). These observations support the

idea that the thymoma can initiate and sustain the B-cell response to certain antigens, such as IFN-α and IL-12.

Cultured neoplastic epithelial cells from thymomas express epidermal growth factor receptor and Ki67, indicative of their growth potential (Gilhus et al., 1995a). Interestingly, CD80 expression is high in thymoma epithelial cells in comparison with those in normal cortex, indicating the potential for these cells to activate T-cell responses (Marx et al., 1994). Moreover, these cells can process and present antigens to class II restricted T cells (Gilhus et al., 1995b), indicating that they express both the appropriate class II molecule and the necessary processing apparatus and accessory molecules. However, functional AChR does not appear to be present in thymoma tissue; the AChR α subunit is present but only at very low levels in the thymoma tissue itself, as identified by polymerase chain reaction (Hara et al 1991; Geuder et al 1992).

The typical MG thymoma contains large numbers of polyclonal developing T-lymphocytes, many double positive for CD4 and CD8 and others with the single markers of maturing T cells (Fujii et al., 1990). The number of T cells is often drastically depleted owing to preceding steroid therapy (Willcox et al., 1989). The remaining lymphocytes might either escape deletion in the disorganized cortical epithelium, or be specifically sensitized to antigens presented and expressed by the thymoma epithelium. Although studies on specific T cells have been difficult, there is no doubt that the thymoma does generate potentially functional T cells. The proportion of both CD4 and CD8 T cells containing TRECs (T-cell receptor excision circles), which gives an indication of the number of T cells recently emigrated to the periphery, is greatly raised in thymoma MG cases and falls after thymomectomy (Figure 48.2) (Buckley et al., 2001a). By contrast, TRECs are only slightly increased in patients with early-onset MG, and are not increased in patients with late-onset MG, indicating that they are not just the result of thymic perturbation. Moreover, they rise again in thymoma cases if the tumor recurs (Figure 48.2). On the other hand, double-negative $CD4^-/CD8^-$ T cells were not abnormal in thymoma cases and did not change after surgery (Reinhardt and Melms, 2000).

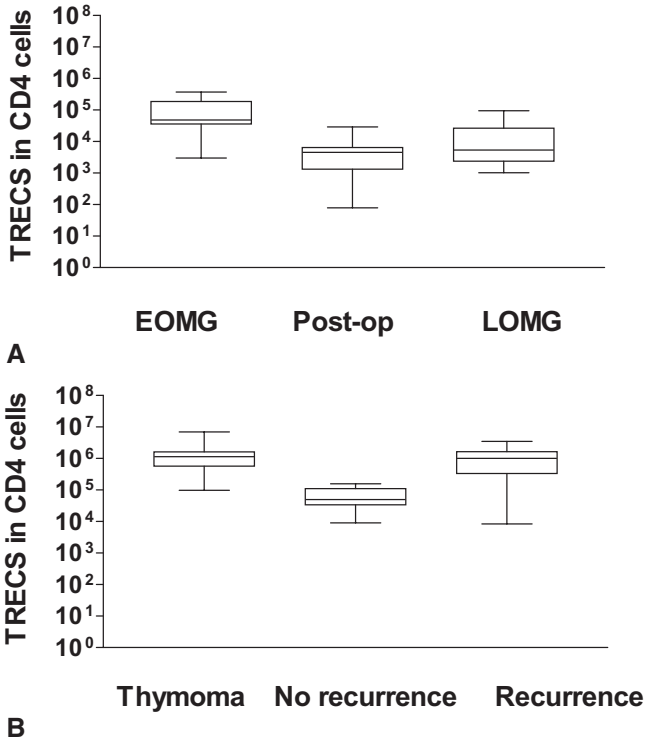


FIGURE 48.2 Thymomas export large numbers of T cells to the periphery. The number of T-cell receptor excision circles (TRECS) in peripheral $CD4^+$ T cells from patients with different forms of myasthenia gravis (MG). *A*, TREC levels in CD4 cells from patients with early-onset MG (EOMG), late-onset MG (LOMG), and appropriate post-operative controls who are age matched for each group. There are no significant differences between patients and controls. *B*, TREC levels in CD4 cells from patients with thymoma and MG on the day of their operation (thymoma), and a mean of 10 years after thymomectomy for patients who either had no evidence of recurrence (no recurrence) or had a recurrence of their tumor (recurrence). The mean ages were equivalent for each group. Results are presented as box and whisker plots.

Neonatal Myasthenia Gravis and Arthrogryposis Multiplex Congenita

A proportion of babies born to MG mothers have transient respiratory and feeding difficulties, owing to transplacental transfer of maternal anti-AChR. These problems may be more common in the offspring of mothers who have a high proportion of antibodies reactive with fetal-type AChR (Vernet der Garabedian et al., 1994), although the fetal form is thought to be replaced by the adult form from around 33 weeks' gestation. Occasionally, neonatal MG occurs without maternal disease or when the mother is anti-AChR negative.

A few cases of severe arthrogryposis multiplex congenital have been found in a number of consecutive pregnancies in mothers with MG. Arthrogryposis is a condition that develops during intrauterine life as a result of lack of fetal movement. The baby is born with fixed joint contractures, which can be associated with inadequate development of lungs and failure to survive after birth. Maternal anti-AChR antibodies, which even at high dilution completely inhibit the function of fetal AChR (see Figure 48.1B) in an *in vitro* assay, but have no affect on adult AChR function, were present in these mothers (Polizzi et al., 2000). Moreover, AChR-specific Fabs were relatively easy to select from the combinatorial libraries prepared from the mothers and were highly specific for fetal AChR (Matthews et al., 2002). These antibodies appeared to be responsible for the complete loss of fetal movement *in utero* and the subsequent deformities that are incompatible with survival after birth. An animal model of this condition has been induced

by injecting the maternal plasmas into pregnant mice (Jacobson et al., 1999b).

Etiology of Acetylcholine Receptor-Positive Myasthenia Gravis

It is clear from the above discussion that there are probably different etiologies for the different subgroups of MG, and that little is still known about the precipitating factors. During the 1980s there were several reports of idiotypic networks involving AChR antibodies, but these findings have not been pursued. Other evidence for molecular mimicry between the *Torpedo* AChR α subunit and microbial proteins, and between human AChR α157 170 and herpes simplex glycoprotein D, have also not been followed up [for a review see Vincent et al. (1998)]. In general, it seems unlikely that cross-reacting antibodies could demonstrate the heterogeneity, high affinity, and specificity that characterize MG anti-AChR. Nevertheless, such a cross-reaction could be an initiating event that leads subsequently to autosensitization against the muscle AChR by a process of determinant spreading. Such a process has been demonstrated in a rabbit model (see Vincent et al., 1998).

Animal Models

The experimental autoimmune myasthenia gravis (EAMG) model was important in stimulating the search for AChR antibodies in MG, but will not be discussed in any detail here [see Lindstrom (2000) and Christadoss et al. (2002) for reviews].

The AChR is very immunogenic. Rats and mice can mount strong immune responses even to syngeneic AChR in the absence of adjuvant (Jermy et al., 1993). It is important to realize that the proportion of antibodies that cross-react between the immunizing protein and the recipient's muscle AChR depends on the closeness of the species, whereas the clinical effects of immunization depend mainly on the susceptibility of the immunized animal's NMJs to changes in AChR numbers. Studies on inbred rodents demonstrated the importance of immunogenetic factors in the susceptibility to EAMG. C57B1/6 mice and AKR mice are the most susceptible mouse strains, correlating with H-2^b and Ig-1^b, respectively, whereas BALB/c strains are relatively resistant (Berman and Patrick, 1980; Christadoss et al., 1979). The basis for these differences in susceptibility, however, is still not clear. It does not relate to the serum antibody levels (Berman and Patrick, 1980), or to intrinsic muscle factors. The I-A^{bm12} mutation in C57B1/6 mice confers resistance to EAMG, probably by changing the T-cell repertoire and preventing recognition of α146–162, a dominant T-cell epitope (Biesecker and Koffler, 1988). Detailed analysis of the role of class II and T-cell receptor (TCR) genes in EAMG is provided elsewhere (Kaul et al., 1994). Recently, EAMG has been induced in mice deficient for cytokines or accessory molecules, or treated with soluble forms of these substances. In general, the results demonstrate the involvement of Th1-dependent mechanisms in the pathogenic immune response to AChR. For instance, not surprisingly, these studies have shown that CD28–CD80 and CD154 (CD40L)–CD40 interactions are important, and that IFN-γ is essential for the full immune response, whereas IL-4 does not play a major role. Mice deficient in IL-6, by contrast, are less likely to develop EAMG and show less germinal center formation (Christadoss et al., 2000).

The experimental model continues to be used for testing approaches to specific treatments for MG, despite the fact that the relevance of the animal model to the human disease must be limited. Many of these approaches are reviewed elsewhere (Christados et al., 2000; Vincent and Drachman, 2002).

Myasthenia Gravis Without Acetylcholine Receptor Antibodies

About 10–15% of all MG patients with generalized symptoms do not have detectable anti-AChRs. Traditionally they have been termed as having "seronegative MG (SNMG)." Their symptoms are similar to those of patients with AChR antibody "seropositive" generalized MG, but there are some subtle differences (Vincent et al., 2003b). An important feature is the relative lack of thymic changes in most of these patients (Willcox et al., 1991; Verma and Oger, 1992). Thymoma is never found, and no HLA association has been identified. Thus, on clinical and pathologic grounds, the disease appears to have several unusual features.

SNMG is clearly an antibody-mediated disease because patients get better with plasma exchange, and passive transfer to mice results in defects in neuromuscular transmission (Vincent et al., 2003b). However, the actual mechanism by which the antibodies cause these defects is not clear. A series of studies indicated that SNMG is likely to be heterogeneous. Some patients have an IgG antibody to MuSK (Figure 48.3) (Hoch et al., 2001; Scuderi et al., 2002). MuSK is the receptor tyrosine kinase that is essential for clustering AChRs during development. Interestingly, the antibodies are not found in patients with AChR antibody-positive MG, in patients with purely ocular MG, or in other control groups, and thus they define a distinct form of MG (McConville et al., 2004). They appear to be found often in patients with marked combined ocular and bulbar symptoms (Scuderi et al., 2002). How these antibodies affect neuromuscular transmission is not clear, but given the role of MuSK during development (Liyanage et al., 2002), it is likely that they interfere with some aspect of MuSK signaling and alter

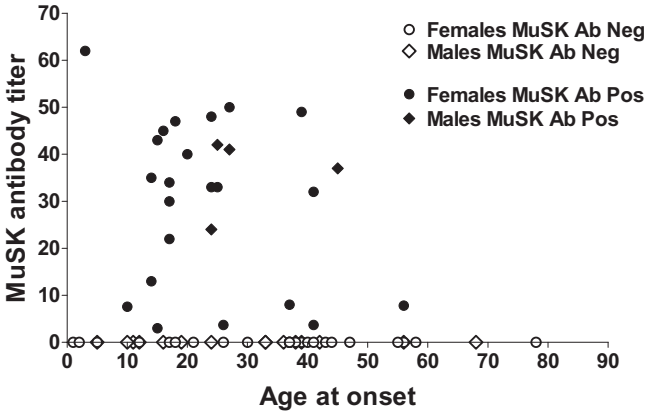


FIGURE 48.3 Muscle-specific kinase (MuSK) antibodies in patients without acetylcholine receptor (AChR) antibodies. MuSK antibody titers (in nmoles of ^{125}I-MuSK precipitated/L of serum, solid symbols) in patients with myasthenia gravis related to sex and age at presentation. MuSK antibodies are found mainly in young women. One patient presented at age 2 years with very high titers. MuSK-antibody negative patients (open symbols) present at any age and are both male and female. Many patients are negative for MuSK antibodies.

AChR localization or stability. Interestingly, the MuSK antibodies are predominantly IgG4 with smaller amounts of IgG1 and IgG2 (McConville et al., 2004). This contrasts with the AChR antibodies (see above), further emphasizing the differences between AChR antibody-positive and MuSK antibody-positive MG.

In some SNMG patients, including those with and without MuSK antibodies, an IgM-containing fraction inhibits AChR function in the muscle-like TE671 cell line. This inhibition is rapid and reversible, and may be linked to AChR desensitization (Plested et al., 2002; Spreadbury et al., 2005), but the target of these antibodies is not yet clear.

Lambert–Eaton Myasthenic Syndrome

In 1957 Eaton and Lambert described a myasthenic syndrome that was electrophysiologically distinct from MG (O'Neill et al., 1988). Subsequent studies showed that the defect was a reduction in the number of packets of ACh released per nerve impulse, contrasting with MG in which the defect is in the number of AChRs. Lambert–Eaton myasthenic syndrome (LEMS) is often associated with small cell lung cancer (SCLC), and is also sometimes found with paraneoplastic cerebellar degeneration and paraneoplastic encephalomyelitis. Thus, LEMS is a member of the expanding family of paraneoplastic neurologic conditions that can be associated with CNS disease (see Chapter 68). However, about 50% of patients never develop a tumor and have an acquired autoimmune disease of unknown etiology.

There are certain features of LEMS that contrast with those in MG. First, there is more prominent involvement of lower limb muscles and less significant involvement of ocular muscles in LEMS. Second, muscle strength improves after maximal voluntary contraction and third, autonomic features, such as dry mouth, constipation and impotence are common.

Pathophysiology

Freeze-fracture electron microscopic studies of motor nerve terminals in healthy human muscle biopsy samples reveal, as in other species, double parallel rows of intramembranous particles (each about 10^{-2} nm in diameter). These particle arrays are associated with the presynaptic active zones that are known to be close to the site of exocytosis of the transmitter (see Figure 48.1). The particles themselves (active zone particles) appear to represent voltage-gated calcium channels that are known to be important for ACh release (see above). Engel and colleagues found a highly significant reduction in the number of active-zone particles and the number of particles per active-zone in LEMS patients, and an increase in the number of clusters of particles (Engel et al., 1989; Engel, 1991).

Autoimmunity

Many LEMS patients, particularly those without a tumor, have other autoimmune disorders or organ-specific autoantibodies (Lennon et al., 1982), and an increased association with HLA-B8 and IgG heavy chain markers (Demaine et al., 1988). The evidence for an antibody-mediated pathogenesis followed the paradigms outlined above. Plasma exchange was clinically effective with the maximal response occurring about 10 days after the treatment. Patients also respond well to immunosuppressive drugs or intravenous immunoglobulin (IVIG) therapy (O'Neill et al., 1988).

Daily injection of LEMS plasmas or IgG fractions into mice reproduced the principal neurophysiologic changes of LEMS (Lang et al., 1983; Kim, 1985), although there was little clinical evidence of disease. However, there were changes in the number and clustering of active-zone particles, similar to those found in patients. Interestingly, complement did not seem to be involved as C3-deficient mice were as susceptible as normal mice (Prior et al., 1985), and purified IgG was as effective as plasma. In most cases, acute incubation in LEMS sera or IgG (1–3 h) did not affect neuromuscular transmission, indicating that the antibodies did not inhibit function directly.

The clustering of the active zone particles suggested that the divalent antibodies were acting by cross-linking adjacent particles (Fukuoka et al., 1987), and the importance of divalent antibodies was confirmed by studies on monovalent Fab′ (Peers et al., 1993).

Using a sensitive immunoelectron microscopy technique, Engel et al. (1989) localized IgG at the sites of presynaptic active zones in mice that had received multiple intraperitoneal doses of LEMS IgG. The amount of IgG detected was small, reflecting the low number of active zone particles and their restricted localization.

Voltage-Gated Calcium Channel Antibodies

The data above strongly suggested that the active zone particles, thought to be voltage-gated calcium channels (VGCC), were the target for the LEMS antibodies. Moreover, it was known that SCLC cells express VGCCs on their surface. Roberts et al. (1985) showed that the function of these VGCCs were reduced when the cells were grown for several days in the presence of LEMS IgG.

The cone snail derived toxin ω-conotoxin (ω-CmTx) MVIIC binds to the P or Q type VGCCs that are involved in ACh release at the NMJ. Solubilized VGCCs prelabeled with ^{125}1 ω-CmTx can be immunoprecipitated by 80–90% of LEMS sera (Lennon et al., 1995; Motomura et al., 1995). A direct relationship between the anti-VGCC antibody titer and clinical severity was demonstrated in a longitudinal study on individuals receiving immunosuppressive therapy, and after a double-blind cross-over trial of IVIG treatment in eight LEMS patients (Bain et al., 1996). Of note, the clinical improvement and fall in VGCC antibodies that followed IVIG treatment were delayed, and there was no evidence that the injected immunoglobulins were anti-idiotypic.

Autonomic dysfunction is common in LEMS, suggesting that LEMS IgG may interfere with neurotransmission at autonomic synapses. Mice injected with LEMS IgG show reduced smooth muscle activity (Waterman et al., 1997). However, there was no obvious clinical effect and it would be interesting to know more about the role of these channels in normal autonomic functions.

These studies in LEMS reflected many of the approaches that had already proved successful in the study of MG, emphasizing the use of experimental clinical and laboratory techniques to investigate neurologic autoimmune diseases.

Acquired Neuromyotonia

Neuromyotonia (NMT), or Isaacs syndrome (Isaacs, 1961), is a rare syndrome of spontaneous and continuous muscle fiber contraction resulting from hyperexcitability of motor nerves. Like MG and LEMS, it can be paraneoplastic when associated with thymoma or SCLC (Newsom-Davis and Mills, 1993).

Clinical Features

Clinical features include muscle stiffness, cramps, myokymia (visible undulation of the muscle), and weakness, most prominent in the limbs and trunk. Increased sweating is common. Pseudomyotonia, a slow relaxation of muscles after contraction, may also be present. For detailed reviews of the clinical and electrophysiologic findings, the reader is referred to Newsom-Davis and Mills (1993) and Hart et al. (2002). Sensory symptoms are present in some patients and CNS symptoms, such as insomnia, hallucinations, delusions, and personality change may also be present, when the condition is usually referred to as Morvan syndrome (see below).

The abnormal muscle activity is the result of neuronal hyperexcitability, which may be generated anywhere along the peripheral motor nerve (Vincent, 2000). The diagnosis can be confirmed by typical findings on electromyography (Newsom-Davis and Mills, 1993), which include high frequency spontaneous discharges, and by the detection of antibodies to VGKCs in some patients.

Autoimmunity and Antibodies to Voltage-Gated Potassium Channels

As in MG and LEMS, NMT may be associated with other autoimmune diseases or other autoantibodies, and is relatively frequently found in patients with thymoma (Hart et al., 2002). CSF analysis occasionally shows a raised total IgG level or oligoclonal bands, suggesting that there is intrathecal IgG synthesis (Newsom-Davis and Mills, 1993).

The evidence for a pathogenic antibody was demonstrated before its target was known. Patients respond to plasma exchange and immunosuppression (Sinha et al., 1991), and some signs of neuronal hyperexcitability can be transferred to mice by injection of plasma or IgG from patients with NMT (Shillito et al., 1995). Importantly, the effects seen were very similar to those with the drugs 3,4-diamino-pyridine or 4-amino pyridine, which block VGKCs.

Antibodies to VGKCs can be detected in about 40% of NMT patients by immunoprecipitation of ^{125}I-α-dendrotoxin-labeled VGKCs extracted from human frontal cortex (Hart et al., 2002), and the level of this antibody is reduced by immunosuppressive treatment. A proportion of the 60% of sera negative for these antibodies may contain antibodies to some other peripheral nerve or motor nerve terminal protein, such as the neuronal α-7 AChR (Vernino and Lennon, 2002).

As for the other diseases considered in this chapter, there is no clear information regarding the etiology of NMT. However, there are anecdotal reports of the condition occurring in association with infections and some patients seem to have a monophasic illness that recovers spontaneously within 1–2 years (A. Vincent, unpublished observations). These observations suggest that there may be specific con-

ditions, probably infections, which can lead to the development of these antibodies.

CNS DISORDERS ASSOCIATED WITH ANTIBODIES

A number of diseases are strongly associated with antibodies to neuronal antigens, and in some of these the antibodies are likely to be pathogenic. This opens a new area of antibody-mediated CNS disorders.

Glutamic Acid Decarboxylase Antibodies with Stiff-Person Syndrome, Cerebellar Ataxia, and Other Disorders

Glutamate acid decarboxylase (GAD) is the enzyme responsible for the synthesis of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), and antibodies to GAD are best known for their association with type 1 diabetes (T1D). Nevertheless, they were first detected in a patient with stiff-person syndrome (SPS) associated with diabetes (Solimena et al., 1988). SPS is a very rare condition characterized by severe muscle stiffness, particularly affecting the spine and lower limbs, with superimposed muscle spasms. The clinical picture appears to result from lack of muscle relaxation that should occur in one set of muscles when the antagonistic muscles are activated.

GAD antibodies can now be detected by immunoprecipitation of ^{35}S-labeled rabbit reticulocyte lysates expressing GAD, or of ^{125}I-recombinant GAD. The levels of GAD antibodies in patients with neurologic diseases are usually much higher than those of patients with T1D (Dalakas et al., 2001b). GAD antibodies are also found in other disorders, particularly encephalomyelitis with rigidity and myoclonus, and sporadic cerebellar ataxia (Meinck et al., 2001).

SPS is associated with other autoimmune diseases and with HLA-DR3 (DRβ1 0301) (Nicholas et al., 1997). In many cases, oligoclonal bands are present in the CSF, and the CSF antibody/CSF IgG index is higher than the serum antibody/serum IgG index, suggesting that some of the GAD antibody is actually made in the CNS (Dalakas et al., 2001b), but generally the serum levels are substantially higher than the CSF levels (Vincent et al., 1997).

The striking association of SPS with high titers of a very specific antibody, which is not present in the great majority of individuals with other neurologic disorders, suggests that the disease is immune-mediated. Moreover, one study showed clinical improvement following administration of IVIG (Dalakas et al., 2001a). However, since GAD is an intracellular antigen, it may not, itself, be the target for the pathogenic immune response. There could be other antibodies directed against cell-surface determinants that are pathogenic.

Rasmussen Encephalitis

Rasmussen encephalitis (RE) is a very rare, chronic inflammatory disease of the brain, usually occurring in young children, and characterized by intractable focal seizures. It typically involves one side of the brain only and often progresses to hemiparesis. It does not respond well to typical anticonvulsant therapy and hemispherectomy is often performed in order to stop the seizures.

A recent study detailed clinical and pathologic evidence from 13 cases with histologically proven RE (Bien et al., 2002b). After a prodromal phase, the acute phase with seizures and focal inflammatory lesions develops. This leads to permanent and stable hemiparesis with cerebral atrophy and decreased seizure frequency. The histopathology of the brains showed perivascular and parenchymal CD3 T-cells, CD68 and HLA-DR positive microglial cells and glial fibrillary acidic protein (GFAP)-positive astrocytes, the majority of which were characterized as "reactive," and neuronal cell density was reduced. In fact, the pathology of RE is not unlike that of multiple sclerosis and is consistent with a T-cell-mediated process (Bien et al., 2002a).

However, there are reports of antibodies to glutamate receptor in RE. Two of three GluR3 immunized rabbits developed seizures (Rogers et al., 1994) and postmortem examination showed inflammatory changes not unlike those in patients. GluR3 antibodies were found in sera from two of four children using Western blot and antibody-binding assays. Most significant was that one child showed a remarkable improvement after plasma exchange (Rogers et al., 1994). This latter finding has been confirmed in other patients, and a number of studies have looked at possible mechanisms by which GluR3 antibodies could cause increased neuronal excitability and seizures (Whitney and McNamarra, 1999).

Although several reports confirm the presence of GluR3 antibodies in patients with RE, some find them also in patients with other forms of intractable epilepsy (Wiendl et al., 2001), and there was little GluR3 antibody in the CSF (Mantegazza et al., 2002). Moreover, other groups have failed to find GluR3 antibodies by a range of techniques (Watson et al., 2004).

Potassium Channel Antibodies in Morvan Syndrome and a New Form of Immune-Responsive Limbic Encephalitis

Antibodies to potassium channels were first identified in patients with acquired NMT (see above), but about 20% of these patients have CNS symptoms, such as depression, anxiety, memory loss or sleep disorders (Heidenreich and Vincent, 1998; Hart et al., 2002). In a few cases, NMT is associated with autonomic dysfunction, including increased sweating, and marked CNS abnormalities, including

insomnia, hallucinations, altered behavior, and cognitive dysfunction, known as Morvan syndrome or Morvan fibrillary chorea.

Only a few cases of Morvan syndrome have been described but several recent cases have been shown to have raised levels of VGKC antibodies, and symptoms improve following plasma exchange (Liguori et al., 2001). Thymoma has been reported in around 20% of cases, sometimes with associated MG. In one case with CNS symptoms of memory loss, confusion and seizures, the seizures and cognitive dysfunction improved slowly over a period of 2 years, while VGKC antibodies spontaneously fell (Buckley et al., 2001c and Vincent et al., 2004). A recent study has reported 10 patients with similar "limbic" symptoms and highly raised VGKC antibodies, several of whom improved quite markedly following immunotherapies (Vincent et al., 2004). Interestingly, some patients have presented first to psychiatric or epilepsy clinics before developing other neurologic features (McKnight et al., 2005).

Importantly, the limbic symptoms are highly similar to those experienced by patients with paraneoplastic limbic encephalitis, which is normally considered to respond very poorly to treatments. In patients with VGKC antibodies, by contrast, tumors are not common, and the response to treatments is often good. How the antibodies gain access to the CNS, why only some patients have peripheral symptoms, and why the hippocampus is the main target will be the topics for further experimental studies.

SUMMARY

Autoimmune diseases of the nervous system are particularly interesting because they include disorders caused by highly specific antibodies directed against equally specific ion channels or receptors. The role of antibodies to the muscle nicotinic AChR in MG is well established and much is now known about the immunology of the disease. The division of MG into groups, depending on antibody status, thymic pathology, and age at onset, has been useful in defining the existence of different clinical subtypes that may result from different etiologies. Treatment includes anticholinesterases, nonspecific immunosuppression and, in appropriate younger patients, thymectomy. Despite much work in animal models of MG, no disease-specific immunosuppressive treatments are currently available. A new form of MG, associated with antibodies to MuSK, has been identified, and found to present some distinct clinical features.

The experimental approaches that led to the successful work on MG have been equally successful in demonstrating other rare diseases caused by, or strongly associated with, antibodies to different ion channels or other neuronal proteins. Antibodies to calcium and potassium ion channels are found in LEMS and acquired NMT, respectively, and to the ganglionic form of AChR in some cases of autonomic neuropathy (see Chapter 17). Antibodies to glutamic-acid decarboxylase (GAD) and to a particular form of glutamate receptor (GluR3) have been detected in SPS and RE, respectively, although their roles in causing these disorders are not yet clear. More recently, antibodies to VGKCs have been detected in a form of limbic encephalitis with memory loss, confusion, and seizures. These patients often improve with immunotherapies, suggesting an antibody-mediated CNS disorder.

The next 10 years should show an explosion of interest in antibody-associated CNS disorders, leading to improved diagnosis and the development of new experimental paradigms to demonstrate the pathogenic relevance of antibodies to CNS antigens.

References

Aarli, J.A. (2001). Titin, thymoma, and myasthenia gravis. *Arch. Neurol. 58*, 869–870.

Andreetta, F., Baggi, F., Antozzi, C., Torchiana, E., Bernasconi, P., Simoncini, O., Cornelio, F., Montegazza, R. (1997). Acetylcholine receptor alpha-subunit isoforms are differentially expressed in thymuses from myasthenic patients. *Am. J. Pathol. 150*, 341–348.

Asher, O., Neumann, D., Witzemann, V., and Fuchs, S. (1990). Acetylcholine receptor gene expression in experimental autoimmune myasthenia gravis. *FEBS Lett. 267*, 231–235.

Baggi, F., Nicolle, M., Vincent, A., Matuo, H., Willcox, N., and Newsom-Davis, J. (1993). Presentation of endogenous acetylcholine receptor epitope by an MHC class II-transfected human muscle cell line to a specific $CD4^+$ T cell clone from a myasthenia gravis patient. *J. Neuroimmunol. 46*, 57–65.

Bain, P.G., Motomura, M., Newsom-Davis, J., Misbah, S.A., Chapel, H.M., Lee, M. I, Vincent, A., and Lang, B. (1996). Effects of intravenous immunoglobulin on muscle weakness and calcium-channel autoantibodies in the Lambert-Eaton myasthenic syndrome. *Neurology 47*, 678–683.

Bao, S., King, N.J., and Dos Remedios, C.G. (1992). Flavivirus induces MHC antigen on human myoblasts: a model of autoimmune myositis? *Muscle Nerve 15*, 1271–1277.

Beeson, D., Jacobson, L., Newsom-Davis, J., and Vincent, A. (1996). A transfected human muscle cell line expressing the adult subtype of the human muscle acetylcholine receptor for diagnostic assays in myasthenia gravis. *Neurology 47*, 1552–1555.

Berman, P.W. and Patrick, J. (1980). Linkage between the frequency of muscular weakness and loci that regulate immune responsiveness in murine experimental myasthenia gravis. *J. Exp. Med. 152*, 507–520.

Bien, C.G., Bauer, J., Deckwerth, T.L., Wiendl, H., Deckert, M., Wiestler, O.D., Schramm, J., Elger, C.E., and Lassmann, H. (2002a). Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's encephalitis. *Ann. Neurol. 51*, 311–318.

Bien, C.G., Widman, G., Urbach, H., Sassen, R., Kuczaty, S., Wiestler, O.D., Schramm, J., and Elger, C.E. (2002b). The natural history of Rasmussen's encephalitis. *Brain 125*, 1751–1759.

Biesecker, G. and Koffler, D. (1988). Resistance to experimental autoimmune myasthenia gravis in genetically inbred rats. Association with decreased amounts of *in situ* acetylcholine receptor-antibody complexes. *J. Immunol. 140*, 3406–3410.

Blalock, A., Mason, M.F., Morgan, H.J., and Riven, S.S. (1939). Myasthenia gravis and tumors of the thymic region. Report of a case in which the tumor was removed. *Ann. Surg. 110*, 544–559.

Bonifati, D.M., Willcox, N., Vincent, A., and Beeson, D. Lack of association between acetylcholine receptor epsilon polymorphisms and early-onset myasthenia gravis. *Muscle Nerve 29*, 436–439.

Buckley, C., Douek, D., Newsom-Davis, J., Vincent, A., and Wilcox, N. (2001a). Mature, long-lived $CD4^+$ and $CD8^+$ T cells are generated by the thymoma in myasthenia gravis. *Ann. Neurol. 50*, 64–72.

Buckley, C., Newsom-Davis, J., Willcox, N., and Vincent, A. (2001b). Do titin and cytokine antibodies in MG patients predict thymoma or thymoma recurrence? *Neurology 57*, 1579–1582.

Buckley, C., Oger, J., Clover, L., Tuzun, E., Carpenter, K., Jackson, M., and Vincent, A. (2001c). Potassium channel antibodies in two patients with reversible limbic encephalitis. *Ann. Neurol. 50*, 73–78.

Burges, J., Wray, D.W., Pizzighella, S., Hall, Z., and Vincent, A. (1990). A myasthenia gravis plasma immunoglobulin reduces miniature endplate potentials at human endplates *in vitro*. DR-DQ associations in subgroups of idiopathic myasthenia gravis. *Muscle Nerve 13*, 407–413.

Buzzard, E.F. (1905). The clinical history and postmortem examination of five cases of myasthenia gravis. *Brain 28*, 438.

Carlsson, B., Wallin, J., Pirskanen, R., Matell, G., and Smith, C.I. (1990). Different HLA DR-DQ associations in subgroups of idiopathic myasthenia gravis. Immunogenetics *31*, 285–290.

Chang, C. and Lee, C. (1962). Isolation of neurotoxins from the venom of *Bungarus multicinctus* and their modes of neuromuscular blocking action. *Arch. Pharmacodyn. Ther. 144*, 241–257.

Christadoss, P., Lennon, V.A., and David, C. (1979). Genetic control of experimental autoimmune myasthenia gravis in mice. I. Lymphocyte proliferative response to acetylcholine receptors is under H-2-linked Ir gene control. *J. Immunol. 123*, 2540–2543.

Christadoss, P., Poussin, M., and Deng, C. (2000). Animal models of myasthenia gravis. *Clin. Immunol. 94*, 75–87.

Compston, D.A., Vincent, A., Newsom-Davis, J., and Batchelor, J.R. (1980). Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain 103*, 579–601.

Conti-Fine, B.M., Navaneetham, D., Karachunski, P.I., Raju, R., Diethelm-Okita, B., Okita, A., Howard, J., Jr., and Wang, Z.Y. (1998). T cell recognition of the acetylcholine receptor in myasthenia gravis. *Ann. N. Y. Acad. Sci. 841*, 283–308.

Croxen, R., Vincent, A., Newsom-Davis, J., and Beeson, D. (2002). Myasthenia gravis in a woman with congenital AChR deficiency due to epsilon-subunit mutations. *Neurology 58*, 1563–1565.

Curnow, J., Corlett, L., Willcox, N., and Vincent, A. Presentation by myoblasts of an epitope from endogenous acetylcholine receptor indicates a potential role in the spreading of the immune response. *J. Neuroimmunol. 115*, 127–134.

Dalakas, M.C., Fujii, M., Li, M., Luthi, B, Kyhos, J., and McElroy, B. (2001a). High-dose intravenous immune globulin for stiff-person syndrome. *N. Engl. J. Med. 345*, 1870–1876.

Dalakas, M.C., Li, M., Fujii, M., and Jacobowitz, D.M. (2001b). Stiff person syndrome: quantification, specificity, and intrathecal synthesis of GAD65 antibodies. *Neurology 57*, 780–784.

Demaine, A., Willcox, N., Welsh, K., and Newsom-Davis, J. (1988). Associations of the autoimmune myasthenias with genetic markers in the immunoglobulin heavy chain region. *Ann. N. Y. Acad. Sci. 540*, 266–268.

Djabiri, F., Caillat-Zucman, S., Gajdos, P., Jais, J. P., Gomez, L., Khalil, J., Charron, D., Bach, J.F., and Garchon, H.J. (1997). Association of the AChRalpha-subunit gene (CHRNA), DQA1*0101, and the DR3 haplotype in myasthenia gravis. Evidence for a three-gene disease model in a subgroup of patients. *J. Autoimmun. 10*, 407–413.

Drachman, D.B., Angus, C.W., Adams, R.N., and Kao, I. (1978). Effect of myasthenic patients' immunoglobulin on acetylcholine receptor turnover: selectivity of degradation process. *Proc. Natl. Acad. Sci. USA 75*, 3422–3426.

Elmqvist, D., Hofmann, W., Kugelberg, J., and Quastel, D. (1964). An electrophysiological investigation of neuromuscular transmission in myasthenia gravis. *J. Physiol. (Lond.) 174*, 417–434.

Engel, A.G. (1991). Review of evidence for loss of motor nerve terminal calcium channels in Lambert-Eaton myasthenic syndrome. *Ann. N. Y. Acad. Sci. 635*, 246–258.

Engel, A.G. and Arahata, K. (1987). The membrane attack complex of complement at the endplate in myasthenia gravis. *Ann. N. Y. Acad. Sci. 505*, 326–332.

Engel, A.G., Lindstrom, J.M., Lambert, E.H., and Lennon, V.A. (1977). Ultrastructural localization of the acetylcholine receptor in myasthenia gravis and in its experimental autoimmune model. *Neurology 27*, 307–315.

Engel, A.G., Nagel, A., Fukuoka, T., Fukunaga, H., Osame, M., Lang, B., Newsom-Davis, J., Vincent, A., Wray, D.W., and Peers, C. (1989). Motor nerve terminal calcium channels in Lambert-Eaton myasthenic syndrome. Morphologic evidence for depletion and that the depletion is mediated by autoantibodies. *Ann. N. Y. Acad. Sci. 560*, 278–290.

Fambrough, D.M., Drachman, D.B., and Satyamurti, S. (1973). Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. *Science 182*, 293–295.

Farrar, J., Portolano, S., Willcox, N., Vincent, A., Jacobson, L., Newsom-Davis, J., Rapaport, B., and McLachlin, S.M. (1997). Diverse Fab specific for acetylcholine receptor epitopes from a myasthenia gravis thymus combinatorial library. *Int. Immunol. 9*, 1311–1318.

Fujii, Y., Hayakawa, M., Inada, K., and Nakahara, K. (1990). Lymphocytes in thymoma: association with myasthenia gravis is correlated with increased number of single-positive cells. *Eur. J. Immunol. 20*, 2355–2358.

Fukuoka, T., Engel, A.G., Lang, B., et al. Lambert-Eaton myasthenic syndrome: I. Early morphological effects of IgG on the presynaptic membrane active zones. *Ann. Neurol. 22*, 193–199.

Garchon, H.J., Djabiri, F., Viard, J.P., Gajdos, P., and Bach, J.F. (1994). Involvement of human muscle acetylcholine receptor alpha-subunit gene (CHRNA) in susceptibility to myasthenia gravis. *Proc. Natl. Acad. Sci. USA 91*, 4668–4672.

Gautel, M., Lakey, A., Barlow, D.P., Holmes, Z., Scales, S., Leonard, K., Labeit, S., Mygland, A., Gilhus, N.E., and Aarli, J.A. (1993). Titin antibodies in myasthenia gravis: identification of a major immunogenic region of titin. *Neurology 43*, 1581–1585.

Geuder, K.I., Marx, A., Witzemann, V., Schalke, B., Kirchener, T., and Muller-Hermelink, H.K. (1992). Genomic organization and lack of transcription of the nicotinic acetylcholine receptor subunit genes in myasthenia gravis-associated thymoma. *Lab. Invest. 66*, 452–458.

Gilhus, N.E., Jones, M., Turley, H., Gatter, K.C., Nagveker, N., Newsom-Davis, J., and Willcox, N. (1995a). Oncogene proteins and proliferation antigens in thymomas: increased expression of epidermal growth factor receptor and Ki67 antigen. *J. Clin. Pathol. 48*, 447–455.

Gilhus, N.E., Willcox, N., Harcourt, G., Nagveker, N., Beeson, D., Vincent, A., and Newsom-Davis, J. (1995b). Antigen presentation by thymoma epithelial cells from myasthenia gravis patients to potentially pathogenic T cells. *J. Neuroimmunol. 56*, 65–76.

Graus, Y.F., de Baets, M.H., Parren, P.W., Berrih-Aknink, S., Wokke, J., van Breda Vriesman, P.J., and Burton, D.R. (1997). Human anti-nicotinic acetylcholine receptor recombinant Fab fragments isolated from thymus-derived phage display libraries from myasthenia gravis patients reflect predominant specificities in serum and block the action of pathogenic serum antibodies. *J. Immunol. 158*, 1919–1929.

Guigou, V., Emilie, D., Berrih-Aknin, S., Fumoux, F., Fougereau, M., and Schiff, C. (1991). Individual germinal centres of myasthenia gravis human thymuses contain polyclonal activated B cells that express all the Vh and Vk families. *Clin. Exp. Immunol. 83*, 262–266.

Guyon, T., Levasseur, P., Truffault, F., Cottin, C., Gaud, C., and Berrih-Aknin, S. (1994). Regulation of acetylcholine receptor alpha subunit

variants in human myasthenia gravis. Quantification of steady-state levels of messenger RNA in muscle biopsy using the polymerase chain reaction. *J. Clin. Invest. 94*, 16–24.

Hara, Y., Ueno, S., Uemichi, T., Takahashi, N., Yorifuji, S., Fujii, Y., and Tarui, S. (1991). Neoplastic epithelial cells express alpha-subunit of muscle nicotinic acetylcholine receptor in thymomas from patients with myasthenia gravis. *FEBS Lett. 279*, 137–140.

Hart, I.K., Maddison, P., Newsom-Davis, J., Vincent, A., and Mills, K.R. (2002). Phenotypic variants of autoimmune peripheral nerve hyperexcitability. *Brain 125*, 1887–1895.

Hawke, S., Matsuo, H., Nicolle, M., Malcherek, G., Melms A., and Willcox, N. (1996). Autoimmune T cells in myasthenia gravis: heterogeneity and potential for specific immunotargeting. *Immunol. Today 17*, 307–311.

Heidenreich, F. and Vincent, A. (1998). Antibodies to ion-channel proteins in thymoma with myasthenia, neuromyotonia, and peripheral neuropathy. *Neurology 50*, 1483–1485.

Hjelmstrom, P., Giscombe, R., Lefvert, A.K., Pirskanen, R., Kockum, I., Landin-Olsson, M., and Sanjeevi, C.B. (1997). TAP polymorphisms in Swedish myasthenia gravis patients. *Tissue Antigens 49*, 176–179.

Hjelmstrom, P., Peacock, C.S., Giscombe, R., Pirskanen, R., Lefvert, A.K., Blackwell, J.M., and Sanjeevi, C.B. (1998). Myasthenia gravis with thymic hyperplasia is associated with polymorphisms in the tumor necrosis factor region. *Ann. N. Y. Acad. Sci. 841*, 368–370.

Hoch, W., McConville, J., Helms, S., Newsom-Davis, J., Melms, A., and Vincent, A. (2001). Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat. Med. 7*, 365–368.

Hohlfeld, R. and Engel, A.G. (1990). Induction of HLA-DR expression on human myoblasts with interferon-gamma. *Am. J. Pathol. 136*, 503–508.

Hohlfeld, R. and Wekerle, H. (1994). The role of the thymus in myasthenia gravis. *Adv. Neuroimmunol. 4*, 373–386.

Isaacs, H.A. (1961). Syndrome of continuous muscle fibre activity. *J. Neurol. Neurosurg. Psychiatry 24*, 319–325.

Jacobson, L., Beeson, D., Tzartos, S., and Vincent, A. (1999a). Monoclonal antibodies raised against human acetylcholine receptor bind to all five subunits of the fetal isoform. *J. Neuroimmunol. 98*, 112–120.

Jacobson, L., Polizzi, A., Morriss-Kay, G., and Vincent, A. (1999b). Plasma from human mothers of fetuses with severe arthrogryposis multiplex congenita causes deformities in mice. *J. Clin. Invest. 103*, 1031–1038.

Janer, M., Cowland, A., Picard, J., Campbell, D., Pontarotti, P., Newsom-Davis, J., Bunce, M., Welsh, K., Demaine, A., Wilson, A.G., and Willcox, N. (1999). A susceptibility region for myasthenia gravis extending into the HLA-class I sector telomeric to HLA-C. *Hum. Immunol. 60*, 909–917.

Jermy, A., Beeson, D., and Vincent, A. (1993). Pathogenic autoimmunity to affinity-purified mouse acetylcholine receptor induced without adjuvant in BALB/c mice. *Eur. J. Immunol. 23*, 973–976.

Jolly, F. (1895). Ueber Myasthenia gravis pseudoparalytica. *Berl. Klin. Wochenschr. 32*, 1–7.

Kao, I. and Drachman, D.B. (1977). Myasthenic immunoglobulin accelerates acetylcholine receptor degradation. *Science 196*, 527–529.

Kaul, R., Shenoy, M., Goluszko, E., and Christadoss, P. (1994). Major histocompatibility complex class II gene disruption prevents experimental autoimmune myasthenia gravis. *J. Immunol. 152*, 3152–3157.

Kim, Y.I. (1985). Passive transfer of the Lambert-Eaton myasthenic syndrome: neuromuscular transmission in mice injected with plasma. *Muscle Nerve 8*, 162–172.

Kuks, J.B., Oosterhuis, H.J., Limburg, P.C., and The, T.H. (1991). Anti-acetylcholine receptor antibodies decrease after thymectomy in patients with myasthenia gravis. Clinical correlations. *J. Autoimmun. 4*, 197–211.

Kusner, L.L., Mygland, M., and Kaminski, H.J. (1998). Ryanodine receptor gene expression thymomas. *Muscle Nerve 21*, 1299–1303.

Lang, B., Newsom-Davis, J., Prior, C., and Wray, D. (1983). Antibodies to motor nerve terminals: an electrophysiological study of a human myasthenic syndrome transferred to mouse. *J. Physiol. 344*, 335–345.

Lang, B., Richardson, G., Rees, J., Vincent, A., and Newsom-Davis, J. (1988). Plasma from myasthenia gravis patients reduces acetylcholine receptor agonist-induced Na^+ flux into TE671 cell line. *J. Neuroimmunol. 19*, 141–148.

Lennon, V.A., Kryzer, T.J., Griesmann, G.E., O'Suillebhain, P.E., Windebank, A.J., Woppmann, A., Miljanich, G.P., and Lambert, E.H. (1995). Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. *N. Engl. J. Med. 332*, 1467–1474.

Lennon, V.A., Lambert, E.H., Whittingham, S., and Fairbanks, V. (1982). Autoimmunity in the Lambert-Eaton myasthenic syndrome. *Muscle Nerve 5*, S21–25.

Levine, G.D. and Rosai, J. (1978). Thymic hyperplasia and neoplasia: a review of current concepts. *Hum. Pathol. 9*, 495–515.

Liguori, R., Vincent, A., Clover, L., Avoni, P., Plazzi, G., Cortelli, P., Baruzzi, A., Carey, T., Gambetti, P, Lubaresi, E., and Montagna, P. (2001). Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. *Brain 124*, 2417–2426.

Lindstrom, J.M. (2000). Acetylcholine receptors and myasthenia. *Muscle Nerve 23*, 453–477.

Lindstrom, J.M., Engel, A.G., Seybold, M.E., Lennon, V.A., and Lambert, E.H. (1976a). Pathological mechanisms in experimental autoimmune myasthenia gravis. II. Passive transfer of experimental autoimmune myasthenia gravis in rats with anti-acetylcholine receptor antibodies. *J. Exp. Med. 144*, 739–753.

Lindstrom, J.M., Seybold, M.E., Lennon, V.A., et al. (1976b). Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology 26*, 1054–1059.

Link, H., Olsson, O., Sun, J., Wang, W.Z., Andersson, G., Ekre, H.P., Brenner, T., Abramsky, O., and Olsson, T. (1991). Acetylcholine receptor-reactive T and B cells in myasthenia gravis and controls. *J. Clin. Invest. 87*, 2191–2195.

Liyanage, Y., Hoch, W., Beeson, D., and Vincent, A. (2002). The agrin/muscle-specific kinase pathway: new targets for autoimmune and genetic disorders at the neuromuscular junction. *Muscle Nerve 25*, 4–16.

Mantegazza, R., Bernasconi, P., Baggi, F., Spreafico, R., Ragona, F., Antozzi, C., Bernardi, G., and Granata, T. (2002). Antibodies against GluR3 peptides are not specific for Rasmussen's encephalitis but are also present in epilepsy patients with severe, early onset disease and intractable seizures. *J. Neuroimmunol. 131*, 179–185.

Marx, A., Schomig, D., Schultz, A., Gattenlohner, S., Jung, A., Kirchner, T., Melms, A., and Muller-Hermelink, H.K. (1994). Distribution of molecules mediating thymocyte–stroma interactions in human thymus, thymitis and thymic epithelial tumors. *Thymus 23*, 83–93.

Marx, A., Wilisch, A., Schultz, A., Greiner, A., Magi, B., Pallini, V, Schalke, B., Toyka, K., Nix, W., Kirchner, T., and Muller-Hemelink, H.K. (1996). Expression of neurofilaments and of a titin epitope in thymic epithelial tumors. Implications for the pathogenesis of myasthenia gravis. *Am. J. Pathol. 148*, 1839–1850.

Matthews, I., Sims, G., Ledwidge, S., Stott, D., Beeson, D., Willcox, N., and Vincent, A. (2002). Antibodies to acetylcholine receptor in parous women with myasthenia: evidence for immunization by fetal antigen. *Lab. Invest. 82*, 1407–1417.

McConville, J., Farrugia, M.E., Beeson, D., Kishore, U., Metcalfe, R., Newsom-Davis, J., and Vincent, A. (2004). Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann. Neurol. 55*, 580–584.

McKnight, K., Jiang, Y., Hart, Y., Cavey, A., Wroe, S., Blank, M., Shoenfeld, Y., Vincent, A., Palace, J., and Lang, B. (2005). Serum anti-

bodies in epilepsy and seizure-associated disorders. *Neurology. 65*, 1730–1736.

Meager, A., Vincent, A., Newsom-Davis, J., and Willcox, N. (1997). Spontaneous neutralising antibodies to interferon—alpha and interleukin-12 in thymoma-associated autoimmune disease. *Lancet 350*, 1596–1597.

Meinck, H.M., Faber, L., Morgenthaler, N., Seissler, J., Maile, S., Butler, M., Solmena, M., DeCamilla, P., and Scherbaum, V.A. (2001). Antibodies against glutamic acid decarboxylase: prevalence in neurological diseases. *J. Neurol. Neurosurg. Psychiatry 71*, 100–103.

Motomura, M., Johnston, I., Lang, B., Vincent, A., and Newsom-Davis, J. (1995). An improved diagnostic assay for Lambert-Eaton myasthenic syndrome. *J. Neurol. Neurosurg. Psychiatry 58*, 85–87.

Muller-Hermelink, H.K. and Marx, A. (2000). Towards a histogenetic classification of thymic epithelial tumours? *Histopathology 36*, 466–469.

Mygland, A., Kuwajima, G., Mikoshiba, K., Tysnes, O.B., Aarli, J.A., and Gilhus, N.E. (1995). Thymomas express epitopes shared by the ryanodine receptor. *J. Neuroimmunol. 62*, 79–83.

Nastuk, W.L., Plescia, O.J., Osserman, K.E. (1960). Changes in serum complement activity in patients with myasthenia gravis. *Proc. Soc. Exp. Biol. Med. 105*, 177–184.

Newsom-Davis, J. and Mills, K.R. (1993). Immunological associations of acquired neuromyotonia (Isaacs' syndrome). Report of five cases and literature review. *Brain 116*, 453–469.

Newsom-Davis, J., Pinching, A.J., Vincent, A., and Wilson, S.G. (1978). Function of circulating antibody to acetylcholine receptor in myasthenia gravis: investigation by plasma exchange. *Neurology 28*, 266–272.

Nicholas, A.P., Chatterjee, A., Arnold, M.M., Claussen, G.C., Zorn, G.L., Jr., and Oh, S.J. (1997). Stiff-persons' syndrome associated with thymoma and subsequent myasthenia gravis. *Muscle Nerve 20*, 493–498.

O'Neill, J.H., Murray, N.M., and Newsom-Davis, J. (1988). The Lambert-Eaton myasthenic syndrome. A review of 50 cases. *Brain 111*, 577–596.

Palmisani, M.T., Evoli, A., Batocchi, A.P., Bartoccioni, E., and Tonali, P. (1994). Myasthenia gravis and associated autoimmune diseases. *Muscle Nerve 17*, 1234–1235.

Patrick, J. and Lindstrom, J. (1973). Autoimmune response to acetylcholine receptor. *Science 180*, 871–872.

Peers, C., Johnston, I., Lang, B., and Wray, D. (1993). Cross-linking of presynaptic calcium channels: a mechanism of action for Lambert-Eaton myasthenic syndrome antibodies at the mouse neuromuscular junction. *Neurosci. Lett. 153*, 45–48.

Pinching, A.J., Peters, D.K., and Davis, J.N. (1977). Plasma exchange in myasthenia gravis. *Lancet. 1*, 428–429.

Plested, C.P., Tang, T., Spreadbury, I., Littleton, E.T., Kishore, U., and Vincent, A. (2002). AChR phosphorylation and indirect inhibition of AChR function in seronegative MG. *Neurology 59*, 1682–1688.

Polizzi, A., Huson, S.M., and Vincent, A. (2000). Teratogen update: maternal myasthenia gravis as a cause of congenital arthrogryposis. *Teratology 62*, 332–341.

Prior, C., Lang, B., Wray, D., and Newsom-Davis, J. (1985). Action of Lambert-Eaton myasthenic syndrome IgG at mouse motor nerve terminals. *Ann. Neurol. 17*, 587–592.

Reinhardt, C. and Melms, A. (2000). Normalization of elevated $CD4^-/CD8^-$ (double-negative) T cells after thymectomy parallels clinical remission in myasthenia gravis associated with thymic hyperplasia but not thymoma. *Ann. Neurol. 48*, 603–608.

Roberts, A., Perera, S., Lang, B., Vincent, A., and Newsom-Davis, J. (1985). Paraneoplastic myasthenic syndrome IgG inhibits $^{45}Ca^{2+}$ flux in a human small cell carcinoma line. *Nature 317*, 737–739.

Rogers, S.W., Andrews, P.I, Gahring, L.C., Whisenand, T., Cauley, K., Crain, B., Hughes, T.E., Heinemann, S.F., and McNamara, J.O. (1994). Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science 265*, 648–651.

Romi, F., Bo, L., Skeie, G.O., Myking, A., Aarli, J.A., and Gilhus, N.E. (2002). Titin and ryanodine receptor epitopes are expressed in cortical thymoma along with costimulatory molecules. *J. Neuroimmunol. 128*, 82–89.

Rose, N.R. and Bona, C. (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today 14*, 426–430.

Roxanis, I., Micklem, K., McConville, J., Newsom-Davis, J., Willcox, N. (2002). Thymic myoid cells and germinal center formation in myasthenia gravis; possible roles in pathogenesis. *J Neuroimmunol. 125*, 185–197.

Salmon, A.M., Bruand, C., Cardona, A., Changeux, J.P., and Berrih-Aknin, S. (1998). An acetylcholine receptor alpha subunit promoter confers intrathymic expression in transgenic mice. Implications for tolerance of a transgenic self-antigen and for autoreactivity in myasthenia gravis. *J. Clin. Invest. 101*, 2340–2350.

Sauerbruch, H., Schumacher, C.H., and Roth, P. (1913). Thymektomie bei einem Fall von Morbus Basedowi mit Myastheine. *Mitteil. Grenzgeb. Med. Chir 25*, 746–765.

Scadding, G.K., Vincent, A., Newsom-Davis, J., and Henry, K. (1981). Acetylcholine receptor antibody synthesis by thymic lymphocytes: correlation with thymic histology. *Neurology 31*, 935–943.

Schluep, M., Willcox, N., Vincent A., Dhoot, G.K., and Newsom-Davis, J. (1987). Acetylcholine receptors in human thymic myoid cells *in situ*: an immunohistological study. *Ann. Neurol. 22*, 212–222.

Scuderi, F., Marino, M., Colonna, L., Mannella, F., Evoli, A., Provenzono, C., and Bartoccioni, E. (2002). Anti-p110 autoantibodies identify a subtype of "seronegative" myasthenia gravis with prominent oculobulbar involvement. *Lab. Invest. 82*, 1139–1146.

Shillito, P., Molenaar, P.C., Vincent, A., Leys, K., Zheng, W., van den Berg, R.J., Plomp, J.J., van Kempen, G.T., Chauplannaz, G., and Wintzen, A.R. (1995). Acquired neuromyotonia: evidence for autoantibodies directed against K^+ channels of peripheral nerves. *Ann. Neurol. 38*, 714–722.

Shiono, H., Wong, Y.L., Matthews, I., Liu, J.L., Zhang, W., Sims, G., Meager, A., Beeson, P., Vincent, A., and Willcox, N. (2003). Spontaneous production of anti-IFN-alpha and anti-IL-12 autoantibodies by thymoma cells from myasthenia gravis patients suggests autoimmunization in the tumor. *Int. Immunol. 15*, 903–913.

Simpson, J.A. (1960). Myasthenia gravis, a new hypothesis. *Scott. Med. J. 5*, 419–436.

Sims, G.P., Shiono, H., Willcox, N., and Stott, D.I. (2001). Somatic hypermutation and selection of B cells in thymic germinal centers responding to acetylcholine receptor in myasthenia gravis. *J. Immunol. 167*, 1935–1944.

Sinha, S., Newsom-Davis, J., Mills, K., Byrne, N., Lang, B., and Vincent A. (1991). Autoimmune aetiology for acquired neuromyotonia (Isaacs' syndrome). *Lancet 338*, 75–77.

Solimena, M., Folli, F., Denis-Donini, S., Comi, G.C., Pozza, G., De Camilli, P., and Vicari, A.M. (1988). Autoantibodies to glutamic acid decarboxylase in a patient with stiff-man syndrome, epilepsy, and type I diabetes mellitus. *N. Engl. J. Med. 318*, 1012–1020.

Somnier, F.E. (1994). Exacerbation of myasthenia gravis after removal of thymomas. *Acta Neurol. Scand. 90*, 56–66.

Somnier, F.E. and Engel, P.J. (2002). The occurrence of anti-titin antibodies and thymomas: a population survey of MG 1970–1999. *Neurology 59*, 92–98.

Spreadbury, I, Kishore, U., Beeson, D., and Vincent, A. Inhibition of acetylcholine receptor function by seronegative myasthenia gravis non-IgG factor correlates with desensitisation. *J. Neuroimmunol. 162*, 149–156.

Stanley, E.F. and Drachman, D.B. (1978). Effect of myasthenic immunoglobulin on acetylcholine receptors of intact mammalian neuromuscular junctions. *Science 200*, 1285–1287.

Strauss, A.J.L., Seegal, B.C., Hsu, K.C., Burkholder, P.M., Nastuk, W.L., and Osserman, K.E. (1960). Immunofluorescence demonstration of muscle binding, complement fixing serum globulin fraction in myasthenia gravis. *Proc. Soc. Exp. Biol. Med 105*, 184–191.

Toyka, K.V., Brachman, D.B., Pestronk, A., and Kao, I. (1975). Myasthenia gravis: passive transfer from man to mouse. *Science 190*, 397–399.

Tzartos, S.J. and Lindstrom, J.M. (1980). Monoclonal antibodies used to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. *Proc. Natl. Acad. Sci. USA 77*, 755–759.

Tzartos, S.J., Seybold, M.E., and Lindstrom, J.M. (1982). Specificities of antibodies to acetylcholine receptors in sera from myasthenia gravis patients measured by monoclonal antibodies. *Proc. Natl. Acad. Sci. USA 79*, 188–192.

Tzartos, S.J., Barkas, T., Cung, M.T., Mamalaki, A., Marraud, M., Orlawski, P., Papanastasiou, D., Sakarelles, C., Sakarelles-Daitsiotis, M., Tsantili, P., and Tsikaris, V. (1998). Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. *Immunol. Rev. 163*, 89–120.

Unwin, N. (2000). The Croonian Lecture 2000. Nicotinic acetylcholine receptor and the structural basis of fast synaptic transmission. *Philos. Trans. R. Soc. Lond. B Biol. Sci. 355*, 1813–1829.

Verma, P.K. and Oger, J.J. (1992). Seronegative generalized myasthenia gravis: low frequency of thymic pathology. *Neurology 42*, 586–589.

Vernet der Garabedian, B., Lacokova, M., Eymard, B., Morel, E., Faltin, M., Zajac, J., Sadovsky, O., Dommergues, M., Tripon, P., and Bach, J.F. (1994). Association of neonatal myasthenia gravis with antibodies against the fetal acetylcholine receptor. *J. Clin. Invest. 94*, 555–559.

Vernino, S. and Lennon, V.A. (2002). Ion channel and striational antibodies define a continuum of autoimmune neuromuscular hyperexcitability. *Muscle Nerve 26*, 702–707.

Vieira, M.L., Caillat-Zucman, S., Gajdos, P., Cohen-Kaminsky, S., Casteru, A., and Bach, J.F. (1993). Identification by genomic typing of non-DR3 HLA class II genes associated with myasthenia gravis. *J. Neuroimmunol. 47*, 115–122.

Vincent, A. (2000). Understanding neuromyotonia. *Muscle Nerve 23*, 655–657.

Vincent, A. and Drachman, D.B. (2002). Myasthenia gravis. *Adv. Neurol. 88*, 159–188.

Vincent, A., Newsom-Davis, J., Newton, P., and Beck, N. (1983). Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. *Neurology 33*, 1276–1282.

Vincent, A., Whiting, P. J., Schluep, M., Heidenreich, F., Lang, B., Roberts, A., Willcox, N., and Newsom-Davis, J. (1987). Antibody heterogeneity and specificity in myasthenia gravis. *Ann. N. Y. Acad. Sci. 505*, 106–120.

Vincent, A., Grimaldi, L. M., Martino, G., Davenport, C., and Todd, I. (1997). Antibodies to ^{125}I-glutamic acid decarboxylase in patients with stiff man syndrome. *J. Neurol. Neurosurg. Psychiatry 62*, 395–397.

Vincent, A., Willcox, N., Hill, M., Curnow, J., MacLennan, C., and Beeson, D. (1998). Determinant spreading and immune responses to acetylcholine receptors in myasthenia gravis. *Immunol. Rev. 164*, 157–168.

Vincent, A., Palace, J., and Hilton-Jones, D. (2001). Myasthenia gravis. *Lancet 357*, 2122–2128.

Vincent, A. (2002). Unravelling the pathogenesis of myasthenia gravis. *Nat. Rev. Immunol. 2*, 797–804. Review.

Vincent, A., Bowen, J., Newsom-Davis, J., and McConville, J. (2003a). Seronegative generalised myasthenia gravis: clinical features, antibodies, and their targets. *Lancet Neurol. 2*, 99–106.

Vincent, A., Clover, L., Buckley, C., Grimley Evans, J., and Rothwell, P.M.; UK Myasthenia Gravis Survey. (2003b). Evidence of underdiagnosis of myasthenia gravis in older people. *J. Neurol. Neurosurg. Psychiatry 74*, 1105–1108.

Vincent, A., Buckley, C., Schott, J.M., Baker, I., Dewar, B.K., Detert, N., Clover, L., Parkinson, A., Bien, C.G., Omer, S., Lang, B., Rossor, M.N., and Palace, J. (2004). Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain 127*, 701–712.

Walker, M.B. (1934). Treatment of myasthenia gravis with physostigmine. *Lancet 1*, 1200–1201.

Waterman, S.A., Lang, B., and Newsom-Davis, J. (1997). Effect of Lambert-Eaton myasthenic syndrome antibodies on autonomic neurons in the mouse. *Ann. Neurol. 42*, 147–156.

Watson, R., Jiang, Y., Bermudez, I., Houlihan, L., Clover, L. McKnight, A., Cross, J.H., Hart, I.K., Roubertie, A., Valmier, J., Hart, Y., Palace, J., Beeson, D., Vincent, A., and Lang, B. (2004). Absence of antibodies to glutamate receptor type 3 (GluR3) in Rasmussen encephalitis. *Neurology 63*, 43–50.

Wekerle, H. and Ketelsen, U.P. (1977). Intrathymic pathogenesis and dual genetic control of myasthenia gravis. *Lancet 1*, 678–680.

Whiting, P.J., Vincent, A., and Newsom-Davis, J. (1986). Myasthenia gravis: monoclonal antihuman acetylcholine receptor antibodies used to analyze antibody specificities and responses to treatment. *Neurology 36*, 612–617.

Whitney, K.D. and McNamara, J.O. (1999). Related articles, autoimmunity and neurological disease: antibody modulation of synaptic transmission. *Annu. Rev. Neurosci. 22*, 175–195.

Wiendl, H., Bien, C.G., Bernasconi, P. et al. (2001). GluR3 antibodies: prevalence in focal epilepsy but no specificity for Rasmussen's encephalitis. *Neurology 57*, 1511–1514.

Willcox, N., Schluep, M., Sommer, N., Campana, D., Janossy, G., Brown, A.N., and Newsom-Davis, J. (1989). Variable corticosteroid sensitivity of thymic cortex and medullary peripheral-type lymphoid tissue in myasthenia gravis patients: structural and functional effects. *Q. J. Med. 73*, 1071–1087.

Willcox, N., Schluep, M., Ritter, M.A., and Newsom-Davis, J. (1991). The thymus in seronegative myasthenia gravis patients. *J. Neurol. 238*, 256–261.

Willis, T. (1672). *De Anima Brutorum* (pp. 404–407). Oxford, England: Teatro Sheldoniano.

Wilson, S., Vincent, A., and Newsom-Davis, J. (1973). Acetylcholine receptor turnover in mice with passively transferred myasthenia gravis. II. Receptor synthesis. *J. Neurol. Neurosurg. Psychiatry 46*, 383–387.

CHAPTER

49

Ocular Disease

GRACE A. LEVY-CLARKE, ZHUQING LI, LEILA I. KUMP, AND ROBERT B. NUSSENBLATT

The Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

HISTORIC BACKGROUND

The concept that the eye harbors autoimmune-inducing or uveitogenic materials has been suggested by many since the beginning of the last century. It was the demonstration by Uhlenhuth (1903) of autoantibody production to the lens that pioneered investigation in this area. Several investigators used homogenates from the eye, which when injected into an animal appeared capable of inducing an intraocular inflammatory response. Noteworthy of mention are Wacker and Lipton (1968) and Faure (1980).

The presence of uveitogenic antigens in the eye that are capable of inducing disease is a well-established concept, proposed as early as 1910 by Elschnig. Since then several antigens have been isolated that are capable of inducing ocular inflammatory disease similar to that seen in humans. Retinal S-antigen or arrestin was isolated and its immunologic properties partially characterized by Wacker et al. (1977). It is one of the most potent uveitogenic antigens defined to date. It causes an immune-mediated, bilateral inflammatory response in the eye, or experimental autoimmune uveitis (EAU), when injected in microgram quantities at a site far from the globe (Pfister et al., 1985). Several other uveitogenic antigens have since been identified, such as interphotoreceptor retinoid-binding protein (IRBP) (Hirose et al., 1986); recoverin (Gery et al, 1994); bovine melanin protein (Chan et al., 1994); rhodopsin (Schalken et al., 1989); phosducin (Lee et al., 1990); RPE 65 (Ham et al., 2002), and tyrosinase proteins (Yamaki et al., 2000).

These studies have broadened our understanding of the ocular immune response and allowed templates to be developed with which newer approaches to immunosuppression can be tested.

CLINICAL FEATURES

The uveal tract can be divided anatomically into the iris, ciliary body, and choroid (Figure 49.1). Uveitis is usually defined as any inflammation of the uveal tract. This inflammation may be an antigen-specific, immune-mediated response or a nonspecific response, which can be elicited by infection, trauma or surgery. In clinical practice, any inflammatory reaction involving the structures of the eye—sclera, cornea, lens, vitreous, retina, retinal pigment epithelium or choroid (see Figure 49.1)—is considered to be uveitis.

Episcleritis is an inflammatory disease involving the tissue that lies superficial to the sclera. Scleritis is a painful and potentially sight-threatening inflammatory disease involving the sclera. Episcleritis is typically more acute and less severe than scleritis and it is often idiopathic; however,

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

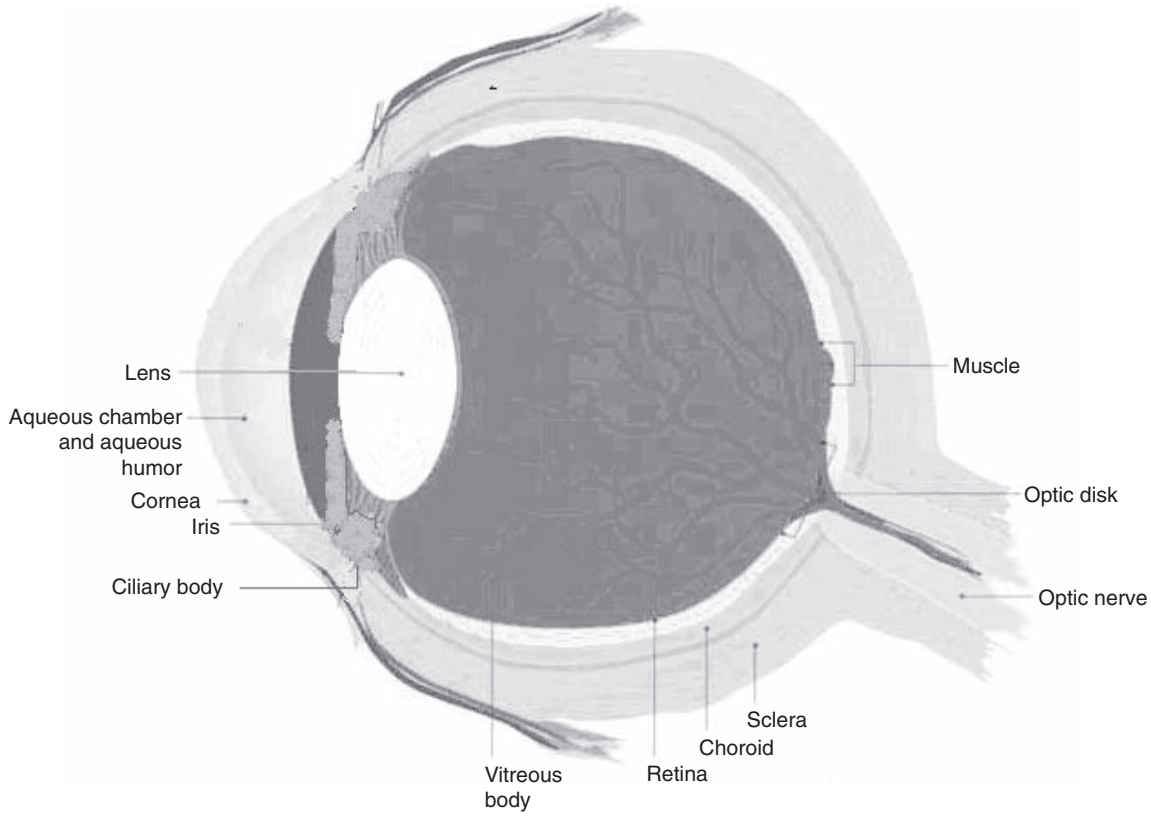


FIGURE 49.1 Key anatomic features of the eye.

many of the diseases associated with scleritis have also been associated with episcleritis. The clinical examination evaluates for the presence of erythema and edema of episclera or sclera (Figure 49.2). The inflamed sclera is usually characterized by a violaceous hue. Many inflammatory systemic disorders are associated with scleritis: rheumatoid arthritis, juvenile idiopathic arthritis, Reiter syndrome, Crohn's disease, ankylosing spondylitis, ulcerative colitis, polymyositis, polyarteritis nodosa, systemic lupus erythematosus (SLE), Wegener's granulomatosis, sarcoidosis, Lyme disease, and Cogan syndrome.

Uveitis can be classified based on anatomic location: anterior, intermediate, posterior, and diffuse or panuveitis. *Anterior uveitis* describes a disease predominantly limited to the anterior segment. In the literature it is also called iritis, iridocyclitis, and anterior cyclitis. There are many inflammatory systemic diseases associated with anterior uveitis, including juvenile idiopathic arthritis, HLA-B27-associated diseases, Behçet's disease, and sarcoidosis. The corneal examination may reveal keratic precipitates (Figure 49.3), small aggregates of inflammatory cells that accumulate on the endothelial surface of the cornea. Examination of the anterior chamber via biomicroscopy will reveal the presence of inflammatory cells and increased protein (flare), resulting from spillover of inflammation from the iris and the ciliary body, graded in a standardized fashion (Nussenblatt and Whitcup, 2004). An accumulation of leukocytes associated with fibrin, layered in the lower angle of the anterior chamber, is called a hypopyon (Figure 49.4), and is commonly associated with Behçet's disease and the HLA-B27-associated uveitidies. Inflammation of the iris may

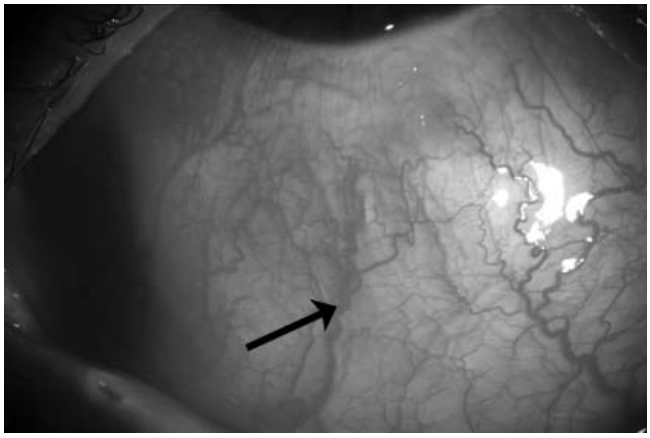


FIGURE 49.2 Slit-lamp photograph of a patient with scleritis of presumed autoimmune etiology, having ruled out infectious etiologies. Arrow indicates dilated episcleral and scleral vessels.

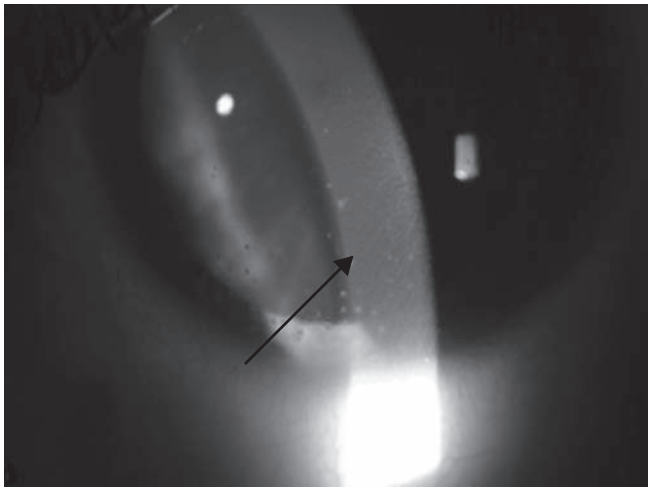


FIGURE 49.3 Slit-lamp photograph of a patient with anterior uveitis with keratic precipitates. Arrow points to corneal endothelium with keratic precipitates.

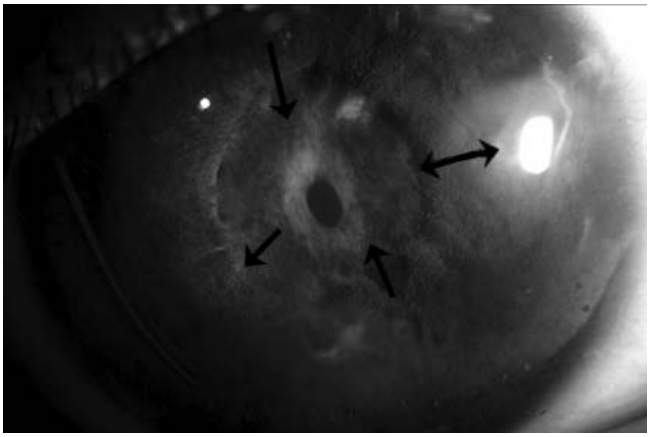


FIGURE 49.5 Slit-lamp photograph of a patient with anterior uveitis, extensive posterior synechiae, and iris adhesions to the anterior lens capsule. Arrows show the areas of adhesions.

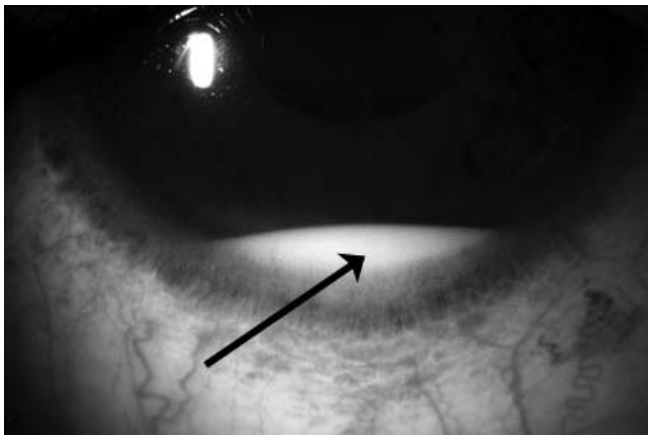


FIGURE 49.4 Slit-lamp photograph of a patient with ocular Behçet's disease and anterior uveitis, with a hypopyon. Arrow points to layer of inflammatory cells in the anterior chamber, the hypopyon.
Reproduced from Levy-Clarke et al. (2005), with permission.

cause synechiae (adhesions) between the iris and the lens capsule (Figure 49.5) or the cornea. It may also develop accumulations of inflammatory cells called nodules on the papillary margin, referred to as Koeppe nodules, or on the iris surface, known as Busacca nodules, commonly seen with sarcoidosis.

Intermediate uveitis designates that subset of uveitis where the vitreous is the major site of inflammation. Inflammation in the vitreous is characterized by increased cells and protein, vitreous haze (Nussenblatt et al., 1985) or pars plana exudates. Retinal inflammation may cause cystoid macular edema and retinal vasculitis. There are specific systemic autoimmune diseases associated with this type of uveitis: multiple sclerosis, sarcoidosis, and Lyme disease. A common form of intermediate uveitis is referred to as pars planitis, which is idiopathic. Syphilis and tuberculosis are common infectious etiologies of intermediate uveitis.

Posterior uveitis refers to inflammation affecting the posterior segment, particularly the retina and the choroid. There are numerous autoimmune diseases that are associated with posterior uveitis: Behçet's disease, SLE, polyarteritis nodosa, Wegener's granulomatosis, sarcoidosis, syphilis, and Vogt–Koyanagi–Harada (VKH) syndrome. There are also many infectious etiologies: ocular histoplasmosis, cytomegalovirus retinitis, acute retinal necrosis (varicella zoster virus, herpes simplex virus), toxoplasmosis, and immune-mediated local ocular disorders: sympathetic ophthalmia, birdshot retinochoroidopathy, and the "white-dot syndromes," which can cause posterior uveitis. In addition, primary intraocular lymphoma can masquerade as an intermediate or posterior uveitis.

Finally, *panuveitis* is a term reserved for inflammation involving all segments of the eye and in which there is no predominant site of inflammation (Nussenblatt and Whitcup, 2004). Typical systemic diseases associated with this form of uveitis are Behçet's disease, sarcoidosis, VKH syndrome, and syphilis.

PATHOLOGIC FEATURES

Uveitis can be classified as granulomatous or nongranulomatous. This is a clinical definition, based on the type of inflammatory cells infiltrating the ocular tissues, as seen by biomicroscopy. It is not based on histopathologic analysis. Biomicroscopy will reveal inflammatory precipitates throughout the eye. The most common type of corneal (keratic) precipitates are nongranulomatous. They are characterized by fine, white-colored lymphocytes, plasma

cells, and pigment. Many etiologic factors may be responsible for this type of inflammation. Granulomatous inflammation forms large, greasy-appearing collections of lymphocytes, plasma cells, and giant cells, also called "mutton-fat" keratic precipitates. It can also cause iris nodules, vitreous inflammatory cells, called "snowballs," and retinal vascular inflammation, called "candle wax drippings." This clinical classification is an important diagnostic clue, because the etiologic agents associated with granulomatous uveitis form a fairly short list, including sarcoidosis, VKH syndrome, syphilis, tuberculosis, and toxoplasmosis.

EPIDEMIOLOGIC FEATURES

Studies of the prevalence of uveitis tend to compare community-based practices with tertiary or university-based referral centers. An analysis of the publication by McCannel et al. (1996) estimates that approximately 20% of community and 18% of university or referral-based uveitis are autoimmune in nature. A similar study by Henderly et al. (1987) gives an estimate of approximately 23% in a university or referral-type practice.

There are many demographic features that offer important clues when evaluating a patient with uveitis. These include age, sex, race, ethnic heritage, and geographic residence. Specific examples include juvenile idiopathic arthritis associated with chronic anterior uveitis seen in children under age 16. One half of the patients in one study reportedly had chronic uveitis by the age of 6 (Key and Kimura, 1975). It is seen in young females with pauci-articular arthritis and a positive antinuclear antibody (ANA) test (Cassidy et al., 1986). The uveitis associated with ankylosing spondylitis is typically a recurrent anterior uveitis seen in young men between the ages of 20 and 30 years (Brewerton et al., 1973). Sarcoidosis affects young adults aged 20–50 years and has a slightly increased prevalence in women. It can affect all races, but African-Americans are 10 times more likely to be affected compared to white persons, and with chronic sarcoidosis they are more likely to develop ocular manifestations than white persons (Jabs and Johns, 1986). VKH syndrome is a multisystem disorder, with ocular, central nervous system, cutaneous, and vestibulo-auditory manifestations; the ocular manifestation is usually a severe, bilateral, granulomatous panuveitis. It is common in Japan and certain parts of Latin America (Sugiura, 1988). We have also noted a fairly high Native-American ancestry among our patients. Finally, Behçet's disease is a multisystem disorder with ocular involvement; it is especially common in the Far East and the Mediterranean basin (Ohno and Matsuda, 1986). The ocular disease may be an anterior and/or posterior uveitis, associated with retinal vasculitis (Figure 49.6).

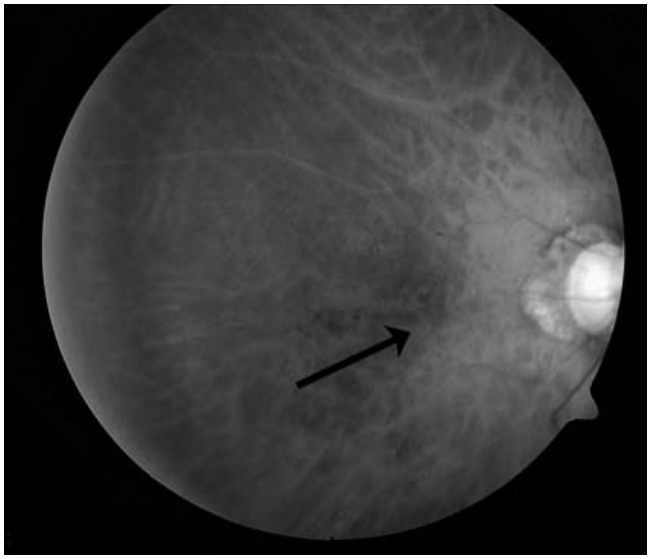


FIGURE 49.6 Fundus photograph of a patient with Behçet's disease and retinal vasculitis. The inferior arcade shows a branch vein occlusion. Arrow points to areas of bleeding in the retina.
Reproduced from Levy-Clarke et al. (2005), with permission.

AUTOIMMUNE FEATURES

Immunity in the eye is deviant and atypical. The eye's ability to regulate local immune responses has been attributed to several factors. First, there are several immunosuppressive substances in the aqueous humor of the eye, such as α-melanocyte-stimulating hormone (α-MSH), vasoactive intestinal peptide (VIP), transforming-growth factor-β (TGF-β), and macrophage-migration-inhibitory factor (MIF), which act to modulate ocular immune responses. Second, there are bone marrow-derived cells in the vascularized tissues of the eye that may capture and present antigens to immune cells. Third, surface markers, such as CD95 ligand (Griffith et al., 1996; Ferguson and Griffith, 1997), CD46 (Bora et al., 1993), and CD59 are expressed in ocular parenchymal tissues; they directly inhibit effector T-cell components of complement, thus saving the eye from tissue damage. Also, inside the eye, both retina and anterior chamber have a well-structured vascular network with blood–ocular barriers that protect the eye from intruding macromolecules.

The ability to discriminate between self and nonself is used for elimination of autoreactive cells. Because the eye is considered to be an immunologically privileged organ, it is believed that expression of ocular autoantigens occurs exclusively within the intraocular environment (Gery and Streilein, 1994). A classic example of organ-specific autoimmune disease caused by autoantigens is sympathetic ophthalmia, a rare form of intra-ocular inflammation in which the fellow eye develops inflammation after penetrat-

ing injury of the opposite eye. The inflammation may develop as early as several days after the penetrating insult to decades later. Typically, it occurs in the first several weeks (4–12 weeks). It is speculated that the injury causes primary immunization to self-antigens. Although the target antigen is still unknown, an experimental animal model has been developed. In this model cutaneous immunization of animals with retinal antigens (arrestin, rhodopsin, interphotoreceptor retinol-binding protein), retinal pigment epithelial-associated antigens, and melanocyte-associated tyrosinase results in an autoimmune uveitis, with features characteristic of sympathetic ophthalmia (Rao, 1997).

Substantial evidence exists that autoimmune diseases may also be initiated or aggravated by infection. This happens presumably when nonspecific polyclonal activation of the immune system, either by virus or immunostimulatory agents such as gram-negative bacterial cell wall components, overwhelms the normal regulatory mechanisms and permits "forbidden clones" of cells to proliferate and cause tissue damage. The molecular mimicry theory states that structural similarity between invading organism and organ tissue components may lead to persistence of the inflammatory response even after the inciting agent has been quickly cleared. It has been postulated as a mechanism for uveitis. The primary amino acid sequence of the hepatitis B virus DNA polymerase shows sequence homology to the retinal S-antigen. An experimental autoimmune uveitis was induced in Lewis rats using synthetic peptides (Singh et al., 1990). Lymph node cells from rats immunized with this synthetic peptide or peptide M in S-antigen showed a significant degree of cross-reaction. As a clinical corollary, some types of idiopathic posterior uveitis have been reported in association with immunization with hepatitis B virus vaccine (Brezin et al., 1995; Baglivo et al., 1996). However, no definitive etiologic link has been made via histopathology or immunohistochemistry.

HORMONAL INFLUENCES

Hormones play a significant role in immunomodulation, yet the exact mechanism is not understood. Certain hormones of pregnancy, such as progesterone, α-fetoprotein, α-2 pregnancy-associated globulin, and early pregnancy factor are thought to have immunomodulating properties (Anon, 1976; Chakraborty and Mandal, 1993; Harness et al., 2003). Other hormones, the levels of which increase during pregnancy, such as corticotrophin-releasing hormone, α-melanocyte stimulating hormone, estrogens, or altered estrogen/androgen ratios, are thought to be immunosuppressive (Ilias and Mastorakos, 2003; Lipton and Catania, 1997). We and other researches have reported that flares of uveitis decrease during pregnancy compared to the nonpregnant state (Rabiah and Vitale, 2003). Many women with chronic recurrent noninfectious uveitis report an association between flares and point in the menstrual cycle. Further studies are needed to more definitively delineate hormonal influences on uveitis.

GENETIC FACTORS

Genetic factors play a significant role in the development of endogenous uveitis. The ability to respond to a specific immune stimulant is genetically determined. Genes for human leukocyte antigens (HLA) are clustered in major histocompatibility complex (MHC) proteins, located on the short arm of chromosome 6. They have been linked to various uveitic syndromes (Table 49.1).

Brewerton et al. (1973) were among the first to observe that a high percentage of white patients with ankylosing spondylitis showed HLA-B27 positivity. Khan et al. (1977) demonstrated that HLA-B7 was associated with ankylosing spondylitis in African-Americans to a greater degree than HLA-B27. It is clear that HLA associations may be different for various ethnic groups. It is possible that different genes initiate responses that finally lead to a common pathway resulting in a particular disease. The reasons for the association between HLA and diseases are unclear. It has been hypothesized that exogenous environmental factors

TABLE 49.1 Association of selected ocular diseases with human leukocyte antigens

Human leukocyte antigen	Ocular disease
HLA-A11	Sympathetic ophthalmia
HLA-A24	Tubulointerstitial nephritis–uveitis syndrome
HLA-A29	Birdshot retinochoroidopathy
HLA-B5	Adamantiades-Behçet's disease
HLA-B7	Presumed ocular histoplasmosis, serpiginous retinochoroidopathy, acute posterior multifocal placoid epitheliopathy, ankylosing spondylitis
HLA-B8	Acute anterior uveitis
HLA-B12	Ocular cicatricial pemphigoid
HLA-B27	Acute anterior uveitis, Reiter syndrome
HLA-B51	Adamantiades–Behçet's disease
HLA-DQw3	Vogt–Koyanagi–Harada (VKH) syndrome
HLA-DR2	Presumed ocular histoplasmosis
HLA-DR4	Rheumatoid arthritis, VKH syndrome, relapsing polychondritis
HLA-DR6	Tubulointerstitial nephritis–uveitis syndrome
HLA-DR15	Intermediate uveitis
HLA-DRB1*0405	Sympathetic ophthalmia
HLA-DRw53	VKH syndrome

may play a role in expression of a disease. For example, it has been suggested that Ia antigens are required for antigen presentation and initiation of the immune response; if Ia expression is inappropriate and other defects of immunosurveillance occur, it may result in a disease (Botazzo et al., 1983).

Most researchers agree that mechanisms of the autoimmune disease of the eye are multifactorial, so several factors must be present to cause the disease, otherwise disease expression would be far more common. MHC plays an important role in determining disease susceptibility. Family studies in patients with uveitis show that susceptibility to particular types of idiopathic uveitis is possibly due to genetic background (Kimura and Hogan, 1963; Hogan et al., 1965; Giles and Tranton, 1980; Augsburger et al., 1981; Culbertson et al., 1983; Doft, 1983; Wetzig et al., 1988; Duinkerke-Eorela et al., 1990; Tejada et al., 1994; Lee, 1995). Genetic predisposition may determine the severity of the disease. A permissive MHC in a nonpermissive background will result in a mild disease or no disease at all (Caspi et al., 1992).

Single-nucleotide polymorphisms (SNPs) are genetic variations found normally in genes that mediate the immune response as opposed to those that control it. For example, one cytokine may have several SNP variations. Some SNPs may change the functioning of the protein. Population studies of disease groups versus controls are able to identify SNPs more commonly seen in disease groups and thus, are associated with these particular autoimmune or neoplastic diseases (Shastry, 2002). A report of 102 patients with ocular Behçet's disease, who were tested for a possible link between a specific tumor necrosis factor (TNF) SNP and the disease, found no TNF gene polymorphisms to be associated with the disease (Verity et al., 1999).

ANIMAL MODELS

Several species of mammals have been used to establish experimental autoimmune uveitis (EAU) models, including guinea pigs (Collins, 1949; Kalsow and Wacker, 1973), rabbits (Wacker and Lipton, 1968), rats (Faure, 1980; de Kozak et al., 1981; Gery and Nussenblatt, 1986), mice (Caspi et al., 1988), and primates (Nussenblatt et al., 1981). Currently, mice and rats are probably the two most commonly used animals for EAU studies due to the availability of inbred and other genetically-modified strains, such as gene knock-out or transgenic animals. Typically, uveitogenic antigens such as S-antigen, interphotoreceptor retinoid-binding protein (IRBP), retinal pigment epithelium-specific 65kDa protein (RPE65) or crude extract of retina/uvea are emulsified with complete Freund adjuvant (CFA) and injected subcutaneously into the animals ("active immunization"). Coinjection of pertussis toxin intravenously or intraperitoneally has been routinely administered to achieve maximum EAU score. EAU can also be induced by adoptively transferring primary T cells from diseased animals with active EAU or uveitogenic cell lines to syngeneic normal recipients. EAU induced by adoptive transfer typically has a shortened clinical course because of the lack of an induction phase compared to the active immunization protocol.

It is noteworthy that despite the fact that both rat and mouse EAU models have been extensively used for studying molecular mechanisms of uveitis, the clinical manifestations of the two models differ. Murine EAU seems to be less acute than rat EAU, with a later onset and longer duration. In addition, pathologic changes in murine EAU primarily involve the posterior segment with little anterior segment involvement, while rat EAU usually manifests inflammation in both anterior and posterior segments (Gery and Nussenblatt, 1986; Caspi et al., 1988). Although it was shown that passive transfer of immune serum from humans induced ocular retinitis in guinea pigs (de Kozak et al., 1976) and there is evidence of involvement of humoral immunity in EAU (Faure, 1980; Gery and Nussenblatt, 1986), humoral immune response-mediated uveitis animal models have not been widely reported or used.

PATHOGENIC MECHANISMS

The establishment of and extensive studies using EAU models have greatly facilitated the understanding of autoimmune uveitis. Despite some evidence of the involvement of humoral immunity (Faure, 1980; Gery and Nussenblatt, 1986), cellular immunity is primarily responsible for the disease (Nussenblatt et al., 1980; Gery and Nussenblatt, 1986; Caspi et al., 1986; Nussenblatt, 1987). The majority of data support the notion that helper T-cells (Th) are the key players in the molecular pathogenesis of EAU models (Caspi, 1999). While the Th1 subset has been shown to play the predominant role, accumulated data also point to a role for the Th2 subset (Caspi, 2002; Kim et al., 2002). In addition, the pertussis toxin, an adjuvant routinely administered to maximize induction of EAU, has been shown to block the induction of EAU by adoptive transfer (Su et al., 2001) underscoring a delicate balance of the immune system in this disease setting. Although limited, some data have also suggested an important role for immunosuppressive regulatory T cells (Tregs) and/or cytokines in EAU. Coadministration of interleukin (IL)-2 could potentiate induction of oral tolerance by IRBP, accompanied by increased levels of IL-10, IL-4, and TGF-β (Rizzo et al., 1994). Induced tolerance to S-antigen after intratesticular injection of S-antigen into the Lewis rat was shown to be associated with the induction of regulatory T cells capable of secreting immunosuppressive cytokines such as IL-4, IL-10, and TGF-β (Yotsukura et al., 1997). An immunosuppressive neuropeptide believed to be associated with immune privilege of the eye, α-melanocyte-

stimulating hormone, was shown to induce $CD4^+CD25^+$ Tregs.

Besides T-cells, other immune cells have also been investigated for their roles in the pathogenesis of EAU. For example, dendritic cells and macrophages have been shown to be important in EAU induction and tissue destruction (Jiang et al., 1999). In addition, natural killer (NK) cells and natural killer T (NKT) cells have also been implied in the pathogenesis of EAU. One report suggested that NK cells might play a detrimental role in EAU (Kitaichi et al., 2002) while another study showed increased NK and NKT cells in association with interferon (IFN)-β amelioration of EAU (Suzuki et al., 2002). Nonetheless, the data point to potential roles for other cellular components of the immune system that could be critical in the pathogenesis of EAU.

Ocular Immune Responses

The ocular immune system is thought to have an innate set of mechanisms for "effector blockade" that protects the ocular tissue from the destructive effects of the secondary effector phase of the immune response arc. This blockade in the anterior chamber is known as anterior chamber-associated immune deviation (ACAID), where immunization by an anterior chamber injection in experimental animals results in an altered form of systemic immunity to that antigen (Streilein, 1993; 1997; Streilein-Stein and Streilein, 2002). The eye also has soluble and membrane-bound inhibitors, such as CD178 (FasL), which can induce apoptosis (Green and Ferguson, 2001). Additionally, the eye appears to have an immunosuppressive microenvironment of chemokines and cytokines (Granstein et al., 1990; Foxman et al., 2002). Finally, it is believed that a state of tolerance can be induced in the eye (Ferguson and Griffiths, 1997; Ferguson et al., 2002).

Tissue Destruction

During ocular inflammatory processes, there is an influx of T cells, B cells, macrophages, and polymorphonuclear leukocytes (PMNs) into the choroids, choriocapillaris, and retina. There is also local synthesis of cytokines by ocular tissue. These changes in the ocular milieu most likely contribute to the ocular tissue damage seen clinically and experimentally. The underlying pathogenesis depends on the disease process involved and thus will vary. Immunohistopathology in ocular sarcoidosis shows a predominance of lymphocytes and macrophages (Chan et al., 1987), while ocular Behçet's disease revealed predominantly lymphocytic infiltration, with some B cells and plasma cells (George et al., 1997). These reports indicate that cell-mediated immunity may play a pivotal role, while others have reported humoral-mediated immunity and also the involvement of immune complex formation (Reinsh and Moyer, 1991).

Macrophages are reported to be important effector cells for retinal tissue destruction in the animal model (Forrester et al., 1998). However, the macrophage-mediated retinal tissue destruction is probably under the control of antigen-specific T cells and the cytokines that T cells release (Jiang et al., 1999). TNF-α is a well-known cytokine capable of tissue destruction either by direct tissue damage or by induction of apoptosis (Selmaj et al., 1991; Zheng et al., 1995), but other factors such as free radicals, e.g., nitric oxide, could also contribute to inflammation-induced retinal damage (Hoey et al., 1997).

IMMUNOLOGIC MARKERS

Recent advances have demonstrated that cytokines are equally important in the pathogenesis of noninfectious uveitis. The Th1 types of cytokines, such as IFN-γ, IL-2, TNF-α, and IL-12 are considered to be the primary factors in the pathogenesis of uveitis. In humans, IFN-γ and IL-2 were found to be elevated in ocular tissues with concomitant infiltrating T cells in uveitic patients (Hooks et al., 1988). IL-6 has been shown to be elevated in the aqueous humor of patients with noninfectious uveitis (Murray et al., 1990; Franks et al., 1992). Higher levels of TNF-α and IL-1 have also been demonstrated in both peripheral sera and aqueous humor samples (Palexas et al., 1992; Sakaguchi et al., 1998). Cytokine profile analysis of peripheral T-cells from uveitic patients suggested that the intracellular level of IFN-γ was increased (Frassanito et al., 2003), but similar analysis of T-cells derived from aqueous humor samples showed decreased IFN-γ compared to those from peripheral blood (Hill et al., 2005).

Data derived from animal EAU studies, however, have provided more consistent insights into the molecular mechanisms of autoimmune uveitis. TNF-α was shown to be detrimental to the development of EAU (Nakamura et al., 1994; Dick et al., 1996; Robertson et al., 2003), while IL-10 and IL-12 were suggested to be protective to the development of EAU (Rizzo et al., 1998; Tarrant et al., 1999). Consistent with findings in humans, IFN-γ was also found in the ocular tissues of EAU animals, and correlated with the disease course (Charitéris et al., 1992). However, treatment with anti-IFN-γ antibody exacerbated EAU, while treatment with rIFN-γ ameliorated the diseases in a murine EAU model (Caspi et al., 1994), leading investigators to conclude that endogenous IFN-γ was protective for EAU. The same investigators also showed that IFN-γ gene-deficient mice developed EAU similar to their wild-type littermates, suggesting that IFN-γ is not necessary for the development of EAU (Jones et al., 1997). To further complicate the issue, an independent study using IFN-γ transgenic rats showed that overexpression of IFN-γ resulted in more severe EAU (Egwuagu et al., 1999). These studies

underscore the complexity of cytokine networks in the pathogenesis of EAU.

TREATMENT AND OUTCOMES

Corticosteroids have been the mainstay of therapy for ocular inflammatory disease since the early 1950s. However, some diseases are steroid-resistant and for others long-term steroid therapy carries the risk of developing unacceptable side effects. For these patients other immunomodulatory agents need to be added as steroid-sparing agents, so that lower steroid doses or none at all can be used. The steroid-sparing agents include alkylating agents (cyclophosphamide, chlorambucil), antimetabolites (azathioprine, methotrexate, mycophenolate mofetil), cyclosporine, tacrolimus, rapamycin, antibodies, and monoclonal antibodies (daclizumab, etanercept, infliximab).

Antibodies and monoclonal antibodies directed against various parts of the immune cascade have not yet been used extensively in the treatment of uveitis, with the exception of daclizumab (Zenapax). This is a humanized anti-Tac monoclonal recombinant antibody. Tac is a subunit of the IL-2 receptor (IL-2R) high-affinity complex. Caspi et al. (1986) demonstrated the presence of high affinity IL-2Rs in animal models of uveitis. A trial of daclizumab at the National Eye Institute (NEI) (Nussenblatt et al., 1999), suggested that there is a benefit from daclizumab therapy for the treatment of noninfectious uveitis. Subsequent trials at the NEI indicated its usefulness for controlled disease, allowing tapering of other immunosuppressive therapy and maintenance of patients on daclizumab only (Nussenblatt and Whitcup, 2004); subsequent trials are underway for pediatric uveitis and to evaluate the efficacy of various doses for noninfectious uveitis.

TNF-α is found during the acute phase of experimental autoimmune uveoretinitis (Kim et al., 2001). Enbrel is a TNF fusion protein that binds to and inactivates TNF, while infliximab is a chimeric monoclonal antibody directed against TNF-α. Our experience with both for uveitis is still developing. We conducted a randomized, placebo-control trial of 12 patients, to investigate the efficacy and safety of etanercept in the treatment of juvenile idiopathic arthritis (JIA)-associated uveitis. In this small pilot study there was no apparent difference in anterior segment inflammation between patients treated with etanercept and placebo (Smith et al., 2005). Smith et al. (2001) evaluated 16 patients, 14 receiving etanercept and 2 infliximab, for ocular and systemic inflammatory disease. All 12 patients with articular disease saw benefits, while only 6 of the 16 with uveitis showed any benefit. We are currently investigating infliximab for the treatment of noninfectious scleritis in an open label feasibility study; initial results appear very promising.

In our treatment approach cytotoxic agents are often used last, after corticosteroids, cyclosporine, and monoclonal antibodies, for sight-threatening intraocular inflammation. Other less widely used therapeutic modalities include intravenous immunoglobulin therapy, oral tolerance, plasmapharesis, and IFN-α. In addition, nonsteroidal anti-inflammatory agents have been used for specific diseases; however, they have not been shown to be effective as a sole agent in the treatment of noninfectious uveitis.

In general, the following therapeutic guidelines can be used:

- Topical corticosteroids for anterior segment disease.
- Periocular corticosteroid for unilateral disease, the presence of intermediate or posterior complications, and in children to avoid systemic complications of corticosteroids.
- Systemic immunosuppressive agents for bilateral, sight-threatening intermediate or posterior uveitis, or panuveitis
- Reserve cytotoxic agents for therapeutic failures.
- For uveitis associated with infectious etiologies, specific agents are indicated, including antivirals and antitoxoplasmosis therapy.

CONCLUDING REMARKS AND FUTURE PROSPECTS

We have learned a great deal since the advent of steroids for uveitis in the early 1950s. Most therapies during the 20th century tended to be monotherapy and combination therapy only began to be used more frequently during the 1990s. Concerns about the side effects of steroids, i.e., osteoporosis, came to the attention of treating physicians relatively early, as did the goals for therapy, including long-term approaches that reduced secondary effects. Because of this the use of local therapy, including intraocular therapy, whether by injection or a time-released device, has become more popular. So where will we be going in the 21st century? Some of the possibilities include pharmacogenetics study of variability in drug responses attributed to hereditary factors in different populations; pharmacogenomics determination and analysis of the genome and its products (RNA and proteins) as they relate to drug response; evaluation of patient genomes to identify which patients are the best candidates for a specific therapy; and finally, using cytokines as a marker for inflammation, as potential therapeutic targets, and to determine the level of quiescence in chronic uveitis.

References

Anon (1976). Progesterone in large amounts has immunosuppressive effect. *JAMA 236*, 905–913.

Augsburger, J.J., Annesley, W.H., Jr., Sergott, R.C., Fekberg, N.T., Bowman, J.H., and Raymond, L.A. (1981). Familial pars planitis. *Ann. Ophthalmol. 13*, 553–557.

Baglivo, E., Safran, A.B., and Borruat, F.X. (1996). Multiple evanescent white dot syndrome after hepatitis B vaccine. *Am. J. Ophthalmol. 122*, 431–432.

Bora, N.S., Gobleman, C.L., Atkinson, J.P., Pepose, J.S., and Kaplan, H.J. (1993). Differential expression of the complement regulatory proteins in the human eye. *Invest. Ophthalmol. Vis. Sci. 34*, 3579–3584.

Bottazzo, G.F., Pujol-Borrel, R., Hanafusa, T., and Feldmann, M. (1983). Role of aberrant HLA-Dr expression and antigen presentation in induction of endocrine autoimmunity. *Lancet 2*, 1115–1119.

Brewerton, D.A., Hart, F.D., Nicholls, A., Cafferey, M., James, D.C., and Sturrock, R.D. (1973). Ankylosing spondylitis and HLA-B27. *Lancet 1*, 904–907.

Brezin, A.P., Massin-Korobelnik, P., Boudin, M., Gauric, A., and Le Hoang, P. (1995). Acute posterior multifocal placoid pigment epitheliopathy after hepatitis B vaccine. *Arch. Ophthalmol. 113*, 297–300.

Caspi, R.R. (1999). Immune mechanisms in uveitis. *Springer Semin. Immunopathol. 21*, 113–124.

Caspi, R.R. (2002). Th1 and Th2 responses in pathogenesis and regulation of experimental autoimmune uveoretinitis. *Int. Rev. Immunol. 21*, 197–208.

Caspi, R.R., Roberge, F.G., McAllister, C.G., el-Saied, M., Kuwabara, T., Gery, I., Hanna, E., and Nussenblatt, R.B. (1986). T cell lines mediating experimental autoimmune uveoretinitis (EAU) in the rat. *J. Immunol. 136*, 928–933.

Caspi, R.R., Roberge, F.G., Chan, C.C., Wiggert, B., Chadar, G.J., Rozenszajn, L.A., Lando, Z., and Nussenblatt, R.B. (1988). A new model of autoimmune disease. Experimental autoimmune uveoretinitis induced in mice with two different retinal antigens. *J. Immunol. 140*, 1490–1495.

Caspi, R.R., Grubbs, B.G., Chan, C.C., Chader, G.J., and Wiggert, B. (1992). Genetic control of susceptibility to experimental autoimmune uveoretinitis in the mouse model: concomitant regulation by MHC and non-MHC genes. *J. Immunol. 148*, 2384–2389.

Caspi, R.R., Chan, C.C., Grubbs, B.G., Silver, P.B., Wiggert, B., Parsa, C.F., Bahmanyar, S., Billiau, A., and Heremans, H. (1994). Endogenous systemic IFN-gamma has a protective role against ocular autoimmunity in mice. *J. Immunol. 152*, 890–899.

Cassidy, J.T., Levinson, J.E., Bass, J.C., Baum, J., Brewer, E.J., Jr., Fink, C.W., Hanson, V., Jacobs, J.C., Masi, A.T., and Schaller, J.G. (1986). A study of classification criteria for a diagnosis of juvenile rheumatoid arthritis. *Arthritis Rheum. 29*, 274–281.

Chakraborty, M. and Mandal, C. (1993). Immunosuppresive effect of human alpha-fetoprotein: a cross species study. *Immunol. Invest. 22*, 329–339.

Chan, C.C., Wetzig, R.P., Palestine, A.G., Kuwabara, T., and Nusenblatt, R.B. (1987). Immunohistopathology of ocular sarcoidosis: report of a case and discussion of immunopathogenesis. *Arch. Ophthalmol. 105*, 1398–1402.

Chan, C.C., Hikita, N., Dastgheib, J. Dastgheib, K., Whitcup, S.M., Gery, I., and Nussenblatt, R.B. (1994). Experimental melanin-protein-induced uveitis in the Lewis rat immunopathologic processes. *Ophthalmology 101*, 1275–1280.

Charteris, D.G. and Lightman, S.L. (1992). Interferon-gamma (IFN-gamma) production in vivo in experimental autoimmune uveoretinitis. Immunology 75, 463–467.

Collins, R.C. (1949). Experimental studies on sympathetic ophthalmia. *Am. J. Ophthalmol. 32*, 1687–1699.

Culbertson, W.W., Giles, C.L., West, C., and Stafford, T. (1983). Familial pars planitis. *Retina 3*, 179–181.

de Kozak, Y., Youn, W.S., Bogossian, M., and Faure, J.P. (1976). Humoral and cellular immunity to retinal antigens in guinea pigs. *Mod. Probl. Ophthalmol. 16*, 51–58.

de Kozak, Y., Sakai, J., Thillaye, B., and Faure, J.P. (1981). S antigen-induced experimental autoimmune uveo-retinitis in rats. *Curr. Eye Res. 1*, 327–337.

Dick, A.D., McMenamin, P.G., Korner, H., Scallon, B.J., Ghrayeb, J., Forrester, J.V., and Sedgwick, J.D. (1996). Inhibition of tumor necrosis factor activity minimizes target organ damage in experimental autoimmune uveoretinitis despite quantitatively normal activated T cell traffic to the retina. *Eur. J. Immunol. 26*, 1018–1025.

Doft, B.H. (1983). Pars planitis in identical twins. *Retina 3*, 32–33.

Duinkerke-Eorela, K.U., Pinkers, A., and Cruysberg, J.R.M. (1990). Pars planitis in father and son. *Ophthal. Pediatr. Genet.11*, 305–308.

Egwuagu, C.E., Szetein, J., Mandi, R.M., Li, W., Chao-Chan, C., Smith, J.A., Charukamnoetkanot, P., and Chepelinsky, A.B. (1999). IFN-gamma increases the severity and accelerates the onset of experimental autoimmune uveitis in transgenic rats. *J. Immunol. 162*, 510–517.

Elschnig, A. (1910). Studien zur sympathischen ophthalmic:die antigen wirkung des augenpigmentes. *Albrecht von Grafes Arch. Ophthalmol 76*, 509–546.

Faure, J.P. (1980). Autoimmunity and the retina. *Curr. Top. Eye Res. 2*, 215–302.

Ferguson, T.A. and Griffith, T.S. (1997). A vision of cell death: Insights into immune privilege. *Immunol. Rev. 156*, 167–184.

Ferguson, T.A., Green, D.R., and Griffith, T.S. (2002). Cell death and immune privilege. *Int. Rev. Immunol. 21*, 153–172.

Foxman, E.F., Zhang, M., Hurst, S.D., Muchamuel, T., Shen, D., Wawrousek, E.F., Chan, C.C., and Gery, I. (2002). Inflammatory mediators in uveitis: differential induction of cytokines and chemokines in Th 1 versus Th2 mediated ocular inflammation. *J. Immunol. 168*, 2483–2492.

Forrester, J.V., Huitinga, I., Lumsden, L., and Dijkstra, C.D. (1998). Marrow-derived activated macrophages are required during the effector phase of experimental autoimmune uveoretinitis in rats. *Curr. Eye Res. 17*, 426–437.

Franks, W.A., Limb, G.A., Stanford, M.R., Ogilvie, J., Wolstencroft, R.A., Crignell, A.H., and Dunande, D.C. (1992). Cytokines in human intraocular inflammation. *Curr. Eye Res. 11*(Suppl), 187–191.

Frassanito, M.A., Dammacco, R., Fusaro, T., Cusmai, A., Guerriero, S., and Sborgia, C. (2003). Combined cyclosporin-A /prednisone therapy of patients with active uveitis suppresses IFN-gamma production and the function of dendritic cells. *Clin. Exp. Immunol. 133*, 233–239.

George, R.K., Chan, C.C., Whitcup, S.M., and Nussenblatt, R.B. (1997). Ocular immunopathology of Behcet's disease. *Surv. Ophthalmol. 42*, 157–162.

Gery, I.M. and Nussenblatt, R.B. (1986). Retinal specific antigens and immunopathologic processes they provoke. *Prog. Ret. Res. 5*, 75–109.

Gery, I. and Streilen, J.W. (1994). Autoimmunity in the eye and its regulation. *Curr. Opin. Immunol. 6*, 938–945.

Gery, I., Chanaud, N.P., III, and Anglade, E. (1994). Recoverin is highly uveitogenic in Lewis rats. *Invest. Ophthalmol. Vis. Sci. 35*, 3342–3345.

Giles, C.L. and Tranton, J.H. (1980). Peripheral uveitis in three children of one family. *J. Pediatr. Ophthalmol. Strabismus 17*, 297–299.

Granstein, R., Satszewski, R., Knisely T., Zeira, E., Nazareno, R., Latina, M., and Albert, D.M. (1990). Aqueous humor contains transforming growth factor beta and a small (<3500 daltons) inhibitor of thymocyte proliferation. *J. Immunol. 144*, 3021–3027.

Green, D.R. and Ferguson, T.A. (2001). The role of *FAS* ligand in immune privilege. *Nat. Rev. Mol. Cell. Biol. 2*, 917–924.

Griffith, T.S., Yu, X., Herndon, J.M., Green, D.R., and Ferguson, T.A. (1996). CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. *Immunity 5*, 7–16.

Ham, D.I., Gentleman, S., Chan C.C., McDowell, J.H., Redmond, T.M., and Gery, I. (2002). RPE65 is highly uveitogenic in rats. *Invest. Ophthalmol. Vis. Sci. 43*, 2258–2263.

Harness, J., Cavanagh, A., Morton, H., and McCombe, P. (2003). A protective effect of early pregnancy factor on experimental autoimmune encephalomyelitis induced in Lewis rats by inoculation with myelin basic protein. *J. Neurol. Sci. 216*, 33–41.

Henderly, D.E., Genstler, A.J., Smith, R.E., and Rao, N.A. (1987). Changing patterns of uveitis. *Am. J. Ophthalmol. 103*, 131–136.

Hill, T., Galatowicz, F., Akerele, T., Lau, C.H., Calder, V., and Lightman, S. (2005). Intracellular T lymphocyte cytokine profiles in the aqueous humour of patients with uveitis and correlation with clinical phenotype. *Clin. Exp. Immunol. 139*, 132–137.

Hirose, S., Kuwabara, T., Nussenblatt, R.B., Wiggert, B., Redmond, T.M., and Gery, I. (1986). Uveitis induced in primates by interphotoreceptor retinoid-binding protein. *Arch. Ophthalmol. 104*, 1698–1702.

Hoey, S., Grabowski, P.S., Ralston, S.H., Forrester, J.V., and Liversidge, J. (1997). Nitric oxide accelerates the onset and increases the severity of experimental autoimmune uveoretinitis through an IFN-gamma-dependent mechanism. *J. Immunol. 159*, 5132–5142.

Hogan, M.J., Kimura, S.J., and O'Connor, G.R. (1965). Peripheral retinitis and chronic cyclitis in children. *Trans. Ophthalmol. Soc. UK 85*, 39–51.

Hooks, J.J., Chan, C.C., and Detrick, B. (1988). Identification of the lymphokines, interferon-gamma and interleukin-2, in inflammatory eye diseases. *Invest. Ophthalmol. Vis. Sci. 29*, 1444–1451.

Ilias, I. and Mastorakos, G. (2003). The emerging role of peripheral corticotrophin-releasing hormone. *J. Endocrinol. Invest. 26*, 364–371.

Jabs, D.A. and Johns, C.J. (1986). Ocular involvement in chronic sarcoidosis. *Am. J. Ophthalmol. 102*, 297–301.

Jiang, H.R., Lumsden, L., and Forrester, J.V. (1999). Macrophages and dendritic cells in IRBP-induced experimental autoimmune uveoretinitis in B10RIII mice. *Invest. Ophthalmol. Vis. Sci. 40*, 3177–3185.

Jones, L.S., Rizzo, L.V., Agarwal, R.K., Tarrant, T.K., Chan, C.C., Wiggert, B., and Caspi, R.R. (1997). IFN-gamma-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. *J. Immunol. 158*, 5997–6005.

Kalsow, C.M. and Wacker, W.B. (1973). Localization of a uveitogenic soluble retinal antigen in the normal guinea pig eye by an indirect fluorescent antibody technique. *Int. Arch. Allergy Appl. Immunol. 44*, 11–20.

Key, S.W., III and Kimura, S.J. (1975). Iridocyclitis associated with juvenile rheumatoid arthritis. *Am. J. Ophthalmol. 80*, 425–429.

Khan, M.A., Kushner, I., Braun, W.E., Zachary, A.A., and Steinberg, A.G. (1977). HLA-B7 and ankylosing spondylitis in American blacks. *N. Engl. J. Med. 297*, 513.

Kim, S.J., Zhang, M., Vistica, B.P., Chan, C.C., Shen, D.F., Wawrousek, E.F., and Gery, I. (2002). Induction of ocular inflammation by T-helper lymphocytes type 2. *Invest. Ophthamol. Vis. Sci. 43*, 758–765.

Kim, H.S., Yoon, S.K., and Joo, C. (2001). The expression of multiple cytokines and inducible nitric oxide synthase in experimental melanin protein induced uveitis. *Ophthal. Res. 33*, 329–355.

Kimura, S.J. and Hogan, M.J. (1963). Chronic cyclitis. *Trans. Am. Ophthalmol. Soc. 61*, 397–413.

Kitaichi, N., Kotake, S., Morohashi, T., Onoe, K, Ohno, S., and Taylor, A.W. (2002). Diminution of experimental autoimmune uveoretinitis (EAU) in mice depleted of NK cells. *J. Leukoc. Biol. 72*, 1117–1121.

Kump, L.I., Cervantes Castaneda, R.A., Androudi, S.N., et al. (2005). Patterns of exacerbations of chronic non-infectious uveitis in pregnancy and puerperium. *Ocular Immunol. Inflammation, unpublished*.

Lee, A.G. (1995). Familial pars planitis. *Ophthalmol. Genet. 16*, 17–19.

Lee, R.H., Fowler, A., McGinnis, J.F., Lolley, R.N., and Craft, C.M. (1990). Amino acid cDNA sequence of bovine phosducin, a soluble phosphoprotein from photoreceptor cells. *J. Biol. Chem. 265*, 15867–15873.

Levy-Clarke, G.A., Kump, L.I., and Nussenblatt, R.B. (2005). Uveitis: from clinical assessment to treatment. *Retinal Physician 2*, 18–23.

Lipton, J.M. and Catania, A. (1997). An anti-inflammatory action of the neuromodulator α-MSH. *Immunol. Today 18*, 140–145.

McCannel, C.A., Holland, G.N., Helm, C.J., Cornell, P.J., Winston, J.V., and Rimmer, T.G. (1996). Causes of uveitis in the general practice of ophthalmology. UCLA Community-Based Uveitis Study Group. *Am. J. Ophthalmol. 121*, 35–46.

Murray, P.I., Hoekzema, R., van Haren, M.A., de Hon, F.D., and Kijlstra, A. (1990). Aqueous humor interleukin-6 levels in uveitis. *Invest. Ophthalmol. Vis. Sci. 31*, 917–920.

Nakamura, S., Yamakawa, T., Sugita, M., Kijima, M., Ishioka, M., Tanake, S., and Ohno, S. (1994). The role of tumor necrosis factor-alpha in the induction of experimental autoimmune uveoretinitis in mice. *Invest. Ophthalmol. Vis. Sci. 35*, 3884–3889.

Nussenblatt, R.B. (1987). Basic and clinical immunology in uveitis. *Jpn. J. Ophthalmol. 31*, 368–374.

Nussenblatt, R.B. and Whitcup, S.M. (2004). *Uveitis: Fundamentals and Clinical Practice*, 3rd edn. Philadelphia: Mosby.

Nussenblatt, R.B., Gery, I., and Wacker, W.B. (1980). Experimental autoimmune uveitis: cellular immune responsiveness. *Invest. Ophthalmol. Vis. Sci. 19*, 686–690.

Nussenblatt, R.B., Kuwabara, T., de Monasterio, F.M., and Wacker, W.B. (1981). S-antigen uveitis in primates. A new model for human disease. *Arch. Ophthalmol. 99*, 1090–1092.

Nussenblatt, R.B., Palestine, A.G., Chan, C.C., and Roberge, F. (1985). Standardization of vitreal inflammatory activity in intermediate and posterior uveitis. *Ophthalmology 92*, 467–471.

Nussenblatt, R.B., Fortin, E., Schiffman, R., Rizzo, L., Smith, J., Van Veldhuisen, P., Sran, P., Yaffe, A., Goldman, C.K., Waldman, T.A., and Whitcup, S.M. (1999). Treatment of noninfectious intermediate and posterior uveitis with humanized anti-Tac mAb: a phase I/II clinical trial. *Proc. Natl. Acad. Sci. USA 96*, 7462–7466.

Nussenblatt, R.B., Thompson, D.J.S., Li, Z., Chan, C.C., Peterson, J.S., Robinson, R.R., Shames, R.S., Nagarajan, S., Tang, M.T., Mailman, M., Velez, G., Roy, C., Levy-Clarke, G.A., Suhler, E.B., Djabilian, A., Sen, H.N., Al-Khatib, S., Ursea, R., Srivastava, S., Bamji, A., Mellow, S., Sran, P., Waldmann, T.A., and Buggage, R.R. (2003). Humanized anti-interleukin-2(IL-2) receptor alpha therapy: Long term results in uveitis patients and preliminary safety and activity data for establishing parameters for subcutaneous administration. *J. Autoimmun. 21*, 283–293; erratum *23*, 93.

Ohno, S. and Matsuda, H. (1986). Studies of HLA antigens in Behçet's disease in Japan. In T. Lehner and C. Barnes (eds.) *Recent Advances in Behçet's Disease* (pp. 11–16). London: Royal Society of Medicine Press.

Palexas, G.N., Sussman, G., and Welsh, N.H. (1992). Ocular and systemic determination of IL-1 beta and tumour necrosis factor in a patient with ocular inflammation. *Scand. J. Immunol. 11*(Suppl), 173–175.

Pfister, C., Chabre, M., Plouet, J., Tuyen, V.V., De Kozak, Y., Faure, J.P., and Kuhn, H. (1985). Retinal S antigen identified as the 48K protein regulating light-dependent phosphodiesterase in rods. *Science 228*, 891–893.

Rabiah, P.K. and Vitale, A.T. (2003). Non-infectious uveitis and pregnancy. *Am. J. Ophthalmol. 136*, 91–98.

Rao, N.A. (1997). Mechanisms of inflammatory response in sympathetic ophthalmia and VKH syndrome. *Eye 11*, 213–216.

Reinsh, C.L. and Moyer, C.F. (1991). Animal models of vasculitis. In A. Churg and J. Churg (eds.), *Systemic Vasculitis, the Biologic Basis* (pp. 31–40). New York: Igaku-Shoin.

Rizzo, L.V., Miller-Rivero, N.E., Chan, C.C., Wiggert, B., Nussenblatt, R.B., and Caspi, R.R. (1994). Interleukin-2 treatment potentiates induction of oral tolerance in a murine model of autoimmunity. *J. Clin. Invest. 94*, 1668–1672.

Rizzo, L.V., Xu, H., Chan, C.C., Wiggert, B., and Caspi, R.R. (1998). IL-10 has a protective role in experimental autoimmune uveoretinitis. *Int. Immunol. 10*, 807–814.

Robertson, M., Liversidge, J., Forrester, J.V., and Dick, A.D (2003). Neutralizing tumor necrosis factor-alpha activity suppresses activation of infiltrating macrophages in experimental autoimmune uveoretinitis. *Invest. Ophthalmol. Vis. Sci. 44*, 3034–3041.

Sakaguchi, M., Sugita, S., Sagawa, K., Itoh, K., and Machizuki, M. (1998). Cytokine production by T cells infiltrating in the eye of uveitis patients. *Jpn. J. Ophthalmol. 42*, 262–268.

Schalken, J.J. Winkens, H.J., van Vugt, A.H.M., De Grip, W.J., and Broekhuyse, R.M. (1989). Rhodopsin induced experimental autoimmune uveoretinitis in monkey. *Br. J. Ophthalmol. 73*, 168–172.

Selmaj, K., Raime, C.S., Farooq, M., Norton, W.T., and Brosnan, C.F. (1991). Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. *J. Immunol. 147*, 1522–1529.

Shastry, B.S. (2002). SNP alleles in human disease and evolution. *J. Hum. Genet. 47*, 561–566.

Singh, V.K., Kalra, H.K., Yamaki, K., Abe, T, Donoso, L.A., and Shinohara, T. (1990). Molecular mimcry between a uveitopathogenic site of S antigen and viral peptides. Induction of experimental autoimmune uveitis in Lewis rats. *J. Immunol. 144*, 1282–1287.

Smith, J.A., Thompson, D.J., Whitcup, S.M., Suhler, E., Clarke, G., Smith, S., Robinson, M., Kim, J., and Barron, K.S. (2005). A randomized, placebo-controlled, double-masked clinical trial of etanercept for the treatment of uveitis associated with juvenile idiopathic arthritis. *Arthritis Care Res. 53*, 18–23.

Smith, J.R., Levinson, R.D., Holland, G.N., Jabs, D.A., Robinson, M.R., Whitcup, S.M., and Rosenbaum, J.T. (2001). Differential efficacy of tumor necrosis factor inhibition in the management of uveitis and associated rheumatic disease. *Arthritis Rheum. 45*, 252–257.

Streilein, J.W. (1993). Immune privilege as the result of local tissue barriers and immunosuppressive microenvironments. *Curr. Opin. Immunol. 5*, 428–432.

Streiline, J.W. (1997). Molecular basis of ACAID. *Ocul. Immunol. Inflamm. 5*, 217–218.

Streilein-Stein, J. and Streilein, J.W. (2002). Anterior chamber associated immune deviation (ACAID): regulation, biological relevance, and implications for therapy. *Int. Rev. Immunol. 21*, 123–152.

Su, S.B., Silver, P.B., Zhang, M., Chan, C.C., and Caspi, R.R. (2001). Pertussis toxin inhibits induction of tissue-specific autoimmune disease by disrupting G protein-coupled signals. *J. Immunol. 167*, 250–256.

Sugiura, S. (1988). Vogt-Koyanagi-Harada disease. *Jpn. J. Ophthalmol. 32*, 334–343.

Suzuki, J., Sakai, J., Okada, A.A., Takeda, E., Usui, M., and Mizuguchi, J. (2002). Oral administration of interferon-beta suppresses experimental autoimmune uveoretinitis. *Graefes Arch. Clin. Exp. Ophthalmol. 240*, 314–321.

Tarrant, T.K., Silver, P.B., Wahlsten, J.L., Rizzo, L.V., Chan, C.C., Wiggert, B., and Caspi, R.R. (1999). Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. *J. Exp. Med. 189*, 219–230.

Tejada, P., Sanz, A., and Criado, D. (1994). Pars planitis in a family. *Int. Ophthalmol. 18*, 111–113.

Uhlenhuth, P. (1903). Zur lehre von der unterscheidung verscheidener eiweissarten mit hilfe spezifischer sera. *Fetschrift zum 60 Geburstag von Robert Koch* (pp. 49–74). Jena: Fischer.

Verity, D.H., Wallace, G.R., Vaughan, R.W., Kondeati, E., Madanat, W., Zureitkat, H., Fayyad, R., Marr, J.E., Kanawati, C.A., and Stanford, M.R. (1999). HLA and tumor necrosis factor (TNF) polymorphism in ocular Behçet's disease. *Tissue Antigen 54*, 264–272.

Wacker, W.B. and Lipton, M.M. (1968). Experimental allergic uveitis. I. Production in the guinea pig and rabbit by immunization with retina in adjuvant. *J. Immunol. 101*, 151–156.

Wacker, W.B., Donoso, L.A., Kalsow, C.M., Yankeelov, J.A., Jr., and Organisciak, D.T. (1977). Experimental allergic uveitis: isolation characterization, localization of a soluble uveitiopathogenic antigen from bovine retina. *J. Immunol. 119*, 1949–1958.

Wetzig, R.P., Chan, C.C., Nussenblatt, R.B., Palestine, A.G., Mazur, D.O., and Mittal, K.K. (1988). Clinical and immunopathological studies of pars planitis in a family. *Br. J. Ophthalmol. 72*, 5–10.

Yamaki, K., Kondo, I., Nakamura, H., Miyano, M., Konno, S., and Sakuragi, S. (2000). Ocular and extraocular inflammation induced by immunization of tyrosinase related protein 1 and 2 in Lewis rats. *Exp. Eye Res. 71*, 361–369.

Yotsukura, J., Huang, H., Singh, A.K., and Shichi, H. (1997). Regulatory cells generated by testicular tolerization to retinal S-antigen: possible involvement of IL-4, IL-10, and TGF-beta in the suppression of experimental autoimmune uveoretinitis. *Cell. Immunol. 182*, 89–98.

Zheng, L., Fisher, G., Miller, R.E., Peschon, J., Lynch, D.H., and Lenardo, M.J. (1995). Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature 377*, 348–351.

CHAPTER

50

Inner Ear Disease

CLAUDIO LUNARDI*

**Department of Clinical and Experimental Medicine, University Hospital, Verona, Italy*

ANTONIO PUCCETTI†

†Institute Giannina Gaslini and Department of Experimental Medicine, University of Genova, Genova, Italy

The hypothesis that a syndrome of sudden sensorineural hearing loss (SNHL) often accompanied by vestibular symptoms might be of autoimmune origin was firstly proposed by McCabe (1979) who based his findings on clinical features, presence of abnormal immunologic tests, and a positive response to immunosuppressive therapy. Since then, a number of syndromes characterized by SNHL with overlapping clinical features have been described and termed in different ways: autoimmune SNHL, immune-mediated inner ear disease (IMIED), idiopathic progressive bilateral SNHL, sudden SNHL, idiopathic SNHL, bilateral immune-mediated Menière's disease, autoimmune vestibulo-cochlear disorders (Rahman et al., 2001a), generating a great confusion in the identification of patients and in the evaluation of different studies. It is still debated whether we can define as autoimmune all the cases of SNHL without any apparent cause. Although most of the authors in the field refer to these cases with the term "autoimmune inner ear disease" or "autoimmune sensorineural hearing loss" (Solares et al., 2003; Mathews and Kumar, 2003), others (Stone and Francis, 2000; Garcia-Berrocal et al., 2003) still prefer the definition of IMIED, since an autoimmune process can not always be identified. Immune-mediated inner ear disease may be a process confined to the inner ear, and antibodies against a vast array of different molecular weight inner ear antigens may be found in a percentage of these patients; in this case, the process can be identified as an organ-specific autoimmune disease. In other cases, IMIED is a feature of a systemic disorder, such as primary vasculitides, or of systemic autoimmune diseases. Indeed a systemic autoimmune disorder can be present in nearly one-third of patients with IMIED.

CLINICAL FEATURES

Immune-mediated inner ear disease is characterized by the presence of rapidly progressive, often bilateral SNHL, and frequently by vertigo, tinnitus, and a sense of aural fullness, sometimes indistinguishable from Menière's disease at the beginning. Immune-mediated inner ear disease usually leads to irreversible damage within hours or days of the onset and involves both ears, often asynchronously. Sometimes the disease shows an initial fluctuating course of remissions and relapses typical of an autoimmune disease. Since the devastating sequelae of IMIED may be avoided with the early institution of aggressive immunosuppression, a prompt diagnosis represents a major goal for the clinician. The clinical manifestations are shared with entities of different etiologies such as vascular, toxic, metabolic, genetic, traumatic, and infective; therefore, all the possible non-immune-mediated causes of SNHL need to be excluded. Menière syndrome, characterized by hearing loss and episodes of vertigo, tinnitus, and aural fullness, may accompany several causes of inner ear inflammation, including IMIED. In the absence of an identifiable cause, this syndrome is termed Menière's disease considered of autoimmune origin at least in 30–40% of the cases (Yoo et al., 2001; Riente et al., 2004). Time course is the most important criterion to differentiate IMIED from Menière's

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

disease—in the latter hearing loss occurs over a long period of time, sometimes of several years. Moreover Menière's disease is usually limited to one ear in the majority of the cases. Due to the many different etiologies which can lead to SNHL and to the absence of specific diagnostic markers, Garcìa-Berrocal et al. (2002a) have proposed the following criterion to correctly assess IMIED as a distinct entity:

1. Major criteria: bilateral involvement, presence of systemic autoimmune disease, positive antinuclear antibodies (ANA), reduced number of naïve T-cells (CD4RA), and recovery of more than 80% of hearing.
2. Minor criteria: unilateral involvement, young/middle aged patient, often female, presence of antibodies against HSP70, and good response to steroid therapy.

The suspicion of IMIED would be supported by the presence of three major criteria or two major and more than two minor criteria. The same authors (Garcìa-Berrocal et al., 2003) used these criteria to characterize and evaluate the response to therapy of 69 patients with recent-onset SNHL of different origin, such as viral, vascular, immune-mediated, and idiopathic. Patients with IMIED had the best and the earliest recovery rate of hearing after therapy, but also a higher rate of recurrence, typical of an autoimmune disorder. However, patients with profound hearing loss (>90 dB) have a low percentage of recoveries, regardless of the etiology. The criteria proposed by Garcìa-Berrocal et al. need to be validated by different groups in a greater number of patients. In particular, we think that the presence not only of ANA but also of other autoantibodies such as anticardiolipin antibodies, antithyroid antibodies, rheumatoid factor, or myeloperoxidase-antineutrophil cytoplasmic antibodies (Bachor et al., 2005; Toubi et al, 2004; Takagi et al., 2004) may suggest the presence of an autoimmune response also against the inner ear. Moreover, we believe that a positive therapeutic response to corticosteroid administration has to be considered a major criterion for the diagnosis of IMIED (Ruckenstein, 2004).

Due to the lack of a reliable diagnostic test, limited positron emission tomography of the inner ear, in association with anti-HSP70 antibody determination, has been proposed as a useful technique for assessing IMIED (Mazlumzadeh et al., 2003).

Association with Systemic Autoimmune Diseases

Hearing loss, both sensorineural and conductive, has been reported in patients with rheumatoid arthritis (Raut et al., 2001; Ozcan et al., 2002; Salvinelli et al., 2004) and with primary Sjögren syndrome (Boki et al., 2001). Anticardiolipin antibodies have been found associated with the presence of SNHL in patients with Sjögren syndrome (Tumiati et al., 1997) and with systemic lupus erythematosus (SLE) (Naarendorp and Spiera, 1998; Green and Miller, 2001; Kastanioudakis et al., 2002). Moreover SNHL can be present in patients with primary antiphospholipid syndrome (Vyse et al., 1994; Chapman et al., 2003). The histopathologic features of temporal bone described in eight subjects affected by SLE showed various degrees of hair-cell loss and atrophy of the organ of Corti, marked cochlear inflammatory infiltrate or formation of fibrous tissue and new bone throughout the cochlea, and degeneration of the spiral ligament, findings very similar to the known features of IMIED (Yoon et al., 1989; Sone et al., 1999). Sensorineural hearing loss has also been reported in progressive systemic sclerosis (Kastanioudakis et al., 2001) and in ulcerative colitis (Kumar et al., 2000).

Association with Primary Vasculitides

Sensorineural hearing loss is often an early symptom of primary vasculitides, which usually affect both the middle and the inner ear. The best example is Wegener granulomatosis (Kempf, 1989; Takagi et al., 2002) characterized by chronic otitis media leading to conductive hearing loss; SNHL is often associated and is related to vasculitis of the inner ear. Similar findings may be present in patients with relapsing polychondritis (Trentham and Le, 1998; Malard et al., 2002) characterized by vasculitis of the labyrinthine artery and inflammation of the cartilage within the inner ear. Sensorineural hearing loss has been described in polyarteritis nodosa (Tsunoda et al., 2001), microscopic polyangiitis (Koseki et al., 1997), Behçet disease (Narvaez et al., 1998; Adler et al., 2002), and Kawasaki disease (Silva et al., 2002).

Cogan Syndrome

Cogan syndrome is a chronic inflammatory disease characterized by three main clinical features: vestibulo-auditory dysfunction, interstitial keratitis, and vasculitis (St. Clair and McCallum, 1999). It occurs primarily in children and young adults and was first described by Cogan in 1945. Systemic manifestations occur in approximately half of the cases (Van Doornum et al., 2001); fever and weight loss are associated with active vasculitis, which can involve the aorta, aortic arch vessels, or medium vessels (Vollersten, 1990). Central and peripheral nervous system abnormalities may be present in up to 50% of the subjects (Bicknell and Holland,1978; Albayram et al., 2001).

Morbidity in Cogan syndrome results from permanent hearing loss and from cardiovascular disease.

Inner ear pathology reveals endolymphatic hydrops, infiltration of the spiral ligament with lymphocytes and plasma cells, degeneration of the sensory receptors and supporting structures of the cochlea and vestibular apparatus, and demyelinization and atrophy of the vestibular and cochlear

branches of the eighth cranial nerve. In some cases extensive new bone formation can be observed.

The cause of the disease is unknown; upper respiratory tract infections may precede the onset of the disease, suggesting an infectious origin (Vollertsen et al., 1986). Autoimmunity has been implicated because of the presence of serum antibodies to a mixture of corneal antigens and inner ear extracts (Helmchen et al., 1999; Disher et al., 1997). We have demonstrated that Cogan syndrome is an autoimmune disease and that DEP1/CD148 is the pathogenetically relevant autoantigen (Lunardi et al., 2002). Using the peptide library approach (Devlin et al.,1990), already applied to the study of systemic sclerosis (Lunardi et al., 2000), we identified a peptide recognized by the sera of all the patients. The peptide is homologous to autoantigens and the major core protein λ1 (Bartlett and Joklik, 1988) of reovirus type III, which causes mild rhinitis and pharyngitis. Peptide-specific IgG antibodies isolated from patients' sera recognized the viral protein, suggesting a viral involvement in the pathogenesis of the disease, possibly through a molecular mimicry mechanism (Zhao et al., 1998).

The peptide showed homology with the high cell density enhanced protein tyrosine phosphatase-1 (DEP1/CD148), which is highly expressed on both endothelial cells (Takahashi et al., 1999) and supporting cells of the inner ear (Kruger et al., 1999) and with connexin-26, a gap-junction protein expressed in the inner ear (Kikuchi et al., 2000). Affinity-purified antibodies against the peptide obtained from the patients recognized CD148 and connexin-26 in human cochlear extracts. DEP1/CD148 is a widespread cell-surface antigen, and its distribution explains the clinical spectrum of Cogan syndrome. Connexin-26 represents a major system of intercellular communication and its loss results in injury to the organ of Corti, leading to hearing loss; moreover, mutations in the connexin-26 gene are responsible for the majority of cases of congenital deafness. Connexin-26 shows homology with connexin-43 and connexin-50, gap-junction proteins present in corneal fibroblasts and epithelium, and this homology may explain the involvement of the eye in the disease. Peptide-specific antibodies were shown to bind human cochlea by immunohistochemistry (Figure 50.1).

To prove that these autoantibodies are pathogenic and that the identified autoantigen is relevant to Cogan syndrome, we induced the clinical features of the disease in animals (Balb/c mice and rabbits New Zealand White (NZW)) following either passive transfer of peptide-specific autoantibodies or active immunization with autoantigen peptides. The animals developed hearing loss as assessed by auditory brainstem responses (Figure 50.2).

We have tested patients with Cogan syndrome and isolated SNHL for the presence of antibodies directed against the DEP1/CD148, reovirus, and connexin-26 peptides. Table 50.1 shows the results obtained so far: sensitivity and specificity were respectively: 93.7% and 75% for anti-CD148 antibodies, 87.5% and 43.8% for antireovirus antibodies and 81.5 and 62.5% for anti-connexin-26 antibodies (Lunardi C. and Puccetti A. unpublished data).

EVIDENCE OF AUTOIMMUNITY

Different antibodies directed against autoantigens that are either specific to the inner ear or widely distributed have been described in patients with SNHL of unknown origin. The presence of antibodies against type II and type IX collagens as well as against other autoantigens in patients with Menière's disease and with IMIED has been reviewed by

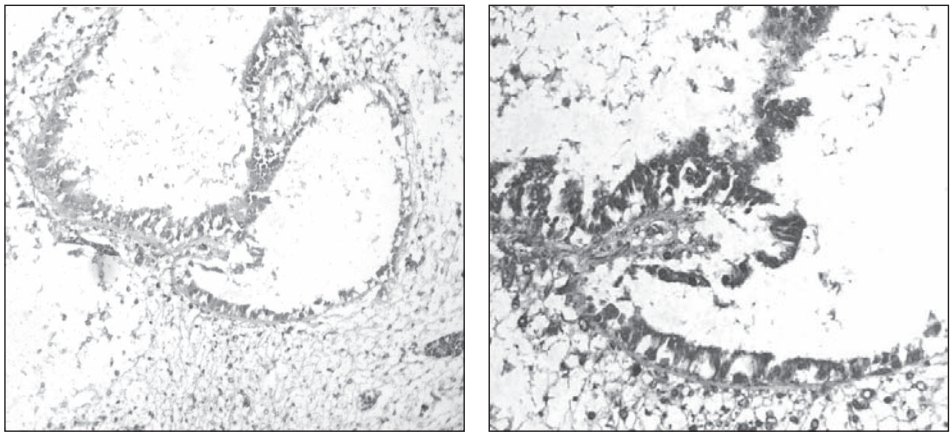


Figure 50.1 Antibodies against the Cogan peptide bind human cochlea. Human cochlea immunostained with antibodies purified against an irrelevant peptide (negative control, *A*) and with antibodies purified against the Cogan peptide (higher magnitude, *B*). (See color plate section).

Reprinted with permission from Elsevier, from Lunardi et al. (2002).

Yoo et al. (2002). The Kresge Hearing Research Institute-3 (KHRI-3) antibody binds to guinea pig inner ear supporting-cell antigen, which has been found to be homologous to the human choline transporter-like protein 2, expressed in the inner ear, making this protein a possible target for autoimmune aggression in IMIED (Nair et al., 2004). Anti-endothelial cell autoantibodies have been reported in some patients with SNHL and may represent a marker of vasculitis or vascular damage of the inner ear, which leads to leukocyte infiltration and local immunoglobulin production (Cadoni et al., 2002; Mathews and Kumar, 2003). The detection of antibodies against the myelin protein P0 (30 kDa) has given conflicting results (Boulassel et al., 2001a; Nuti et al., 2002; Passali et al., 2004). Besides antibodies against the 30 kDa protein, Boulassel et al. (2001a) reported also the presence of antibodies against a 42 kDa protein identified as β-actin and against a 68 kDa protein, identified as HSP70 by some authors (Billings et al., 1995; Bloch et al., 1999), but not by others (Yeom et al., 2003). The Western blot test for HSP70 has a very low sensitivity (Garcia-Berrocal et al., 2002b); however, its positivity seems to correlate with steroid responsiveness in subjects with IMIED (Hirose et al., 1999). The 58 kDa inner ear protein recognized by the sera of some patients with SNHL, has been identified as cochlin, a molecule highly expressed in the cochlea (Boulassel et al., 2001b). Cochlin and β-tectorin have been used to induce SNHL in animal models (see below). Table 50.2 summarizes the putative autoantigens believed or proved to be involved in the pathogenesis of IMIED.

We can conclude that sera of patients with SNHL recognize a large array of proteins of variable molecular weight,

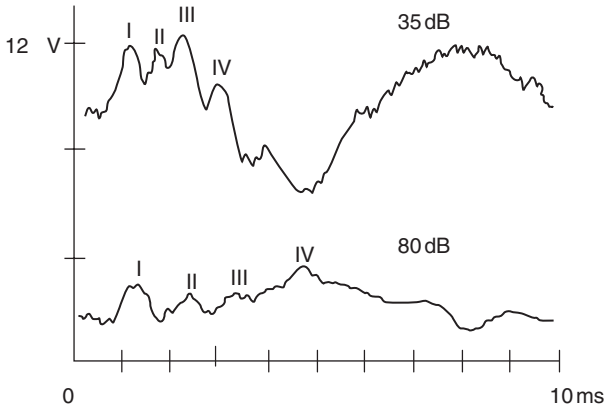


Figure 50.2 Grand average of auditory brainstem responses (ABRs) obtained from the same 6 mice before *(upper trace)* and 1 week after *(lower trace)* the third injection of purified antibodies directed against the Cogan peptide. A higher stimulus intensity (80 versus 35 dB) was needed to obtain much smaller and delayed responses (lower trace), consistent with hearing loss. The traces shown were obtained with above threshold stimuli in order to clearly identify the single components. The delay of waves II, III, and IV suggests an impaired transmission along the auditory pathway from the acoustic nerve to the midbrain. Data represent amplitude of the response in microvolt (μV) (y axis) versus time elapsed from stimulus in milliseconds (x axis). The time of stimulus delivery (click) is coincident with the 0 of the x axis; no pre-stimulus baseline is shown.

Reprinted with permission from Elsevier, from Lunardi et al. (2002).

TABLE 50.1 Anti-CD148, antireovirus, and anticonnexin-26 antibodies in patients with Cogan syndrome and sensorineural hearing loss (SNHL)

	Number of patients with		
	Anti-CD148	**Antireovirus**	**Anticonnexin-26**
Cogan syndrome	15/16	14/16	13/16
SNHL	6/16	9/16	6/16

Sensitivity and specificity, respectively: 93.7% and 75% for anti-CD148 antibodies, 87.5% and 43.8% for antireovirus antibodies, and 81.5 and 62.5% for anticonnexin-26 antibodies (Lunardi C. and Puccetti A., unpublished data).

TABLE 50.2 Putative autoantigen targets in immune-mediated inner ear disease (IMIED) and Cogan syndrome

	Molecular weight (kDa)	**Reference**	**Induces hearing loss in animals**	**Reference**
IMIED				
Collagen type II		Yoo et al., 2002	No	Harris et al., 1986
Collagen type IX		Yoo et al., 2002	?	–
Myelin protein P0	30	Boulassel et al., 2001a	No	Boulassel et al., 1998
β-actin	42	Boulassel et al., 2001a	?	–
HSP70	68	Billings et al., 1995; Bloch et al., 1999	No	Billings et al., 1998
Choline transporter-like protein 2		Nair et al., 2004	?	–
Cochlin	58	Boulassel et al., 2001b	Yes	Solares et al., 2004
β-tectorin	43	Boulassel et al., 2001b	Yes	Solares et al., 2004
Cogan syndrome				
DEP1/CD148		Lunardi et al., 2002	Yes	Lunardi et al., 2002
Connexin-26		Lunardi et al., 2002	Yes	Lunardi et al., 2002

only a few of which have been identified so far. However, there is no direct proof that any of the antibodies directed against such autoantigens may be immunopathogenic and cochleopathic in IMIED. Indeed, the majority of them could not induce SNHL in immunized animals (see below).

The first evidence for an implication of autoreactive T-cells was reported by McCabe and McCormick (1984) who observed leukocyte migration inhibition by T cells exposed to inner ear extracts. More recently, Lorenz et al. (2002) reported an increased number of inner ear-specific IFN-γ producing T cells in the peripheral blood of patients with SNHL believed to be of autoimmune origin. These findings indicated that pro-inflammatory T cells specific for as yet unknown inner ear antigens may play a role in the development and progression of inner ear autoimmunity.

ANIMAL MODELS

A major barrier in understanding the pathophysiology of IMIED derives from the paucity of inner ear tissue available. This problem underlines the importance of animal models for this disorder, which may provide insights into pathogenesis, diagnosis, and treatment of IMIED. Immunization with the better characterized putative autoantigens, such as collagen type II, HSP70, and myelin P0, have failed to elicit hearing loss (Harris et al., 1986; Billings et al., 1998; Boulassel et al., 2001c). Attempts to develop animal models of IMIED have been made by immunizing guinea pigs with either isologous or bovine inner ear homogenates (Harris, 1987; Yamanobe and Harris, 1993; Gong et al., 2002); this model was hampered by the vast array of immunologically active components involved, making it impossible to identify the specific self-antigens involved in disease initiation and progression. The use of fractions of inner ear proteins has partially addressed this problem (Tomiyama, 2002), which will be solved by the availability of recombinant antigens (Billings, 2004). Gloddeck et al. (1999) induced SNHL in naïve Lewis rats following passive transfer of activated T cells specific for bovine inner ear extract, demonstrating the role of T cells in the initiation and pathogenesis of SNHL. A recent experimental confirmation of the importance of T cells was recently provided by Solares et al. (2004), who showed that SWXJ mice immunized with peptides derived from two proteins of the inner ear, cochlin and β-tectorin, had significant hearing loss. Two selected peptides elicited a $CD4^+$ T-cell response of the Th1-like phenotype. Moreover, the passive transfer of peptide-activated $CD4^+$ T cells into naïve SWXJ recipients induced leukocytic infiltration of the inner ear and hearing loss. However, it is not clear whether cochlin and β-tectorin are implicated in IMIED in humans, or how accurately this model reflects events occurring in the spontaneous disease (Billings, 2004).

The administration of monoclonal antibody (KHRI-3) directed either against the guinea pig inner ear supporting cell antigen (Nair et al., 1995) or against the type II collagen fragment CB11 induced SNHL with loss of hair cells, inflammatory cell migration, and endolymphatic hydrops (Matsuoka et al., 2002) suggesting that these antibodies also play a critical role in autoimmunity of the inner ear.

TREATMENT

Since IMIED may result in severe deafness and vestibular dysfunction, patients must be treated aggressively and immediately after the onset of the symptoms. The mainstay of therapy is high dose corticosteroids (1 mg/kg/day) (Chen et al., 2003) continued for at least 2 weeks and then for another 2 weeks in case of improvement. The steroid is then tapered over a period of between 2 and 3 months, and in some cases maintained at low dosage for a long period (e.g. methylprednisolone 4 mg/day). In case of deterioration of symptoms or of no significant improvement during the first 2 weeks of treatment, other immunosuppressive agents are added such as cyclophosphamide (CYP), 2 mg/kg/day, or methotrexate (MTX), 7.5–20 mg/wk. Because of the well-known side effects of CYP, clinicians prefer the use of MTX; however, despite the preliminary favorable responses (Kilpatrick et al., 2000; Matteson et al., 2001; Rahman et al., 2001a; Matteson et al., 2003), the only controlled trial published so far does not support the efficacy of long-term MTX in maintaining the improvement achieved with glucocorticoid therapy (Harris et al., 2003). Since MTX is slow acting, its effect may start too late in a disease that rapidly leads to hearing loss. McCabe (1989) reported the promising results obtained with the CYP-prednisolone combination therapy. We have used oral methylprednisolone and CYP pulse therapy in two particularly rapid and severe cases; this therapy blocked and reverted the hearing loss.

The results obtained with steroids and aggressive immunosuppression are variable (Broughton et al., 2004; Loveman et al., 2004; Ruckenstein, 2004) depending on the characteristics of the patients included, on the severity of the hearing loss at the beginning of the treatment, and on how early the therapy is started.

Etanercept, a tumor necrosis factor-α receptor blocker, has been used in a few cases with apparent efficacy; the addition of leflunomide or of other immunosuppressive agents was necessary to maintain the results obtained in some patients (Rahman et al., 2001b). However recent reports do not support the efficacy of etanercept (Choi et al., 2004; Cohen et al., 2004). There are reports on the utility of plasmapheresis (Luetje, 1989; Luetje and Berliner, 1997), therapeutic apheresis of low-density lipoproteins (LDL) (Bosch, 2003), and fibrinogen (Suzuki et al., 2003).

Suckfull et al. (2002) reported the beneficial effects of a single fibrinogen/LDL apheresis compared with conventional infusion treatment and prednisolone for 10 days. In controlled studies, the use of antiviral therapy with valacyclovir (Tucci et al., 2002) and acyclovir (Uri et al., 2003; Westerlaken et al., 2003) in addition to steroids was not more beneficial than steroids alone. Finally, there are recent reports on the utility of low-molecular-weight heparins (Yue et al., 2003; Mora et al., 2004) and antioxidants (Joachims et al., 2003) in addition to the usual therapy, and on the efficacy of intravenous infusion of tissue plasminogen activator (Mora et al., 2003) alone. In case of permanent severe bilateral hearing loss, the auditory function may be partially replaced with a cochlear implant—an electrical prosthesis with electrodes inserted into the cochlea through mastoidectomy (Cohen et al., 1993). Cochlear implants have been successfully used in patients with Cogan syndrome (Minet et al., 1997; Cinnamon et al., 1997). Of the 16 patients with Cogan syndrome we are following at the moment, eight (age range 7–25 years) have undergone multichannel cochlear implant, resulting in a great improvement of their quality of life, without any complication.

CONCLUDING REMARKS

It is now evident that the inner ear is not an "immunologically privileged" site and may mount an immune response against both foreign and self-antigens. The association of IMIED with systemic autoimmune diseases provides evidence that autoimmunity can damage the inner ear, but it does not address organ-specific disease. Antibodies directed against different inner ear antigens have been identified in some patients; however, they are neither diagnostic nor correlate with disease state. In the future the major goals for research in this field will be: 1) the identification of pathogenetically relevant autoantigen(s); 2) the development of a highly specific diagnostic test; and 3) a better knowledge of the immunopathologic mechanisms in an organ as inaccessible as the inner ear.

Acknowledgments

We are grateful to Dr. C. Bason, Dr. D. Peterlana and to Prof. R. Corrocher.

References

Adler, Y.D., Jovanovic, S., Jivanjee, A., Krause, L., and Zouboulis, C.C. (2002). Adamantiades-Behçet's disease with inner ear involvement. *Clin. Exp. Rheumatol. 20,* S40–42.

Albayram, M.S., Wityk, R., Yousem, D.M., and Zinreich, S.J. (2001). The cerebral angiographic findings in Cogan syndrome. *Am. J. Neuroradiol. 22,* 751–754.

Bachor, E., Kremmer, S., Kreuzfelder, E., Jahnke, K., and Seidahmadi, S. (2005). Antiphospholipid antibodies in patients with sensorineural hearing loss. *Eur. Arch. Otorhinolaryngol. 262,* 622–626.

Bartlett, J.A. and Joklik, W.K. (1988). The sequence of the reovirus serotype 3 L3 genome segment which encodes the major core protein lambda 1. *Virology 167,* 31–37.

Bicknell, J.M. and Holland, J.V. (1978). Neurologic manifestations of Cogan's syndrome. *Neurology 28,* 278–288.

Billings, P., Keithley, E.M., and Harris, J.P. (1995). Evidence linking the 68 kilodalton antigen identified in progressive sensorineural hearing loss patient sera with heat shock protein 70. *Ann. Otol. Rhinol. Laryngol. 104,* 181–188.

Billings, P., Shin, S.O., and Harris, J.P. (1998). Assessing the role of anti-hsp70 in cochlear function. *Hear. Res. 126,* 210–212.

Billings, P. (2004). Experimental autoimmune hearing loss. *J. Clin. Invest. 113,* 1114–1117.

Bloch, D.B., Gutierrez, J.A., Guerriero, Jr., V., Rauch, S.D., and Bloch, K.J. (1999). Recognition of a dominant epitope in bovine heat-shock protein 70 in inner ear disease. *Laryngoscope 109,* 621–625.

Boki, K.A., Ioannidis, J.P., Segas, J.V., Maragkoudakis, P.V., Petrou, D., Adamopoulos, G.K., and Moutsopoulos, H.M. (2001). How significant is sensorineural hearing loss in primary Sjogren's syndrome? An individually matched case-control study. *J. Rheumatol. 28,* 798–801.

Bosch, T. (2003). Recent advances in therapeutic apheresis. *J. Artif. Organs 6,* 1–8.

Boulassel, M.R., Deggouj, N., Tomasi, J.P., and Gersdorff, M. (2001a). Inner ear autoantibodies and their targets in patients with autoimmune inner ear diseases. *Acta Otolaryngol. 121,* 28–34.

Boulassel, M.R., Tomasi, J.P., Deggouj, N., and Gersdorff, M. (2001b). COCH5B2 is a target antigen of anti-inner ear antibodies in autoimmune inner ear diseases. *Otol. Neurotol. 22,* 614–618.

Boulassel, M.R., Guerit, J.M., Denison, S., de Tourtchaninoff, M., Wenderickx, L., Botterman, N., Deggouj, N., Gersdorff, M., and Tomasi, J.P. (2001c). No evidence of auditory disfunction in guinea pigs immunized with myelin P0 protein. *Hear. Res. 152,* 10–16.

Broughton, S.S., Meyerhoff, W.E., and Cohen, S.B. (2004). Immune-mediated inner ear disease: 10-year experience. *Semin. Arthritis Rheum. 34,* 544–548.

Cadoni, G., Fetoni, A.R., Agostino, S., DeSantis, A., Manna, R., Ottaviani, F., and Paludetti, G. (2002). Autoimmunity in sudden sensorineural hearing loss: possible role of anti-endothelial cell autoantibodies. *Acta Otolaryngol. Suppl. 548,* 30–33.

Chapman, J., Rand, J.H., Brey, R.L., Levine, S.R., Blatt, I., Khamashta, M.A., and Shoenfeld, Y. (2003). Non-stroke neurological syndromes associated with antiphospholipid antibodies: evaluation of clinical and experimental studies. *Lupus 12,* 514–517.

Chen, C.Y., Halpin, C., and Rauch, S.D. (2003). Oral steroid treatment of sudden sensorineural hearing loss: a ten year retrospective analysis. *Otol. Neurotol. 24,* 728–733.

Choi, H., Poe, D., Wise, C., Rahman, M., and Matteson, E.L. (2004). Etanercept therapy for immune-mediated cochleovestibular disorders: an open-label, pilot clinical trial. *Arthritis Rheum. 50* (Suppl.), S235 [abstract 512].

Cinnamon, U., Kronenberg, J., Hildesheimer, M., and Taitelbaum, R. (1997). Cochlear implantation in patients suffering from Cogan's syndrome. *J. Laryngol. Otol. 111,* 928–930.

Cogan, D. (1945). Syndrome of nonsyphilitic interstitial keratitis and vestibuloauditory symptoms. *Arch Ophthalmol. 33,* 144–149.

Cohen, N.L., Waltzman, S.B., Fisher, S.G., and the Department of Veterans Affair Cochlear Implant Study Group (1993). A prospective, randomized study of cochlear implants. *N. Engl. J. Med. 328,* 233–237.

Cohen, B., Weisman, M., Shoup, A., and Harris, J. (2004). A pilot study of Etanercept in autoimmune inner ear disease. *Arthritis Rheum.* 50 (Suppl.), S236 [abstract 513].

Devlin, J.J., Panganiban, L.C., and Devlin, P.E. (1990). Random peptide libraries: a source of specific protein binding molecules. *Science 249,* 404–406.

Disher, M.J., Ramakrishnan, A., Nair, T.S., Miller, J.M., Telian, S.A., Arts, H.A., Sataloff, R.T., Altschuler, R.A., Raphael, Y., and Carey, T.E. (1997). Human autoantibodies and monoclonal antibody KHRI–3 bind to a phylogenetically conserved inner-ear supporting cell antigen. *Ann. N.Y. Acad. Sci. 830,* 253–267.

Garcìa-Berrocal, J.R., and Ramìrez-Camacho, R. (2002a). Sudden sensorineural hearing loss: supporting the immunologic theory. *Ann. Otol. Rhinol. Laryngol. 111,* 989–997.

Garcìa-Berrocal, J.R., Ramìrez-Camacho, R., Arellano, B., and Vargas, J.A. (2002b). Validity of the Western blot immunoassay for heat shock protein-70 in associated and isolated immunorelated inner ear disease. *Laryngoscope 112,* 304–309.

Garcìa-Berrocal, J.R., Ramìrez-Camacho, R., Millàn, I., Gorriz, C., Trinidad, A., Arellano, B., and Lobo, D. (2003). Sudden presentation of immune-mediated inner ear disease: characterization and acceptance of a cochleovestibular dysfunction. *J. Laringol. Otol. 117,* 775–779.

Gloddek, B., Gloddek, J., and Arnold, W. (1999). A rat T cell line that mediates autoimmune disease of the inner ear in the Lewis rat. *ORL J. Otorhinolaryngol. Relat. Spec. 61,* 181–187.

Gong, S.S., Yu, D.Z., and Wang, J.B. (2002). Relationship between three inner ear antigens with different molecular weights and autoimmune inner ear disease. *Acta Otoralyngol. 122,* 5–9.

Green, L. and Miller, E.B. (2001). Sudden sensorineural hearing loss as a first manifestation of systemic lupus erythematosus: association with anticardiolipin antibodies. *Clin. Rheumatol. 20,* 220–222.

Harris, J.P., Woolf, N.K., and Ryan, A.F. (1986). A re-examination of experimental type II collagen autoimmunity: middle and inner ear morphology and function. *Ann. Otol. Rhinol. Laryngol. 95,* 176–180.

Harris, J.P. (1987). Experimental autoimmune sensorineural hearing loss. *Laryngoscope 97,* 63–76.

Harris, J.P., Weisman, M.H., Derebery, J.M., Espeland, M.A., Gantz, B.J., Gulya, A.J., Hammerschlag, P.E., Hannley, M., Hughes, G.B., Moscicki, R., Nelson, R.A., Niparko, J.K., Rauch, S.D., Telian, S.A., and Brookhouser, P.E. (2003). Treatment of corticosteroid-responsive autoimmune inner ear disease with methotrexate: a randomized controlled trial. *JAMA 290,* 1875–1883.

Helmchen, C., Arbusow, V., Jager, L., Strupp, M., Stocker, W., and Schulz, P. (1999). Cogan's syndrome: clinical significance of antibodies against inner ear and cornea. *Acta Otoryngol. 119,* 528–536.

Hirose, K., Wener, M.H., and Duckert, L.G. (1999). Utility of laboratory testing in autoimmune inner ear disease. *Laryngoscope 109,* 1749–1754.

Joachims, H.Z., Segal, J., Golz, A., Netzer, A., and Goldenberg, D. (2003). Antioxidants in treatment of idiopathic sudden hearing loss. *Otol. Neurotol. 24,* 572–575.

Kastanioudakis, I., Ziavra, N., Politi, E.N., Exarchakos, G., Drosos, A.A., and Skevas A. (2001). Hearing loss in progressive systemic sclerosis patients: a comparative study. *Otolaryngol. Head Neck Surg. 124,* 522–525.

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,* 103–107.

Kempf, H.G. (1989). Ear involvement in Wegener's granulomatosis. *Clin. Otorinolaryngol. 14,* 451–456.

Kikuchi, T., Kimura, R.S., Paul, D.L., Takasaka, T., and Adams, J.C. (2000). Gap junction systems in the mammalian cochlea. *Brain Res. Rev. 32,* 163–166.

Kilpatrick, J.K., Sismanis, A., Spencer, R.F., and Wise, C.M. (2000). Low dose oral methotrexate management of patients with bilateral Menière's disease. *Ear Nose Throat J. 79,* 82–83.

Koseki, Y., Suwa, A., Nojima, T., Ishijama, K., Nakajima, A., Tanabe, M., Yamada, T., Goto, M., Yasui, T., and Inada, S. (1997). A case of microscopic polyangiitis accompanied by hearing loss as the initial sign of the disease. *Ryumachi 37,* 804–809.

Kruger, R.P., Goodyear, R.J., Legan, P.K., Warchol, M.E., Raphael, Y., Cotanche, D.A., and Richardson, G.P. (1999). The supporting-cell antigen: a receptor-like protein tyrosine phosphatase expressed in the sensory epithelia of the avian inner ear. *J. Neurosci. 19,* 4815–4827.

Kumar, B.N., Smith, M.S.H., and Walsh, R.M. (2000). Sensorineural hearing loss in ulcerative colitis. *Clin. Otolaryngol. 25,* 143–145.

Lorenz, R.R., Solares, C.A., Williams, P., Sikora, J., Pelfrey, C.M., Hughes, G.B., and Tuohy, V.K. (2002). Interferon-gamma production to inner ear antigens by T cells from patients with autoimmune sensorineural hearing loss. *J. Neuroimmunol. 130,* 173–178.

Loveman, D.M., de Comarmond, C., Cepero, R., and Baldwin, D.M. (2004). Autoimmune sensorineural hearing loss: clinical course and treatment outcome. *Semin. Arthritis Rheum. 34,* 538–543.

Luetje, C.M. (1989). Theoretical and practical implications for plasmapheresis in autoimmune inner ear. *Laryngoscope 99,* 1137–1146.

Luetje, C.M. and Berliner, K.I. (1997). Plasmapheresis in autoimmune inner ear disease: long-term follow-up. *Am. J. Otol. 18,* 572–576.

Lunardi, C., Bason, C., Navone, R., Millo, E., Da monte, G., Corrocher, R., and Puccetti, A. (2000). Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. *Nat. Med. 6,* 1183–1186.

Lunardi, C., Bason, C., Leandri, M., Navone, R., Lestani, M., Millo, E., Benatti, U., Cilli, M., Beri, R., Corrocher, R., and Puccetti, A. (2002). Autoantibodies to inner ear and endothelial antigens in Cogan's syndrome. *Lancet 360,* 915–921.

Malard, O., Hamidou, M., Toquet, C., Bailleuil, S., Bordure, P., and Beauvillain De Montreuil, C. (2002). Relapsing polychondritis revealed by ENT symptoms: clinical characteristics in three patients. *Ann Otolarynol. Chir. Cervicofac. 119,* 202–208.

Mathews, J. and Kumar, B.N. (2003). Autoimmune sensorineural hearing loss. *Clin. Otolaryngol. 28,* 479–488.

Matsuoka, H., Kwon, S.S., Yazawa, Y., Barbieri, M., and Yoo, T.J. (2002). Induction of endolymphatic hydrops by directly infused monoclonal antibody against type II collagen CB11 peptide. *Ann. Otol. Rhinol. Laryngol. 111,* 587–592.

Matteson, E.L., Fabry, D.A., Facer, G.W., Beatty, W., Driscoll, C.L., Strome, S.E., and McDonald, T.J. (2001) Open trial of methotrexate as treatment for autoimmune hearing loss. *Arthritis Rheum. 45,* 146–150.

Matteson, E.L., Fabry, D.A., Strome, S.E., Driscoll, C.L., Beatty, C.W., and McDonald, T.J. (2003). Autoimmune inner ear disease: diagnostic and therapeutic approaches in a multidisciplinary setting. *J. Am. Acad. Audiol. 14,* 225–230.

Mazlumzadeh, M., Lowe, V.J., Mullan, B.P., Fabry, D.A., McDonald, T.J., and Matteson, E.L. (2003). The utility of positron emission tomography in the evaluation of autoimmune hearing loss. *Otol. Neurotol. 24,* 201–204.

McCabe, B.F. (1979). Autoimmune sensorineural hearing loss. *Ann. Otol. Rhinol. Laringol. 88,* 585–589.

McCabe, B.F. and McCormick, K.J. (1984). Tests for autoimmune disease in otology. *Am. J. Otol. 5,* 447–449.

McCabe, B.F. (1989). Autoimmune inner ear disease: therapy. *Am. J. Otol. 10,* 196–197.

Minet, M., Deggouj, N., and Gersdorff (1997). Cochlear implantation in patients with Cogan's syndrome: a review of four cases. *Eur. Arch. Otorhinolaryngol. 254,* 459–462.

Mora, R., Barbieri, M., Mora, F., Mora, M., and Yoo, T.J. (2003). Intravenous infusion of recombinant tissue plasmonogen activator for

treatment of patients with sudden and/or chronic hearing loss. *Ann. Otol. Rhinol. Laryngol. 112,* 665–670.

Mora, R., Mora, F., Passali, F.M., Cordone, M.P., Crippa, B., and Barbieri, M. (2004). Restoration of immune-mediated sensorineural hearing loss with sodium enoxaparin: a case report. *Acta Otolaryngol. Suppl. 552,* 25–28.

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.

Nair, T.S., Raphael, Y., Dolan, D.F., Parrett, T.J., Perlman, L.S., Brahmbhatt, V.R., Wang, Y., Hou, X., Ganjei, G., Nuttall, A.L. (1995). Monoclonal antibody induced hearing loss. *Hear. Res. 83,* 101–113.

Nair, T.S., Kozma, K.E., Hoefling, N.L., Kommareddi, P.K., Ueda, Y., Gong, T.W., Lomax, M.I., Lansford, C.D., Telian, S.A., Satar, B., Arts, H.A., El-Kashlan, H.K., Berryhill, W.E., Raphael, Y., and Carey, T.E. (2004). Identification and characterization of choline transporter-like protein 2, an inner ear glycoprotein of 68 and 72 kDa that is the target of antibody-induced hearing loss. *J Neurosci. 24,* 1772–1779.

Narvaez, J., Valverde-Garcia, J., Alegre-Sancho, J.J., Juanola, X., Clavaguera, M.T., and Roig-Escofet, D. (1998). Sudden cochlear hearing loss in a patient with Behcet's disease. *Rev. Rhum. Engl. Ed. 65,* 63–64.

Nuti, D., Bigini, C., Salerni, L., Gaudini, E., and Passali, G.C. (2002). Use of mammalian inner ear antigen for the diagnosis of autoimmune sudden loss of vestibular function. *Acta Otolaryngol. Suppl. 548,* 34–37.

Oczan, M., Karakus, M.F., Gunduz, O.H., Tuncel, U., and Sahin, H. (2002). Hearing loss and middle ear involvement in rheumatoid arthritis. *Rheumatol. Int. 22,* 16–19.

Passali, D., Damiani, V., Mora, R., Passali, F.M., Pssali, G.C., and Bellussi, L. (2004). P0 antigen detection in sudden hearing loss and Menière's disease: a new diagnostic marker? *Acta Otolaryngol. 124,* 1145–1148.

Rahman, M.U., Poe, D.S., and Choi, H.K. (2001a). Autoimmune vestibulocochlear disorders. *Curr. Opin. Rheumatol. 13,* 184–189.

Rahman, M.U., Poe, D.S., and Choi, H.K. (2001b). Etanercept therapy for immune-mediated cochleovestibular disorders: preliminary results in a pilot study. *Otol. Neurotol. 22,* 619–624.

Raut, V.V., Cullen, J., and Cathers, G. (2001). Hearing loss in rheumatoid arthritis. *J. Otolaryngol. 30,* 289–294.

Riente, L., Bongiorni, F., Nacci, A., Migliorini, P., Segnini, G., Delle Sedie, A., Ursino, F., Tommasi, S., and Fattori, B. (2004). Antibodies to inner ear antigens in Menière's disease. *Clin. Exp. Immunol. 135,* 159–163.

Ruckenstein, M.J. (2004). Autoimmune inner ear disease. *Curr. Opin. Otolaryngol. Head Neck Surg. 12,* 426–430.

Salvinelli, F., Cancilleri, F., Casale, M., Luccarelli, V., Di Peco, V., D'Ascanio, L., De Martino, A., and Denaro, V. (2004). Hearing thresholds in patients affected by rheumatoid arthritis. *Clin. Otolaryngol. 29,* 75–79.

Silva, C.H., Roscoe, I.C., Fernandes, K.P., Novaes, R.M., and Lazari, C.S. (2002). Sensorineural hearing loss associated to Kawasaki disease. *J. Pediatr. 78,* 71–74.

Solares, C.A., Hughes, G.B., and Tuohy, V.K. (2003). Autoimmune sensorineural hearing loss: an immunologic perspective. *J. Neuroimmunol. 138,* 1–7.

Solares, C.A., Edling, A.E., Johnson, J.M., Baek, M., Hirose, K., Hughes, G.B., and Tuohy, V.K. (2004). Murine autoimmune hearing loss mediated by CD4+ T cells specific for inner ear peptides. *J. Clin. Invest. 113,* 1210–1217.

Sone, M., Schachern, P.A., Paparella, M.M., and Morizono, N. (1999). Study of systemic lupus erythematosus in temporal bones. *Ann. Otol. Rhonol. Laryngol. 108,* 338–344.

St. Clair, E.W. and McCallum, R.M. (1999). Cogan's syndrome. *Curr. Opin. Rheumatol. 11,* 47–52.

Stone, J.H. and Francis H.W. (2000). Immune-mediated inner ear disease. *Curr. Opin. Rheumatol. 12,* 32–40.

Suckfull, M. and the Hearing Loss Study Group (2002). Fibrinogen and LDL apheresis in treatment of sudden hearing loss: a randomized multicentre trial. *Lancet 360,* 1811–1817.

Suzuki, H., Furukawa, M., Kumagai, M., Takahashi, E., Matsuura, K., Katori, Y., Shimomura, A., and Kobayashi, T. (2003). Defibrinogenation therapy for idiopathic sudden sensorineural hearing loss in comparison with high-dose steroid therapy. *Acta Otolatyngol. 123,* 46–50.

Takagi, D., Nakamaru, Y., Maguchi, S., Furuta, Y., and Fukuda, S. (2002). Otologic manifestations of Wegener's granulomatosis. *Laryngoscope 112,* 1684–1690.

Takagi, D., Nakamaru, Y., Maguchi, S., Furuta, Y., and Fukuda, S. (2004). Clinical features of bilateral progressive hearing loss associated with myeloperoxidase-antineutrophil cytoplasmic antibody. *Ann. Otol. Rhinol. Laryngol. 113,* 388–393.

Takahashi, T., Takahashi, K., Mernaugh, R., Drozdoff, V., Sipe, C., Schoecklmann, H., Robert, B., Abrahamson, D.R., and Daniel, T.O. (1999). Endothelial localization of receptor tyrosine phosphatase, ECRTP/DEP1, in developing and mature renal vasculature. *J. Am. Soc. Nephrol. 10,* 2135–2145.

Tomiyama, S. (2002). Experimental autoimmune labyrinthitis: assessment of molecular size of autoantigens in fractions of inner ear proteins eluted on the Mini Whole Gel Eluter. *Acta Otolaryngol. 122,* 692–697.

Toubi, E., Ben-David, J., Kessel, A., Hals, K., Sabo, E., and Luntz, M. (2004). Immune-mediated disorders associated with idiopathic sudden sensorineural hearing loss. *Ann. Otol. Rhinol. Laryngol. 113,* 445–449.

Trentham, D. and Le, C.H. (1998). Relapsing polychondritis. *Ann. Intern. Med. 129,* 114–122.

Tsunoda, K., Akaogi, J., Ohya, N., and Murofushi, T. (2001). Sensorineural hearing loss as the initial manifestation of polyarteritis nodosa. *J. Laryngol. Otol. 115,* 311–312.

Tucci, D.L., Farmer, J.C. Jr, Kitch, R.D., and Witsell, D.L. (2002). Treatment of sudden sensorineural hearing loss with systemic steroids and valacyclovir. *Otol. Neurotol. 23,* 301–308.

Tumiati, B., Casoli, P., and Parmeggiani, A. (1997). Hearing loss in Sjogren's syndrome. *Ann. Inter. Med. 126,* 450–453.

Uri, N., Doweck, I., Cohen-Kerem, R., and Greenberg, E. (2003). Acyclovir in the treatment of idiopathic sudden sensorineural hearing loss. *Otolaryngol. Head Neck Surg. 128,* 544–549.

Van Doornum, S., McColl, G., Walter, M., Jennens, I., Bhathal, P., and Wicks, I.P. (2001). Prolonged prodrome, systemic vasculitis, and deafness in Cogan's syndrome. *Ann. Rheum. Dis. 60,* 69–71.

Vollertsen, R.S., McDonald, T.J., Younge, B., Banks, P.M., Stanson, A.W., and Ilstrup, D.M. (1986). Cogan's syndrome: 18 cases and a review of the literature. *Mayo Clin. Proc. 61,* 344–361.

Vollertsen, R. (1990). Vasculitis and Cogan's syndrome. *Rheum. Dis. Clin. North Am. 16,* 433–438.

Vyse, T., Luxon, L.M., and Walport, M.J. (1994). Audiovestibular manifestations of the antiphospholipid syndrome. *J. Laryngol. Otol. 108,* 57–59.

Westerlaken, B.O., Stokroos, T.J., Dhooge, I.J., Wit, H.P., and Albers, F.W. (2003). Treatment of idiopathic sudden sensorineural hearing loss with antiviral therapy: a prospective, randomised, double-blind clinical trial. *Ann. Otol. Rhinol Laryngol. 112,* 993–1000.

Yamanobe, S. and Harris, J. (1993) Inner ear-specific autoantibodies. *Laryngoscope 103,* 319–325.

Yeom, K., Gray, J., Nair, T.S., Arts, H.A., Telian, S.A., Disher, M.J., El-Kashlan, H., Aataloff, R.T., Fisher, S.G., and Carey, T.E. (2003). Antibodies to HSP-70 in normal donors and autoimmune hearing loss patients. *Laryngoscope 113,* 1770–1776.

Yoo, T.J., Shea, J. Jr, Ge, X., Kwon, S.S., Yazawa, Y., Sener, O., Mora, F., Mora, R., Mora, M., Barbieri, M., and Du, X. (2001). Presence of

autoantibodies in the sera of Menière's disease. *Ann. Otol. Rhinol. Laryngol*. *110,* 425–429.

Yoo, T.J., Du, X., and Known, S.S. (2002). Molecular mechanism of autoimmune hearing loss. *Acta Otolaringol. Suppl. 548,* 3–9.

Yoon, T., Paparella, M., and Schachem, P. (1989). Systemic vasculitis: a temporal bone hystopathologic study. *Laryngoscope 99,* 600–609.

Yue, W.L., Li, P.Y., Qi, P.Y., Li, H.J., and Zhou, H. (2003). Role for low-molecular-weight heparins in the treatment of sudden hearing loss. *Am. J. Otolaryngol. 24,* 328–333.

Zhao, Z.S., Granucci, F., Yeh, L., Schaffer, P.A., and Cantor, H. (1998). Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science 279,* 1344–1347.

CHAPTER

51

Celiac Disease

LUDVIG M. SOLLID,* ØYVIND MOLBERG,*† AND KNUT E. A. LUNDIN*‡

*Institute of Immunology, †Department of Rheumatology, and ‡Department of Medicine, University of Oslo University Hospital, Oslo, Norway

Celiac disease (also called gluten-sensitive enteropathy or celiac sprue) has autoimmune components of which the highly disease-specific antibodies to the enzyme tissue transglutaminase (TG2) are particularly striking. Notwithstanding, celiac disease is most often categorized as a food hypersensitivity or intolerance disorder. The disease is precipitated in genetically susceptible individuals by the ingestion of wheat gluten and similar proteins of other cereals. Some historic findings that have led to our current understanding of celiac disease as a chronic inflammatory disorder with autoimmune components are listed in Box 51.1. If the critical role of gluten were not known, celiac disease would likely have been considered as a classical autoimmune disease.

Among the chronic inflammatory disorders with autoimmune components, celiac disease stands out as a particularly good model since the precipitating antigen is known and the affected organ—the small intestine—is easily accessed. Insight into the pathogenesis of this disorder is relevant for a large group of diseases for which the genetic and environmental components are poorly characterized.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Clinical Features and Associated Disorders

Celiac disease may present in early childhood soon after the introduction of gluten-containing food. Previously, a dramatic and even fatal clinical picture with diarrhea, anorexia, failure to thrive, abdominal distention, and growth retardation was regarded as typical. Today, most children present with a milder, pauci- or mono-symptomatic disease that

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Box 51.1
Important historical achievements in the study of celiac disease pathogenesis

First description of a condition resembling celiac disease by Aretheus from Cappodocia (second century AD)
First precise description of the disorder (Gee, 1888)
Suggestion that banana diet can cure the disease (Haas, 1932)
Wheat gluten identified as a causative agent (Dicke, 1950)
Villous atrophy and crypt hyperplasia found to be pathognomonic for the disease (Paulley, 1954)
Celiac disease of childhood and adult nontropical sprue share the same pathogenesis (Rubin et al., 1960)
Familial clustering of biopsy-proven celiac disease (MacDonald et al., 1965)
First diagnostic criteria of pediatric celiac disease from ESPGAN (Weijers et al., 1970)
Celiac disease found to be an HLA-associated disorder (Falchuk et al., 1972; Stokes et al., 1972)
Existence of autoantibodies to reticulin detected (Alp and Wright, 1971; Seah et al., 1971)
Cell-mediated immunity to gliadin in celiac disease intestinal biopsy specimens (Ferguson et al., 1975)
HLA-DQ2 heterodimer (DQA1*05/DQB1*02) identified as the primary HLA susceptibility factor (Sollid et al., 1989)
Criteria for histological classification of the mucosal lesion (Marsh, 1992)
Isolation of gliadin-specific, HLA-DQ2 restricted T cells from intestinal biopsies (Lundin et al., 1993)
Celiac disease autoantigen identified as tissue transglutaminase (Dieterich et al., 1997)
Role of tissue transglutaminase (TG2) for making gluten peptides more immunogenic by deamidation recognized (Molberg et al., 1998; van de Wal et al., 1998a)

resembles the picture in adults (Mäki et al., 1988). Many of the symptoms associated with the milder disease form, like chronic fatigue, joint pain, and neuropsychological problems do not directly point to a small intestinal disorder (Murray, 1999; Fasano and Catassi, 2001; Farrell and Kelly, 2002), and neither do complications like osteoporosis, reduced fertility, peripheral neuropathy, epilepsy with cerebral calcifications or the blistering skin disorder dermatitis herpetiformis (Ciacci et al., 1996; Hin et al., 1999; Collin and Reunala, 2003; Fasano et al., 2003; Hadjivassiliou et al., 2003; Sanders et al., 2003). The changes in the phenotype have been paralleled by an increase in incidence, which is mostly attributable to better diagnosis and identification of the pauci- or mono-symptomatic cases. The coexistence of (auto)immune diseases is striking, particularly type 1 diabetes (Green et al., 1962; Cronin et al., 1997; Koletzko et al., 1998), Sjögren syndrome (Collin et al., 1994), autoimmune thyroid disorders (Collin et al., 1994; Counsell et al., 1994) connective tissue disease (Collin et al., 1994), and IgA deficiency (Mawhinney and Tomkin, 1971; Cataldo et al., 1997; Heneghan et al., 1997). One study indicated that prolonged gluten exposure in patients with celiac disease increases the risk of contracting autoimmune diseases (Ventura et al., 1999), but this has not been confirmed (Sategna et al., 2001). Like many of the autoimmune disorders, pediatric and adult celiac disease show a gender bias with a female-to-male ratio of approximately 2 : 1 (Ciacci et al., 1995; Ivarsson et al., 2003b).

Pathologic Features

The diagnosis of celiac disease is defined by typical gluten-induced alterations of the small intestinal mucosa. These changes can, according to the criteria introduced by Marsh (1992), be classified into three stages: the infiltrative, the hyperplastic, and the destructive lesion. In the infiltrative lesion the mucosal architecture is normal, but there is an increased infiltration of intraepithelial lymphocytes (IELs) in the villous epithelium. The hyperplastic lesion is similar to the infiltrative lesion, but in addition has hypertrophic crypts. The last stage, the destructive lesion, is now often subgrouped into partial, subtotal or total villous atrophy (Marsh 3A–C); the latter corresponding to the classic flat lesion (Oberhuber et al., 1999). The classic flat lesion, in addition to the increased IELs, is characterized by swelling of the lamina propria and infiltration of $CD4^+$ $\alpha\beta$ T-cells, plasma cells, macrophages/dendritic cells, mast cells, and neutrophils in the same compartment. Typical examples of small intestinal biopsies of celiac disease patients are shown in Figure 51.1.

Several molecules with immune function have altered expression in the celiac lesions (Figure 51.2). Particularly striking is the epithelial expression of HLA class II molecules, with strong expression of DR and DP molecules but little or no expression of DQ molecules (Marley et al., 1987; Scott et al., 1987). Another interesting molecule with increased expression in untreated celiac disease (and other autoimmune diseases, including type 1 diabetes) is the tight junction modulator zonulin (Fasano et al., 2000; Wang et al., 2000). This upregulation of zonulin is potentially important as it leads to an increased intercellular transport across the epithelium.

Epidemiologic Features

Celiac disease is primarily a disease of white persons. More than half the cases are now diagnosed in adult life,

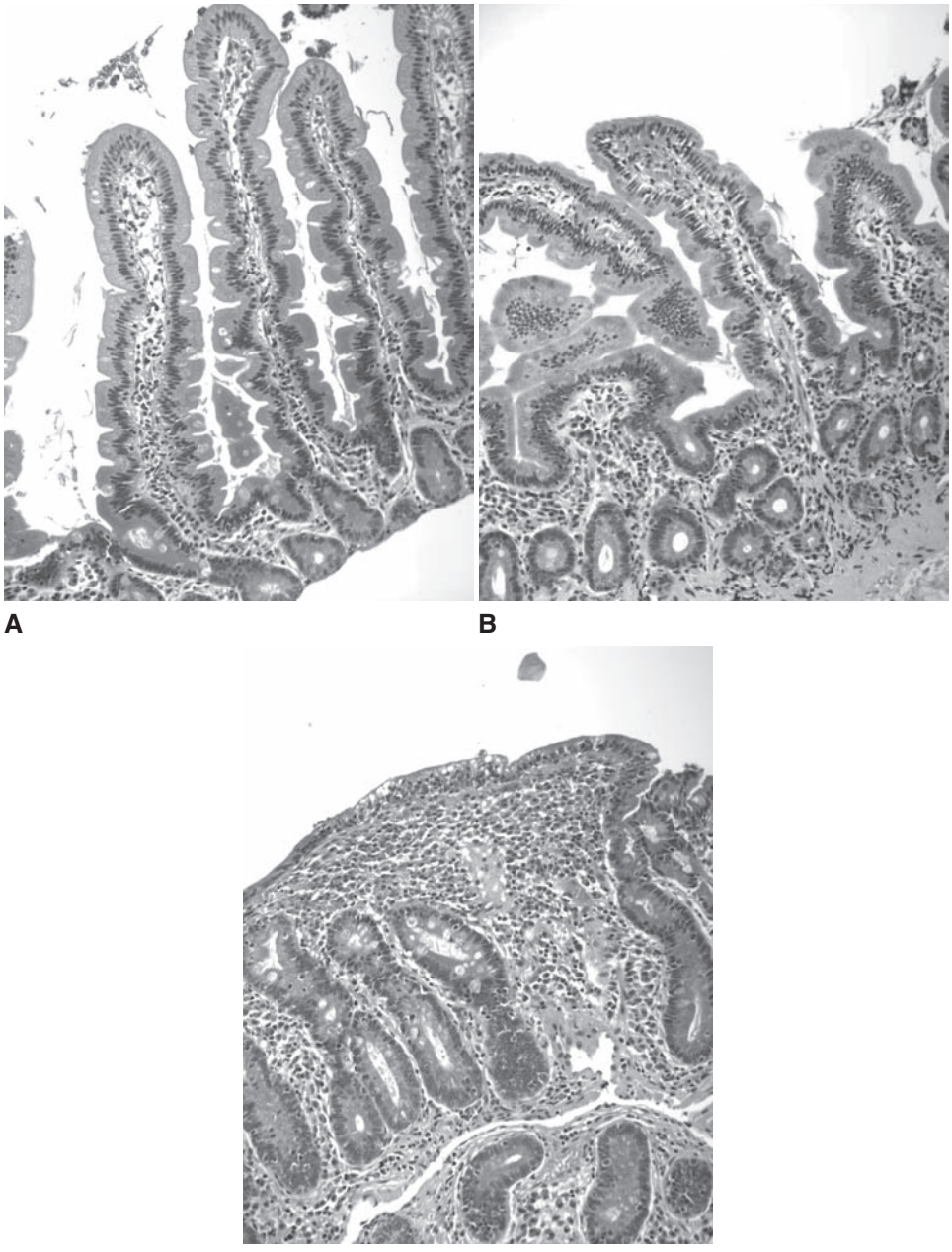


FIGURE 51.1 Histology of small intestinal biopsies. *A*, Normal intestine with long, slim villi and discrete crypts. *B*, Celiac disease Marsh 1 lesion (often referred to as an infiltrative lesion). The epithelium is seeded by intraepithelial lymphocytes (IELs) but the villi are not significantly shortened and there is little crypt hyperplasia. The patient had a positive endomysium test and clinical celiac disease. *C*, Celiac disease Marsh 3C lesion. No villi are seen and there is predominant crypt hyperplasia. Note the marked increase of intraepithelial lymphocytes. The different stages of villous structure that define the Marsh 2, 3A (partial villous atrophy), and 3B (subtotal villous atrophy) lesions are not shown. See color plate section.

Courtesy of I. N. Farstad, Rikshospitalet.

even after the age of 60 years. Many of these cases may have had undetected celiac disease in childhood, whereas in others the disease appears to have started later. The natural history and timing of conversion to seropositivity and/or mucosal inflammation are not fully understood.

Until recently, celiac disease was considered a fairly rare disorder, except in some European countries. Recent serologic screening studies have, however, shown a uniform prevalence throughout Europe of around 1 in 130 to 1 in 300 in the USA (Fasano et al., 2003). Even higher prevalences

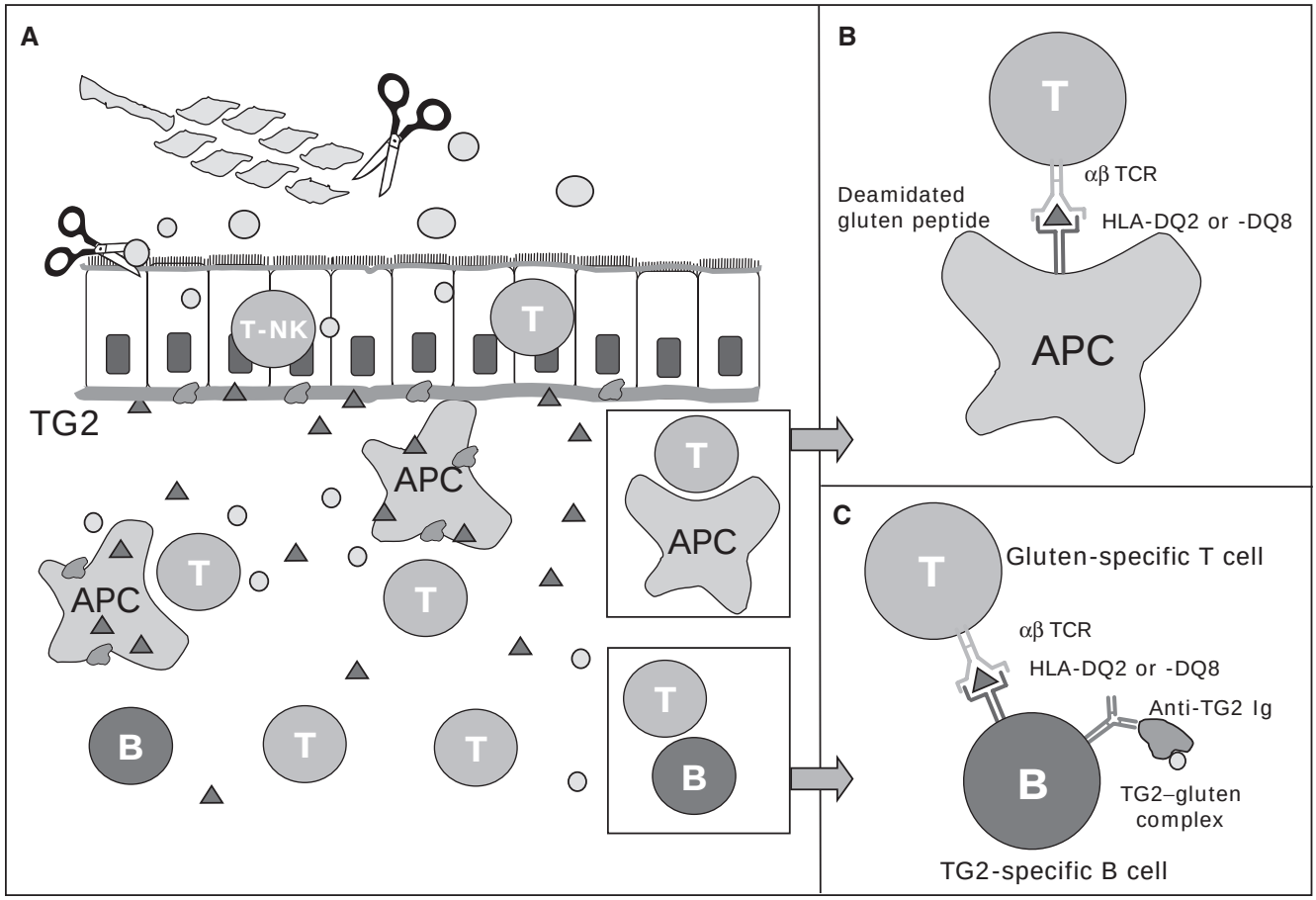


FIGURE 51.2 The celiac small intestinal lesion. *A*, Depiction of the intestinal mucosa with emphasis on the factors taking part in the development and control of celiac disease. The parts of the gluten proteins that are resistant to processing by luminal and brush border enzymes will survive digestion, and can be transported across the epithelial barrier as polypeptides. Gluten peptides are deamidated by tissue transglutaminase (TG2), which, in the intestinal mucosa, is mainly located extracellularly in the subepithelial region, but is also found in the brush border. TG2 may also be expressed by antigen-presenting cells (APCs) like macrophages and dendritic cells. $CD4^+$ T cells in the lamina propria recognize predominantly deamidated gluten peptides, presented by HLA-DQ2 or -DQ8 molecules on the cell surface of the APC. *B*, HLA-DQ2 and -DQ8 molecules have preference for binding peptides with negatively-charged amino acids (DQ2 in positions P4, P6, and P7; and DQ8 in positions P1 and P9) and thereby bind gluten peptides deamidated by TG2 (illustrated by conversion of small circles to triangles) with increased affinities. *C*, Model of how gluten-reactive T cells control the formation of antibodies to TG2 with intramolecular help. During the deamidation reaction, gluten peptides and TG2 form enzyme–substrate intermediates that are fairly stable (thiolester linkage). Such complexes of gluten and TG2 bound by surface immunoglobulin (Ig) of TG2-specific B cells will be endocytosed, and deamidated gluten peptides will be released for binding to DQ2 or DQ8 molecules. After transport to the cell surface of the HLA molecules with bound peptides, gluten-reactive T cells can recognize the deamidated gluten peptides and thereby provide T-cell help to the TG2-specific B cell. TCR, T-cell receptor.

(1 in 100) have been found in Finnish and UK schoolchildren (Mäki et al., 2003; Bingley et al., 2004) and among 45–76-year olds in the UK (West et al., 2003).

Between 1985 and 1995 there was an epidemic of celiac disease in Sweden among children younger than 2 years with a three-fold increase in incidence (Ivarsson et al., 2000). The sharp rise and subsequent abrupt decrease in incidence was likely related to changes in infant feeding habits, including the amount of gluten given, the age at introduction of gluten to the diet, and whether or not breastfeeding was ongoing when gluten was introduced. Notably, the risk for celiac disease was reduced in those children who were breastfed at the time of gluten introduction (Ivarsson et al., 2002).

AUTOIMMUNE FEATURES

Autoantibodies

Untreated celiac disease patients (on a wheat-containing diet) usually have increased levels of antibodies against wheat gluten, several other food antigens, and autoantigens present in the mucosa. The autoantibodies in celiac disease are primarily directed against the Ca^{2+}-activated (extracellular) form of the enzyme TG2 (Dieterich et al., 1997), although antibodies to calreticulin and actin are also found (Clemente et al., 2000; Sanchez et al., 2000, Granito et al., 2004). The antibodies to TG2 are both of the IgA and IgG isotypes, but the IgA antibodies, which are primarily produced in the intestinal mucosa (Marzari et al., 2001), demonstrate the highest disease specificity.

It is uncertain whether autoantibodies play a role in the pathogenesis of celiac disease. A weak inhibitory effect of the autoantibodies on certain TG2-catalyzed reactions has been reported (Esposito et al., 2002; Dieterich et al., 2003). This may be relevant to lesion formation. TG2 is involved in the formation of active transforming growth factor (TGF)-β by cross-linking of the TGF-β-binding protein (Nunes et al., 1997). Indirect inhibition of TGF-β activation may have broad effects, including dysregulation of the enterocytes and immune cells. Outside of its enzymatic activity, TG2 is involved in the attachment and motility of fibroblasts and monocytes via interactions with integrins and fibronectin (Akimov and Belkin, 2001; Balklava et al., 2002). Hence, the celiac villous atrophy could be caused by TG2 autoantibodies disturbing the migration of fibroblasts and epithelial cells from the crypts to the tips of the villi (Halttunen and Mäki, 1999). Autoantibodies to TG2 may also be involved in the extra-intestinal manifestations of celiac disease, although the mechanisms are not understood. One interesting observation comes from celiac patients with dermatitis herpetiformis. It appears that these patients, in addition to their anti-TG2 antibodies, have antibodies that target TG3, a transglutaminase that is uniquely found in the dermal papillae of dermatitis herpetiformis patients (Sardy et al., 2002).

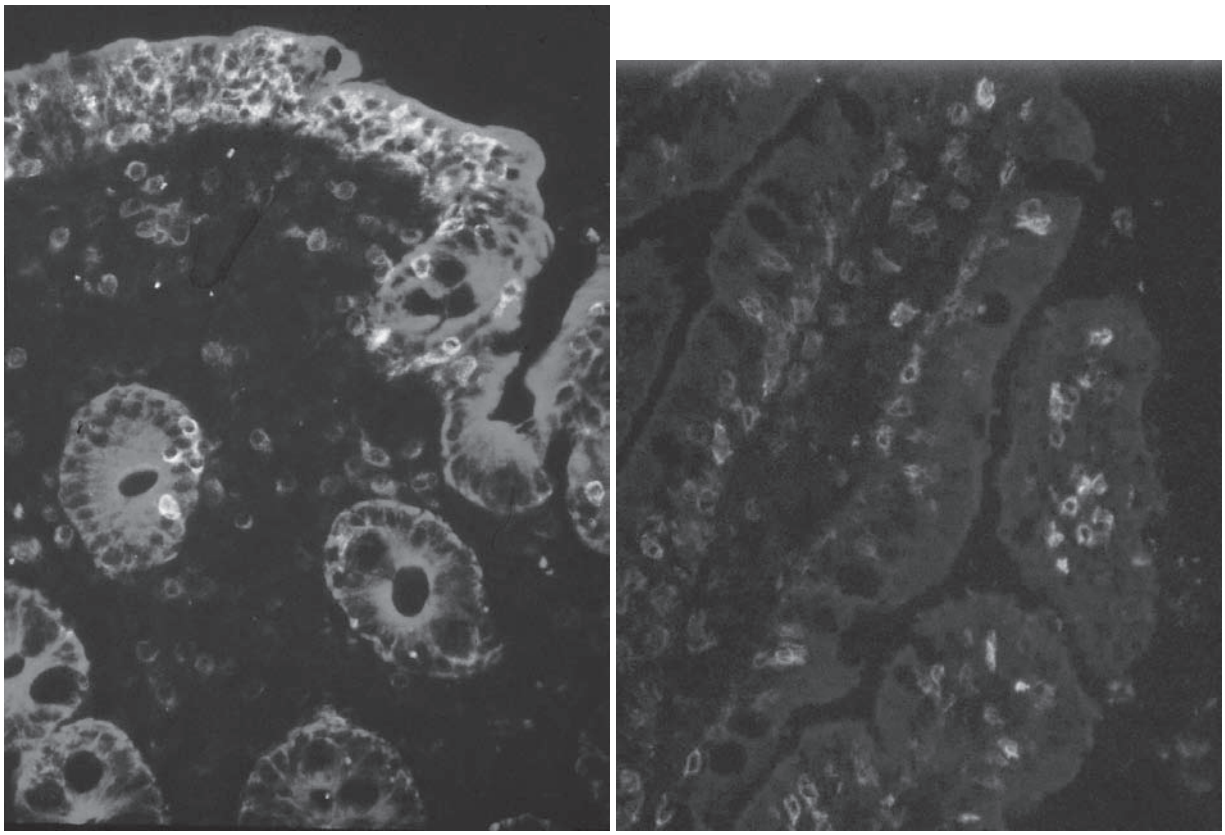
The mechanism underlying the formation of the autoantibodies in celiac disease is not understood. The production of anti-TG2 IgA antibodies is likely to be dependent on cognate T-cell help to facilitate isotype switching of autoreactive B cells (Sollid et al., 1997). Autoreactive T cells specific for TG2 could provide the necessary help for B-cell production of anti-TG2 IgA, but since TG2 is expressed in the thymus such cells are unlikely to survive thymic selection. An alternative explanation could be that the complexes of gluten and TG2, likely in the form of thiolester-linked enzyme substrate intermediates (Fleckenstein et al., 2004), permit the gluten-reactive T cells to provide help to the TG2-specific B cells by a mechanism of intramolecular help (see Figure 51.2C). This model can explain why the serum TG2 antibodies in celiac disease disappear when patients are put on a gluten-free diet. When the gluten goes, so does the T-cell help needed for the B cells to switch isotype and differentiate into plasma cells (Sollid et al., 1997).

Autoreactive Intra-epithelial Lymphocytes

Untreated celiac disease is characterized by an increased density of proliferating T-cell receptor (TCR) $\alpha\beta^+$ $CD8^+CD4^-$ and TCR $\gamma\delta^+$ $CD8^-CD4^-$ cells in the villous epithelium. All these IELs express the epithelial homing marker CD103 (integrin $\alpha_E\beta_7$) (Jabri et al., 2000). In contrast to the TCR $\alpha\beta^+$ $CD8^+$ IELs that return to normal when gluten is removed from the diet, the TCR $\gamma\delta^+$ IELs remain at an elevated level. A typical immunohistochemistry slide demonstrating some of these TCR subsets is shown in Figure 51.3. Many of the IELs coexpress innate [natural killer (NK) cell] receptors recognizing nonclassical HLA molecules. IELs expressing CD94/NKG2, a receptor for HLA-E, are increased in active celiac disease (Jabri et al., 2000), and many IELs express the NK receptor NKG2D (Roberts et al., 2001) and/or the Vγ1Vδ1 γδ TCR (Halstensen et al., 1989) (Figure 51.3B). Both these receptors recognize the non-classical MHC molecules expressed by stressed enterocytes (Groh et al., 1998; Bauer et al., 1999). The innate immune system has developed mechanisms to eliminate stressed cells (Gleimer and Parham, 2003). Killing of MIC-expressing stressed enterocytes by NKG2D-expressing IELs or by Vγ1Vδ1 γδ T cells may be an important autoimmune component of celiac disease. The expansion and activation of IELs in celiac disease seems to be driven by interleukin (IL)-15 (Jabri et al., 2000; Mention et al., 2003), but the mechanism leading to this increased expression is poorly understood.

GENETIC FEATURES

The high prevalence of celiac disease (10%) among first-degree relatives indicates that the susceptibility to this disease is strongly influenced by inherited factors (Ellis, 1981). Familial clustering is stronger in celiac disease than in most other chronic inflammatory diseases with a multifactorial etiology (Risch, 1987). The strong genetic influence in celiac disease is further supported by a very high concordance rate (around 75%) in monozygotic twins (Greco et al., 2002). Both HLA and non-HLA genes contribute to the genetic predisposition, and assuming a multiplicative model of disease genetics, it has been estimated that the overall importance of non-HLA genes is greater than that of HLA genes (Petronzelli et al., 1997; Risch, 1987). This estimation is, however, uncertain as increased sharing of environmental factors within families may lead



A B

FIGURE 51.3 Typical features of celiac disease lesions as illustrated by immunohistochemistry. *A*, Marsh 3C lesion. $CD3^+$ T cells are stained green and the epithelium is stained red. The striking and typical finding is the heavy infiltration of T cells within the epithelium. *B*, Marsh 1 lesion. $CD3^+$ T cells are stained green, T-cell receptor (TCR) $\gamma\delta^+$ cells are stained red and the epithelium is stained blue. There is a high proportion of intraepithelial lymphocytes (IELs) expressing both CD3 and TCR $\gamma\delta$ (seen as yellow cells), but the majority of $CD3^+$ IELs is TCR $\gamma\delta^-$ (i.e., TCR $\alpha\beta^+$). The significance of the infiltration of T cells in the lamina propria and the epithelium is discussed in the text. See color plate section.

Courtesy of I. N. Farstad and Helge Scott, Rikshospitalet.

to an overestimation of the role of the non-HLA linked genes.

HLA Genes

The current data suggest that the overall susceptibility to develop celiac disease is associated with two conventional DQ molecules: primarily to DQ(α1*05,β1*02) (=DQ2) and less pronounced to DQ(α1*03,β1*0302) (=DQ8). DQ molecules bind peptides and present these to $CD4^+$ helper T cells (Th) carrying the $\alpha\beta$ TCR. This genetic evidence points towards a central role for $CD4^+$ T-cells in controlling disease development.

Most celiac disease patients carry the DR3–DQ2 haplotype (the DRB1*0301–DQA1*0501–DQB1*0201 haplotype), or are DR5–DQ7/DR7–DQ2 heterozygotes (i.e., they carry the DRB1*11/12–DQA1*0505–DQB1*0301/DRB1*07–DQA1*0201–DQB1*0202 haplotypes) (Keuning et al., 1976; Ek et al., 1978; Mearin et al., 1983; Tosi et al., 1983; Trabace et al., 1984). Celiac disease patients with these DR–DQ haplotype combinations thus share the same functional DQ molecule on the cell surface, encoded by genes carried in the cis (e.g., DQA1*05 and DQB1*02 carried on the same haplotype) or trans position (e.g., DQA1*05 carried on a different haplotype from DQB1*02) (Figure 51.4) (Sollid et al., 1989). Celiac disease patients who are DQA1*05 and DQB1*02 negative frequently carry the DRB1*04–DQA1*03–DQB1*0302 haplotype (i.e., DR4–DQ8 haplotype). Taken together, the genetic and functional data favor DQ8 as the primary disease-susceptibility determinant in these patients. The very few remaining celiac disease patients who are neither DQ2 (DQA1*05/DQB1*02) nor DQ8 (DQA1*03/DQB1*0302) carry either the α or the β chain of the DQ2 heterodimer (i.e., DQA1*05 or DQB1*02) (Karell et al., 2003).

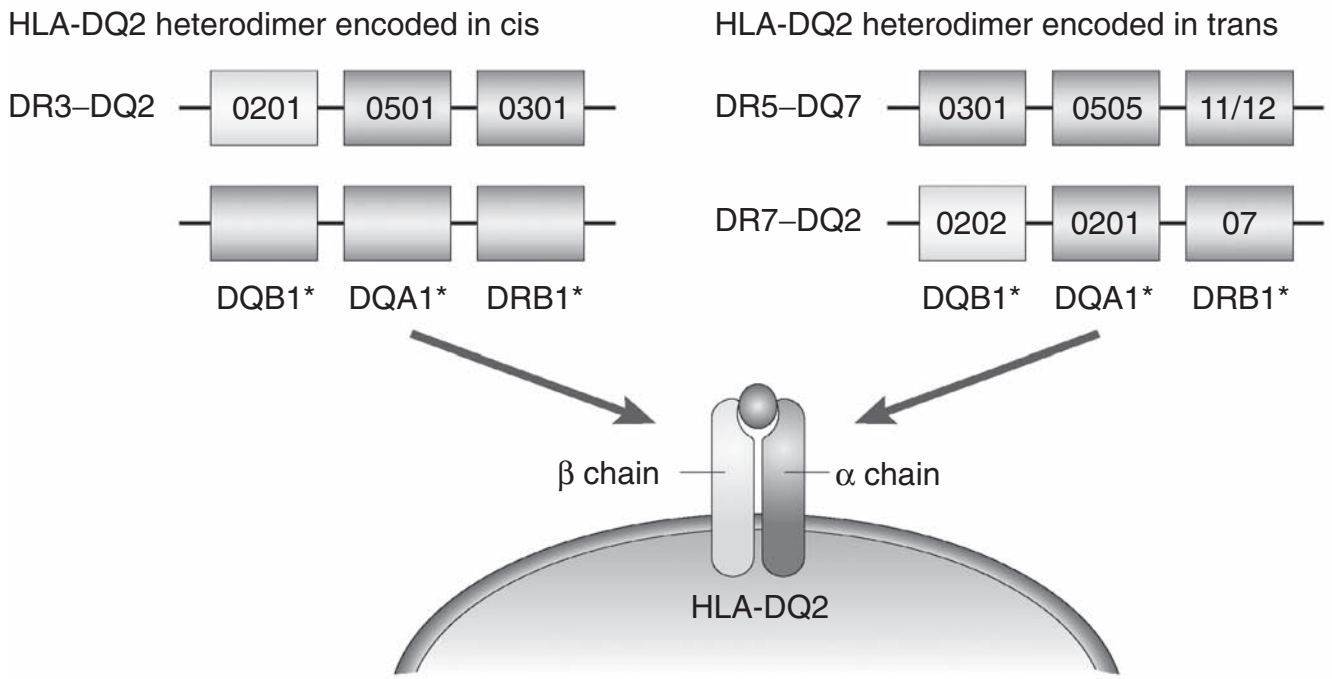


FIGURE 51.4 HLA association in celiac disease. The majority of celiac disease patients expresses a particular HLA-DQ2 (DQA1*05/DQB1*02) heterodimer. This HLA molecule is either encoded in the cis position in individuals who are DR3-DQ2, or in the trans position in individuals who are DR5–DQ7/DR7–DQ2 heterozygous. The polypeptides encoded by DQA1*0501 and DQA1*0505 differ by one residue in the leader peptide, whereas those encoded by DQB1*0201 and DQB1*0202 differ by one residue in the membrane proximal domain. It is unlikely that these substitutions have functional consequences.

Courtesy of Nature Publishing Group.

Non-HLA Genes

Much less is known about the non-HLA genes in this disorder. There are several reports that imply involvement of the gene for the negative costimulatory molecule CTLA4, or a neighboring gene (such as those encoding CD28 or ICOS). However, the overall effect of this gene is small (Holopainen et al., 1999; Naluai et al., 2000; King et al., 2002; Popat et al., 2002).

Several genome searches for risk factors have been performed in celiac disease (Houlston et al., 1997; Greco et al., 1998; 2001; King et al., 2000; Holopainen et al., 2001; Naluai et al., 2001; Liu et al., 2002; van Belzen et al., 2003) With the exception of the HLA genes, there is relatively little consensus between the results, which most likely indicates that each of the non-HLA genes has a relatively modest effect. Gene–gene interactions and disease heterogeneity in the outbred human population could also produce such a picture. The region that has most consistently been linked to celiac disease is on the long arm of chromosome 5 (5q31–33) (Greco et al., 2001; Naluai et al., 2001; Liu et al., 2002). In a meta-analysis of data from several European populations, this region reached genome-wide significance, leaving little doubt that a susceptibility gene exists in this region (Babron et al., 2003). There is also accumulating evidence for a susceptibility factor on chromosome 11q (Greco et al., 1998) and chromosome 19p13 (van Belzen et al., 2003).

ENVIRONMENTAL INFLUENCES

Wheat Gluten and Similar Proteins of Barley, Rye, and Oats

Due to their high content of proline and glutamine residues, the proteins of wheat gluten are collectively referred to as prolamines. They are usually classified into α-, γ- and ω-gliadins and high and low molecular weight (HMW and LMW) glutenins (Shewry et al., 2003). The number of unique prolamine proteins is enormous due to the hexaploid nature of the wheat genome, multiple encoding loci, and allelic variation. A single wheat cultivar may express more than 100 different gliadin proteins (Anderson, 2001). The high content of proline residues makes the prolamines particularly resistant to gastrointestinal digestion. This has important implications for their immunogenicity for T cells.

Clinical observations suggest that the prolamins of barley and rye are also toxic for celiac disease patients (Farrell and

Kelly, 2002). Given the similarities between the prolamine genes of barley, rye, and wheat this is to be expected (Vader et al., 2003). With the oat prolamins the situation is more complex. Several feeding studies have suggested that oats are safe for celiac disease patients (Janatuinen et al., 1995; Srinivasan et al., 1996; Hoffenberg et al., 2000; Janatuinen et al., 2002; Størsrud et al., 2003), but it is now clear that some celiac patients are oat intolerant (Lundin et al., 2003). Interestingly, the celiac lesions of these patients harbor T cells specific for modified peptides derived from oat prolamins (Arentz-Hansen et al., 2004). These oat epitopes closely resemble gliadin T-cell epitopes (see below).

Other Environmental Factors

A possible role of infections in the development of celiac disease was indicated in a recent report from Sweden. In children younger than 2 years, a positive correlation was found between celiac disease risk and being born during the summer, which may be related to the fact that children born in the summer are first exposed to dietary gluten during the winter when infections are more prevalent (Ivarsson et al., 2003a). In addition, case–control data were presented which indicated that celiac patients experience 3 or more episodes of infection more than referents. Adenovirus 12 was proposed as a candidate factor because one of its proteins displays partial linear homology over 12 amino acids with an α-gliadin (Kagnoff et al., 1984). Subsequent epidemiologic studies have, however, not confirmed this hypothesis.

ANIMAL MODELS

Different approaches have been taken to establish an animal model of celiac disease, but none have demonstrated mucosal immunopathology and genetic features mimicking the human disorder. So far, it has not been possible to induce gluten-sensitive enteropathy in mice made transgenic for the HLA-DQ2 (Chen et al., 2002; 2003) or HLA-DQ8 (Black et al., 2002). Possibly, the failure of gluten to induce enteropathy in mice may relate to nonpermissive background genes of the mouse strains used so far. Notably, a fraction of HLA-DQ8 transgenic mice on a nonobese diabetic (NOD) background develops a dermatitis herpetiformis-like skin disorder after systemic immunization with gluten, but no apparent enteropathy (Marietta et al., 2004). Food-induced enteropathy can, however, be induced also in rodents, as demonstrated in hen egg lysozyme TCR transgenic mice orally fed with hen egg lysozyme and a Cox-2 inhibitor (Newberry et al., 1999). Of interest for celiac disease, transgenic mice overexpressing human IL-15 in intestinal epithelial cells develop intestinal inflammation confined to the proximal small intestine with reduced villus length, marked infiltration of lymphocytes, and vacuolar degeneration of the villus epithelium (Ohta et al., 2002).

PATHOGENETIC MECHANISMS

Effects of Gluten Challenge on the Intestinal Mucosa

Co-culturing celiac biopsies with gluten proteins has, over the past 30 years, provided useful insights into the pathogenesis of celiac disease. The first of these organ culture studies demonstrated that the small intestinal epithelium of untreated celiac patients improved in the absence of gluten (Townley et al., 1973; Browning and Trier, 1969). Later, the model was used to link gluten exposure directly to T-cell activation when Halstensen et al. (1993) showed that gluten challenge of biopsies from treated celiac patients induced CD25 (IL-2 receptor α chain) expression on lamina propria T cells (Figure 51.5). One advantage of the organ culture system is that it has allowed semikinetic analyses on the effects of gluten. These studies have suggested two different waves of immune activation: a rapid wave that occurs within hours, including overexpression of HLA-DR molecules on enterocytes, and a later wave dominated by activation of resident T cells (Maiuri et al., 1996). Peptide 31–43 of a particular α-gliadin, A-gliadin, which prevents the increase in enterocyte height seen during culture of intestinal biopsies from untreated celiac disease patients (De Ritis et al., 1988), was recently demonstrated to rapidly activate factors in the innate immune system in biopsies of treated celiac disease patients (Maiuri et al., 2003).

Studies of biopsies taken from patients following oral challenge with gluten proteins have also provided important results. Marsh (Marsh, 1992; Marsh and Crowe, 1995) orally challenged a large number of well-treated patients with gluten and sampled biopsies at several time points thereafter. Morphologic changes were induced in all patients, and the mucosal response was directly related to the amount of gluten given. At higher doses, mucosal damage was severe but the mucosa recovered rapidly (within 24–72 h).

Gluten-Reactive $CD4^+$ T Cells

T cells specific for various gluten proteins can be isolated from the small intestinal biopsies of the majority of celiac disease patients (Lundin et al., 1993; Molberg et al., 1997; Troncone et al., 1998; van de Wal et al., 1998b; Ellis et al., 2003), but not from biopsies obtained from disease controls (Molberg et al., 1997). In contrast, both patients and many control subjects have gliadin-reactive T cells in their peripheral blood. These T cells use many different HLA molecules for antigen presentation (DR, DQ, and DP) (Gjertsen et al., 1994; Jensen et al., 1995), and their reactivity is not enhanced by deamidation of the gliadin (Molberg et al., 1998). This implies that many individuals can be immunologically sensitized to gluten without having small intestinal

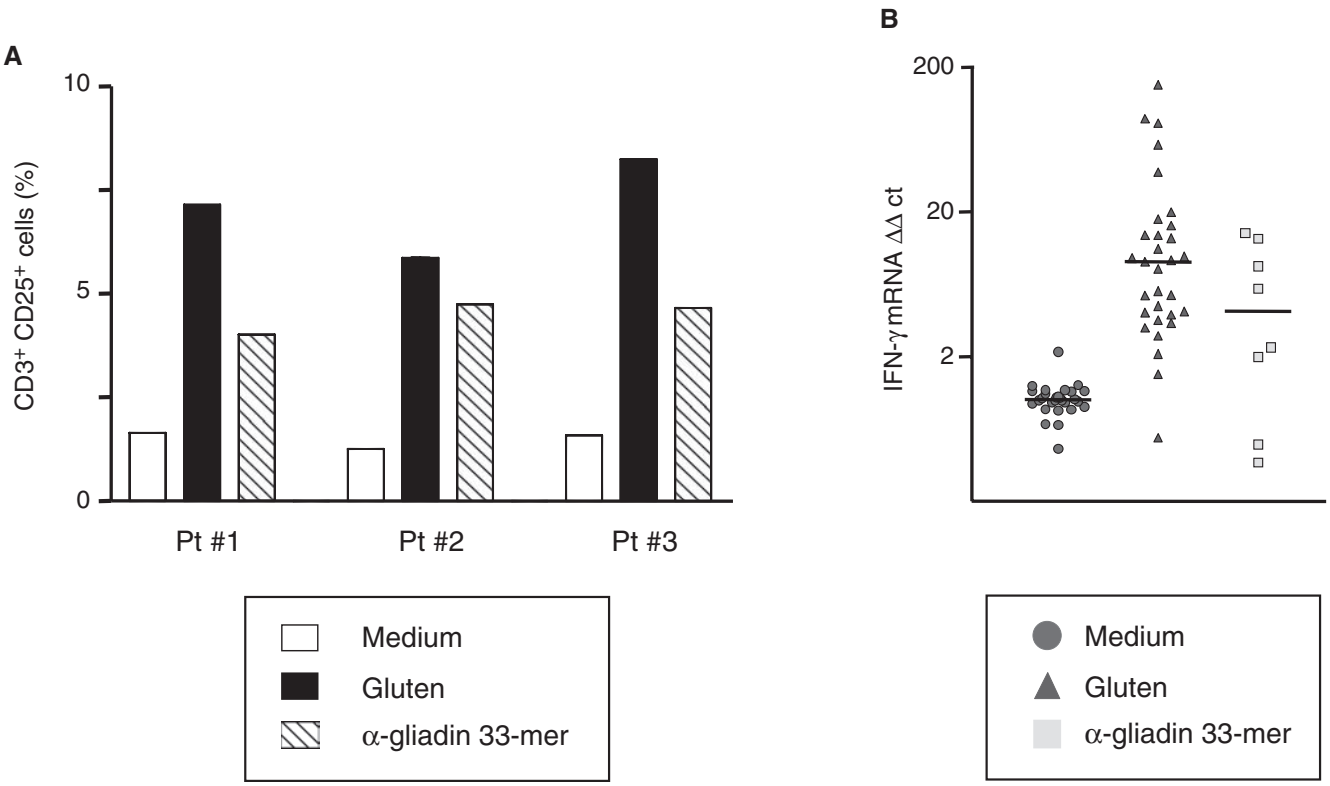


FIGURE 51.5 Gluten-induced activation of T cells within intestinal biopsies of celiac disease patients. *A*, T-cell activation, as evaluated by induction of CD25 expression, following a 24-h *ex-vivo* stimulation of celiac biopsies with a chymotryptic digest of bread wheat gluten with the immunodominant 33-mer peptide fragment of the α-2 gliadin. The figure shows data from three treated adult celiac disease patients with good T-cell reactivity (S. Tollefsen et al., unpublished data). *B*, Immune activation, as evaluated by induction of IFN-γ mRNA, following a 24-h *ex-vivo* stimulation of celiac biopsies with the same antigens as in *A*. Each point represents one individual biopsy specimen. All the biopsies were collected from treated celiac disease patients (S. Tollefsen et al., unpublished data).

pathology. The gluten-specific T cells derived from the intestinal celiac lesions display a remarkable feature; they are invariably restricted by the disease-associated DQ2 or DQ8 molecules (see Figure 51.2A) (Lundin et al., 1993; 1994). Most gluten proteins, including α-gliadins, γ-gliadins, and LMW and HMW glutenins appear to be recognized by these DQ2- and DQ8-restricted T cells, although with variable frequency (Lundin et al., 1997; Sjöström et al., 1998; van de Wal et al., 1999; Anderson et al., 2000; Arentz-Hansen et al., 2000; 2002; Vader et al., 2002b; Ellis et al., 2003; Molberg et al., 2003). The intestinal T-cell responses to gluten are thus characterized by their heterogeneity. The existence of multiple epitopes is related to the enormous heterogeneity of gluten proteins (see above).

Role of Tissue Transglutaminase

The role of the TG2 enzyme in the pathogenesis of celiac disease is not completely understood, but clearly encompasses more than simply being the target of the autoantibodies in the disease. There are high levels of active TG2 in the inflamed lesions of untreated celiac disease patients (Bruce et al., 1985) and the gluten proteins are excellent substrates for TG2 (Larre et al., 1993). Most importantly, however, the T cells within the celiac lesions predominantly recognize gluten peptides modified by TG2 (van de Wal et al., 1998a; Molberg et al., 1998; 2001; Arentz-Hansen et al., 2000; Vader et al., 2002b).

TG2 catalyzes a post-translational transamidation or deamidation of specific glutamine residues within its substrate proteins (Folk, 1983; Lorand and Graham, 2003). In the transamidation reaction, the glutamine becomes cross-linked to a protein-bound lysine or a polyamine, whereas the deamidation reaction results in conversion of the glutamine to glutamic acid. The enzyme displays regional selectivity for particular glutamine residues in the gliadin peptides, and the spacing between the targeted glutamine and proline residues seems particularly important for the specificity. Good targets are QXP and QXXF (Y, W, M, L, I, V), whereas a Q followed directly by a P will not be targeted (Fleckenstein et al., 2002; Piper et al., 2002; Vader et al., 2002a). The enzyme is thus involved in the selection of

DQ2-restricted T-cell epitopes. The fact that the glutamic acid residues created by TG2-mediated deamidation play a crucial role in DQ2/DQ8 binding and T-cell recognition provides indirect evidence that TG2 is involved in the mucosal deamidation of gluten peptides (see Figures 51.2A and B). Direct evidence for the *in situ* deamidation of gluten proteins by TG2 was provided by a study of celiac biopsies challenged with digests of gliadin (Molberg et al., 2001). The T-cell lines derived from these biopsies displayed only weak reactivity to gliadins, but responded efficiently to the TG2-treated variants of the same proteins.

T-Cell Epitopes of Gluten Proteins

There exist multiple T-cell epitopes in gluten proteins (Table 51.1), and T cells with several distinct specificities can be isolated from small intestinal biopsies of a single patient. Notwithstanding, there is a hierarchy within the epitopes that have been characterized. For instance, among the α-gliadins there are epitopes that are immunodominant and are recognized by T-cells from almost all patients (Arentz-Hansen et al., 2000), whereas reactivity to other epitopes can only be found in a minority of patients. T cells specific for these α-gliadin epitopes become detectable in the peripheral blood of treated celiac disease patients following an *in vivo* challenge with gluten (Anderson et al., 2000). Interestingly, a total of 6 copies of 3 of the immunodominant epitopes of α-gliadin (DQ2-α-I, DQ2-α-II, and DQ2-α-III) are expressed in some gliadins within a 33-mer fragment that is resistant to degradation by all gastric, pancreatic, and intestinal brush border membrane proteases (Shan et al., 2002). This 33-mer fragment is superstimulatory to T cells. We believe that a final and unambiguous definition of all DQ2- and DQ8-restricted gluten epitopes recognized by gut T cells will be difficult. While many of the epitopes are dissimilar when aligned according to their DQ2-binding motif, some have high degrees of similarity. In fact, T-cell clones exist that cross-react with similar sequences of α- and γ-gliadins, and with sequences of LMW glutenins and γ-gliadins (Vader et al., 2002b).

Recent experiments using organ culture assays have demonstrated that the gluten epitopes recognized by the intestinal T-cells from DQ2- and DQ8-positive patients induce immune activation *in situ* (Martucci et al., 2003; Mazzarella et al., 2003). Recently, *in vivo* instillation of a peptide containing the DQ2-α-I and DQ2-α-II epitopes has been shown to induce morphologic changes in the intestinal mucosa (Fraser et al., 2003). These changes were more pronounced than those seen in a previous *in vivo* challenge study that used a peptide derived from a distinct α-gliadin (A-gliadin 31–49) (Sturgess et al., 1994).

Despite the absence of a single pathogenic motif, the gluten epitopes recognized by gut T cells are generally very rich in proline and glutamine residues (see Table 51.1). In

TABLE 51.1 HLA-DQ2 restricted T-cell epitopes of gluten proteins

T-cell epitope	Epitope defined from	Native sequence of 9-mer core region of epitope[§]	
α-I (Var1)	α-Gliadin, recombinant*	PFPQPQLPY	Crystal[¶]
α-III (Var2 of α-I)	α-Gliadin, recombinant*	PYPQPQLPY	TCC[¶]
α-II	α-Gliadin, recombinant*	PYPQPQLPY	DQ-TCC**
Glia-α20	α-Gliadin, peptide[†]	FRPQQPYPQ	TCC[¶]
γ-I	γ-Gliadin, natural[‡]	PQQSFPQQQ	DQ-TCC**
γ-II (Glia-γ30)	γ-Gliadin, natural[‡]	IQPQQPAQL	TCC[¶]
γ-III	γ-Gliadin, recombinant*	QQPQQPYPQ	TCC[¶]
γ-IV	γ-Gliadin, recombinant*	SQPQQQFPQ	TCC[¶]
γ-VI	γ-Gliadin, recombinant*	QQPFPQQPQ	TCC[¶]
Glia-γ2	γ-Gliadin, unknown	PYPQQPQQP	
Glu-5 (Var1)	Not defined, natural[‡]	QIPQQPQQF	TCC[¶]
Glu-5 (Var2)	Not defined, natural[‡]	QLPQQPQQF	TCC[¶]
Glt-17 (Var1)	LMW glutenin, peptide[†]	PFSQQQQPV	TCC[¶]
Glt-17 (Var2)	LMW glutenin, peptide[†]	PFSQQQQPI	TCC[¶]

LMW, low molecular weight.

*Identified from fragments of recombinant gliadin protein digests.

[†]Identified from panels of synthetic peptides.

[‡]Identified from fragments of natural gliadin or gluten protein digests.

[§]Core 9-mer region interacting with DQ2 is shown. T-cells usually require additional flanking residues for recognition. Glutamine residues deamidated by tissue transglutaminase are underlined.

[¶]Defined by the crystal structure of the peptide–DQ2 complex.

[¶]Register defined from minimal fragment recognized by specific T-cell clones (TCCs) and binding motif of DQ2.

**Register defined from minimal fragment recognized by specific T-cell clones (TCCs) and DQ2-binding assay.

several of the epitopes, proline residues are found in four of the nine residues in the core region (Sollid, 2000). The key role of prolines in the epitopes is underscored by the observation that DQ2-restricted T-cell epitopes in α- and γ-gliadins often cluster in regions with the highest density of proline residues (Arentz-Hansen et al., 2002). This clustering probably reflects the resistance to gastrointestinal digestion by proline, the importance of proline for the fine specificity of TG2, and the increased immunogenicity imposed by multivalency.

Presentation of Gluten Peptides by DQ2 or DQ8

Stable binding of peptides in the membrane distal groove of the HLA molecules is achieved by multiple hydrogen bonds between the amino acids of HLA and the peptide main chain atoms. Amino acid residues that differ between the polymorphic variants of HLA molecules contribute to the formation of specific binding pockets that can accommodate side chains of peptide amino acids, so-called anchor residues. The peptide interactions with HLA mainly take place in a core region of nine residues. Within this region, side chains of peptide amino acids in positions P1, P4, P6, P7, and P9 dock into pockets of the class II binding site. DQ2 has a unique preference for binding peptides with negatively charged side chains at the three middle anchor positions (Johansen et al., 1996; van de Wal et al., 1996; 1997; Vartdal et al., 1996). The recently solved X-ray crystal structure of DQ2 complexed with the DQ2-α-I epitope (QLQPFPQPELPY) provides a clue to explaining why DQ2 is predisposing to celiac disease by presenting gluten peptides (Figure 51.6) (Kim et al., 2004). The glutamic acid at P6, which is formed by tissue transglutaminase catalyzed deamidation, is an important anchor residue as it participates in an extensive hydrogen-bonding network involving Lys-β71 of DQ2. It is likely that Lys-β71 is important for the binding preference of negatively-charged residues at the P4 and P7 pockets in other peptides by electrostatic interactions (Kim et al., 2004). The α-I peptide–DQ2 complex retains critical hydrogen bonds between the HLA molecule and the peptide backbone despite the presence of many proline residues in the peptide that are unable to participate in amide-mediated hydrogen bonds. The positioning of proline residues so that they do not interfere with backbone hydrogen bonding results in a reduction of available HLA binding registers for gluten peptides, and impairs the likelihood of establishing favorable side chain interactions. The HLA association in celiac disease can thus be explained by a superior ability of DQ2 (and DQ8) to bind the biased repertoire of proline-rich gluten peptides that have survived gastrointestinal digestion and that have been deamidated by TG2. It is also known that MHC molecules are important for determining the repertoire of peripheral T cells during maturation in the thymus. A thymic effect of the same DQ molecules on the TCR repertoire selection is, however, not excluded by these results.

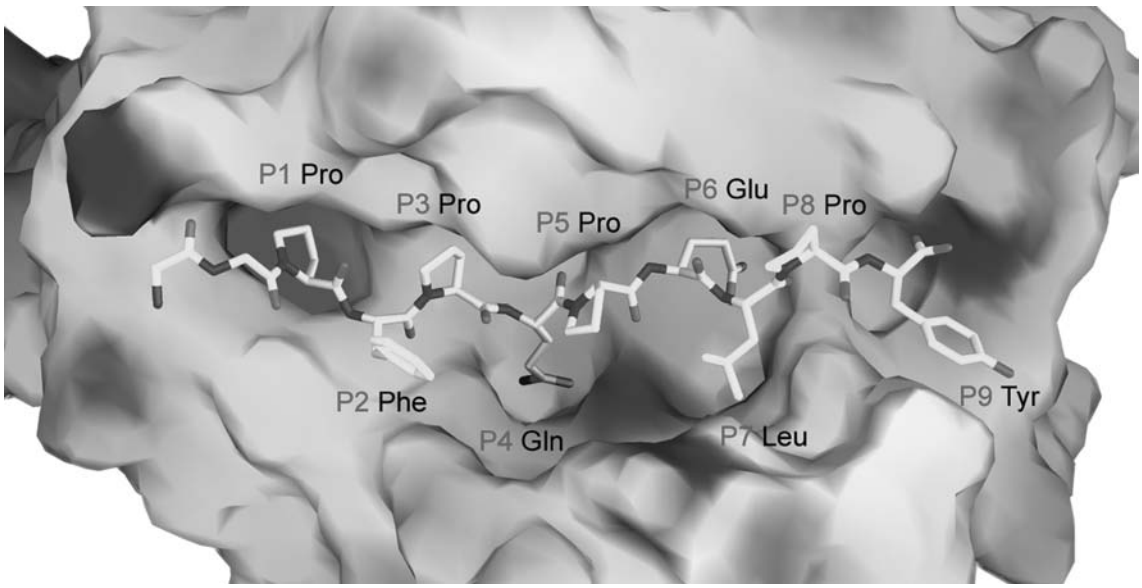


FIGURE 51.6 Three-dimensional structure of the binding site of HLA-DQ2 complexed with a deamidated gliadin peptide (DQ2-α-I epitope—carbon, yellow; nitrogen, blue; oxygen, red). The electrostatic potential surface of DQ2 is shown as red (negative) and blue (positive). Courtesy of the Proceedings of the National Academy of Sciences of the United States of America.

Musosal Antigen-Presenting Cells

The activation of gluten-reactive T-cells by stimulating biopsies with gluten implies that antigen-presenting cells (APCs) exist within the biopsy, but the nature of these cells has not been identified. Just beneath the epithelium in the normal mucosa is a high number of macrophage/dendritic-like cells that stain positive for CD68. It is conceivable that these cells are involved in sampling of luminal antigens. The expression of HLA class II, ICAM-1, and CD25 molecules is increased in these macrophage/dendritic-like cells, suggesting that they are activated in the disease state (Sturgess et al., 1990; Halstensen et al., 1993).

Effector Mechanisms Leading to Mucosal Alterations

The celiac lesion is characterized by increased enterocyte proliferation in the crypts, partly driven by keratinocyte growth factor (KGF) produced by stromal cells (Salvati et al., 2001) and apoptotic loss of surface epithelial cells (Moss et al., 1996; Maiuri et al., 2001). The apoptosis of the epithelium can be secondary to the mucosal inflammation and can involve cytotoxic IELs.

Despite the lack of detailed knowledge of the effector mechanisms involved in the creation of the celiac lesion, there is a large body of evidence that suggests that inflammatory cytokines, particularly interferon (IFN)-γ, are involved. Messenger RNA for IFN-γ is abundant in biopsies taken from untreated patients and is rapidly induced when biopsies from treated patients are challenged *ex vivo* with gluten (Nilsen et al., 1998) (see Figure 51.5B). IFN-γ is also produced by the intestinal, gluten-specific $CD4^+$ T cells (Nilsen et al., 1998), and by IELs (Forsberg et al., 2002; Olaussen et al., 2002). The induction of IFN-γ is probably mediated by IL-18 and IFN-α rather than IL-12 (Nilsen et al., 1998; Monteleone et al., 2001a; Salvati et al., 2002). Interestingly, there are several reports on the occurrence of clinical celiac disease in patients receiving IFN-α therapy for malignancy or viral hepatitis (Bardella et al., 1999; Cammarota et al., 2000; Monteleone et al., 2001a). IFN-α has also been shown to induce activation of Th1 cells in a fetal gut explant model of T-cell-driven enteropathy (Monteleone et al., 2001b).

IL-15 appears to be another important cytokine in celiac disease. In active celiac disease there is increased expression of IL-15, both in the lamina propria (Maiuri et al., 2000) and in the epithelium (Mention et al., 2003). Enterocyte-bound IL-15 can activate and expand IELs (Mention et al., 2003). In addition to the effect on IELs, IL-15 has been shown to induce epithelial CD95(Fas) expression promoting apoptosis of epithelial cells (Maiuri et al., 2000). The expression of IL-15 increases following *in vitro* gluten challenge of celiac biopsies, and it has been demonstrated that certain gluten peptides can directly activate the innate immune system, leading to IL-15 production (Maiuri et al., 2003). This innate effect of gluten is only seen in celiac disease patients, questioning whether some part of the adaptive immune system may be involved in the production of IL-15.

The activation of Th1 cells, which recognize gluten peptides presented by DQ2 or DQ8 molecules, can be considered to be a key event in the development of celiac disease, and this could explain the dominant genetic role of HLA. The products of the other predisposing genes may participate in pathway(s) that lead to lesion formation. The minor genetic effects of the non-HLA genes may indicate a lack of critical checkpoints along these pathways in susceptible individuals, or that there are several different pathways that lead to lesion formation. The role of the innate immune system and whether there are genetic polymorphisms in any innate immune genes that predispose to celiac disease development need to be studied further.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

Serologic Markers

Accumulating evidence suggests that more than 95% of untreated celiac disease patients have high titers of serum IgA antibodies directed against the human TG2 enzyme (Dieterich et al., 1998; Sulkanen et al., 1998; Sblattero et al., 2000; Hansson et al., 2002; Wong et al., 2002).The development of enzyme-linked immunosorbent assays (ELISAs) with human recombinant TG2 as antigen has allowed both the routine detection of anti-TG2 antibodies in clinical practice and large population-based screening for celiac disease. These ELISA tests are now rapidly replacing the laborious detection of anti-endomysium IgA antibodies (i.e., anti-TG2), which was based on immunofluoresence staining of endomysial TG2 in tissue sections. In addition to the anti-TG2 antibodies, untreated celiac disease patients also have increased titers of IgA antibodies against gluten and gliadin, but these antibodies are less disease specific.

Some celiac disease patients undoubtedly present with negative serologies (Dahele et al., 2001; Kaukinen et al., 2002b), and an intestinal biopsy should therefore always be taken when there is high clinical suspicion of celiac disease (Farrell and Kelly, 2002). Notably, false-positive anti-TG2 tests do also occur, particularly in patients with inflammatory bowel disease (Carroccio et al., 2002; Dahele et al., 2001). A recent report from Finland identified some children who were positive for anti-TG2 in archival serum samples but who later were seronegative (Mäki et al., 2003). It is not known if these children had transient celiac disease, but this is clearly a possibility. Anti-TG2 antibodies of the IgG

isotype are useful in evaluating patients with selective IgA deficiency, but should otherwise not be tested for (Collin, 1999; Murray, 1999; Fasano and Catassi, 2001).

Other Immunologic Markers

As almost all celiac disease patients carry DQ2 or DQ8, HLA typing can thus be used to exclude the diagnosis with high probability. However, as these HLA variants are also present in a large proportion of the healthy population, this test has low positive predictive value (Kaukinen et al., 2002a). Immunohistochemical staining of intestinal biopsies with antibodies against TCR γδ and other T-cell markers are of clinical benefit in selected cases (Jarvinen et al., 2003).

TREATMENT AND OUTCOME

The current treatment for celiac disease is a life-long gluten exclusion diet. The disease is largely a benign disorder, particularly in patients detected by screening (West et al., 2003). Although celiac disease patients overall have an increased relative risk for non-Hodgkin lymphoma of the gastrointestinal tract, the absolute risk is low and lower than previously anticipated (Askling et al., 2002; Catassi et al., 2002). A large Italian study it was found that the overall mortality rate in celiac disease patients was twice that in controls (Corrao et al., 2001). This increased mortality was accounted for by increased death rates in the first 3 years after diagnosis. The gluten-free diet has been considered to be protective against the development of malignancy, but this notion was not supported by a recent study from the US (Green et al., 2003).

The most frequent reason for no or incomplete clinical improvement is poor dietary compliance (Ciacci et al., 2002), but it is clear that some patients do have refractory disease that does not respond to an adequate diet. The clinical evaluation and treatment of such patients are difficult (Ryan and Kelleher, 2000). A majority of patients with refractory celiac disease have monoclonal expansions of IELs, which in some patients progress to overt enteropathy-associated T-cell lymphoma (Cellier et al., 2000).

Novel Therapeutic Options

Many patients cope easily with the gluten-free diet. Others find that the dietary restrictions are laborious and have negative impact on their quality of life. Better alternatives are thus called for. It is promising that the greater insight into the molecular mechanisms involved in the intestinal T-cell reactivity to gluten seems to be uncovering novel targets for therapy. There are already some attractive possibilities. The activation of $CD4^+$ gluten-reactive T cells is a critical checkpoint in the development of celiac disease, and interference with this step should be an effective way to control the disease. One possibility, which is basically an extension of today's treatment with a gluten-free diet, is to produce cereals with bread-making properties but that are devoid of T-cell epitopes, either through breeding programs or transgenic technology. Another possibility is enzyme supplementation with the aim of either destroying T-cell epitopes directly or facilitating their gastrointestinal proteolysis (Figure 51.7A) (Hausch et al., 2002; Shan et al., 2002). Prolylendopeptidases are particularly attractive enzymes as they target the proline-rich regions of gluten that harbor the T-cell epitopes. TG2 is a target for intervention because of the critical role it plays in generating gluten T-cell epitopes (Figure 51.7B). Analogs of gluten peptide-containing "warheads" that function as irreversible inhibitors of human TG2 have been designed (Hausch et al., 2003). One possible problem with this approach is that TG2 inhibitors may have unacceptable side effects, as TG2 is involved in many different physiologic processes, including programmed cell death (Aeschlimann and Paulsson, 1994). TG2 inhibitors may also not be specific for TG2, but may affect the function of other transglutaminases. Another strategy would be to aim directly at the gluten-reactive T cells and eliminate or anergize them *in situ* by coadministering gluten and agents that alter the outcome of T-cell activation, such as antibodies to CD154 (Monk et al., 2003) or CD3 molecules (Chatenoud, 2003) (Figure 51.7C). Alternatively, it may be possible to silence gluten-specific T cells directly using soluble dimers of HLA–peptide complexes, as such dimers have been shown to induce the apoptosis of antigen-specific T cells as a result of inappropriate stimulation (Appel et al., 2001). However, the increasing number of characterized gluten epitopes would complicate this approach. The central role of DQ2 and DQ8 in presenting gluten peptides offers yet another target for intervention. Blocking the binding-sites of these HLA molecules would prevent presentation of disease-inducing gluten peptides (Figure 51.7D). The challenge with this approach will be to find an efficient way to target and block the binding sites of DQ molecules, which are continuously synthesized by APCs. Whatever new therapeutic modality is introduced in celiac disease, it will have to prove better than the current gluten-free diet regime with regard to its long-term safety and outcome. This fact must be taken into consideration when devising new treatments.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Considerable progress has been made in recent years in the understanding of the molecular basis for celiac disease, but several new questions have emerged. Many of these

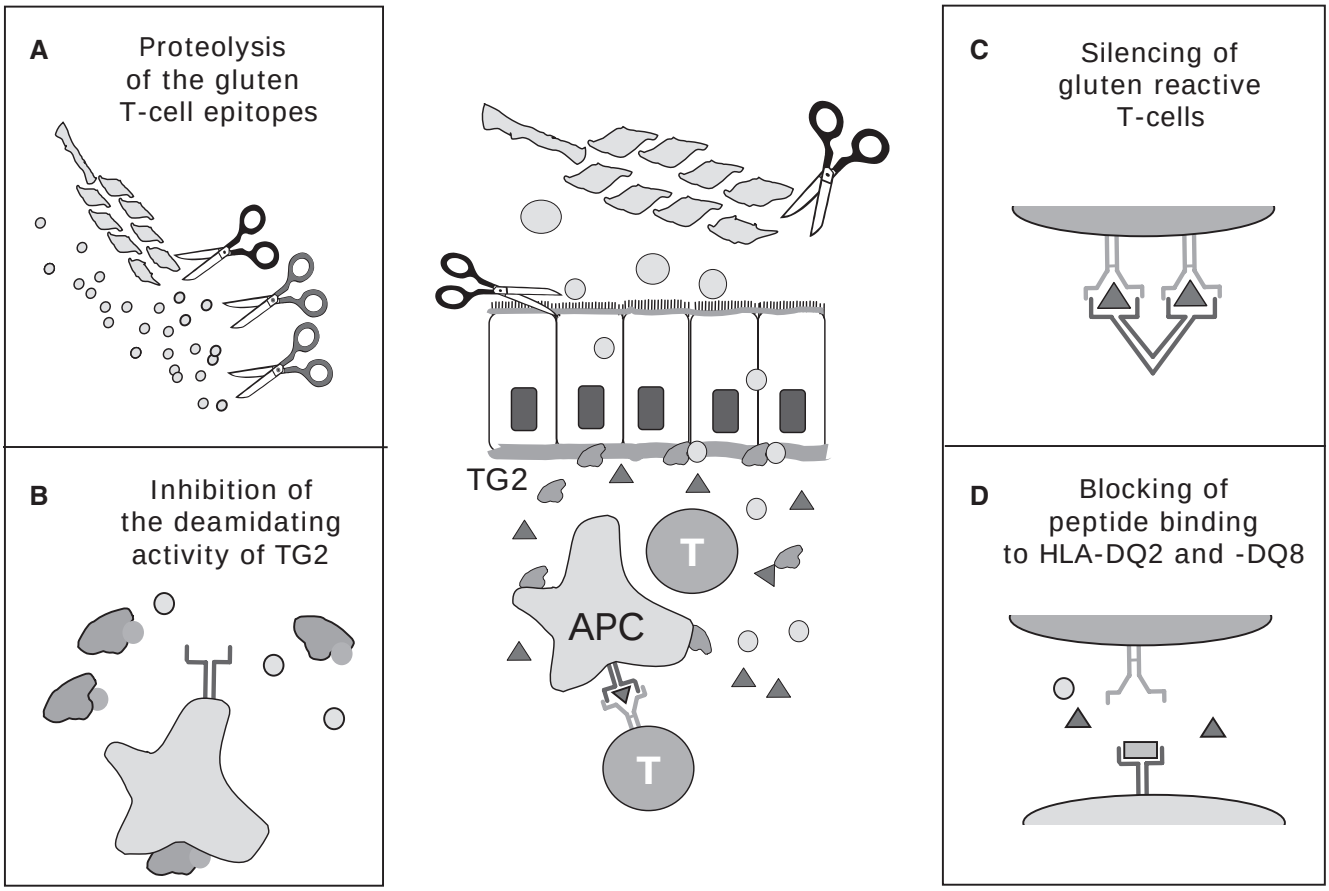


FIGURE 51.7 New insight into the pathogenesis of celiac disease provides rational targets for interfering with the disease process. *A*, Peptidase supplementation can prevent immunogenic peptide from being presented to T cells by direct destruction or by facilitating the gastrointestinal proteolysis. Prolylendopeptidases that cleave adjacent to proline residues are particularly attractive as these enzymes will target proline-rich peptides that are especially resistant to proteolysis and which often contain HLA-DQ2-restricted T-cell epitopes. *B*, Inhibition of the deamidating activity of tissue transglutaminase (TG2). Inhibitors of TG2 will prevent formation of the deamidated gluten peptides that are particularly stimulatory to T cells. *C*, Silencing of gluten-reactive T cells. This can be done in a variety of ways from oral challenge with gluten together with infusion of antibodies to CD154 or CD3 or the generation of multivalent and soluble HLA–peptide complexes (depicted) that are unable to provide all signals required for T-cell stimulation and which induce T-cell apoptosis. *D*, Blocking of peptide presentation by HLA molecules to gluten reactive T-cells by compounds that fill up the binding sites of DQ2 and DQ8 molecules without activating T-cells. This will prevent binding of gluten peptides to these HLA molecules and thereby T-cell activation. APC, Antigen-presenting cell.

relate to the autoimmune aspects of the disease. Is celiac disease an autoimmune disorder? To what extent are the autoimmune components of celiac disease involved in the disease development? How relevant are the findings of T-cell recognition of post-translationally-modified peptides and autoantibody formation driven by an exogenous food antigen for other autoimmune disorders? Can it be that other autoimmune disorders are driven by immune responses to foreign, not yet identified, antigens? Finally, is it true that a gluten-free diet can protect against development of associated autoimmune diseases, and if so, can this knowledge be used to understand autoimmunity in general? We are sure that studies of celiac disease will continue to provide interesting answers.

Acknowledgments

Studies in the authors' laboratory are funded by grants from the Research Council of Norway, the European Commission (BMH4-CT98-3087, QLRT-1999-00037, QLRT-2000-00657), the Norwegian Cancer Society, Medinnova, Freia Chokolade Fabriks Medicinske Fond, the Jahre Foundation, and EXTRA funds from the Norwegian Foundation for Health and Rehabilitation.

References

Aeschlimann, D. and Paulsson, M. (1994). Transglutaminases: protein cross-linking enzymes in tissues and body fluids. *Thromb. Haemost. 71*, 402–415.

Akimov, S.S. and Belkin, A.M. (2001). Cell surface tissue transglutaminase is involved in adhesion and migration of monocytic cells on fibronectin. *Blood 98*, 1567–1576.

Alp, M.H. and Wright, R. (1971). Autoantibodies to reticulin in patients with idiopathic steatorrhoea, coeliac disease, and Crohn's disease, and their relation to immunoglobulins and dietary antibodies. *Lancet 2*, 682–685.

Anderson, O.D. (2001). Genetic variations of gliadins—new approaches for the treatment of coelic disease. In C. Bachman and B. Koletzko (eds), *Proceedings of the 50th Nestle Workshop: Genetic Expression and Nutrition*. Philadelphia: Lippincott, Williams & Wilkins.

Anderson, R.P., Degano, P., Godkin, A.J., Jewell, D.P., and Hill, A.V. (2000). *In vivo* antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T-cell epitope. *Nat. Med. 6*, 337–342.

Appel, H., Seth, N.P., Gauthier, L., and Wucherpfennig, K.W. (2001). Anergy induction by dimeric TCR ligands. *J. Immunol. 166*, 5279–5285.

Arentz-Hansen, H., Körner, R., Molberg, Ø., Quarsten, H., Vader, W., Kooy, Y.M., Lundin, K.E.A., Koning, F., Roepstorff, P., Sollid, L.M., and McAdam, S.N. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J. Exp. Med. 191*, 603–612.

Arentz-Hansen, H., McAdam, S.N., Molberg, Ø., Fleckenstein, B., Lundin, K.E.A., Jorgensen, T.J., Jung, G., Roepstorff, P., and Sollid, L.M. (2002). Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues. *Gastroenterology 123*, 803–809.

Arentz-Hansen, H., Flackenstein, B., Molberg, Ø., Scott, H., Koning, F., Jung, G., Roepstorff, P., Lundin, K.E.A., and Sollid, L.M. (2004). The molecular basis for oat intolerance in celiac disease patients. PLoS Med. 1: e1.

Askling, J., Linet, M., Gridley, G., Halstensen, T.S., Ekström, K., and Ekbom, A. (2002). Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. *Gastroenterology 123*, 1428–1435.

Babron, M.C., Nilsson, S., Adamovic, S., Naluai, A.T., Wahlström, J., Ascher, H., Ciclitira, P.J., Sollid, L.M., Partanen, J., Greco, L., and Clerget-Darpoux, F. (2003). Meta and pooled analysis of European coeliac disease data. *Eur. J. Hum. Genet. 11*, 828–834.

Balklava, Z., Verderio, E., Collighan, R., Gross, S., Adams, J., and Griffin, M. (2002). Analysis of tissue transglutaminase function in the migration of Swiss 3T3 fibroblasts: the active-state conformation of the enzyme does not affect cell motility but is important for its secretion. *J. Biol. Chem. 277*, 16567–16575.

Bardella, M.T., Marino, R., and Meroni, P.L. (1999). Celiac disease during interferon treatment. *Ann. Intern. Med. 131*, 157–158.

Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J.H., Lanier, L.L., and Spies, T. (1999). Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science 285*, 727–729.

Bingley, P.J., Williams, A.J., Norcross, A.J., Unsworth, D.J., Lock, R.J., Ness, A.R., and Jones, R.W. (2004). Undiagnosed coeliac disease at age seven: population based prospective birth cohort study. *BMJ 328*, 322–323.

Black, K.E., Murray, J.A., and David, C.S. (2002). HLA-DQ determines the response to exogenous wheat proteins: a model of gluten sensitivity in transgenic knockout mice. *J. Immunol. 169*, 5595–5600.

Browning, T.H. and Trier, J.S. (1969). Organ culture of mucosal biopsies of human small intestine. *J. Clin. Invest. 48*, 1423–1432.

Bruce, S.E., Bjarnason, I., and Peters, T.J. (1985). Human jejunal transglutaminase: demonstration of activity, enzyme kinetics and substrate specificity with special relation to gliadin and coeliac disease. *Clin. Sci. 68*, 573–579.

Cammarota, G., Cuoco, L., Cianci, R., Pandolfi, F., and Gasbarrini, G. (2000). Onset of coeliac disease during treatment with interferon for chronic hepatitis C. *Lancet 356*, 1494–1495.

Carroccio, A., Vitale, G., Di Prima, L., Chifari, N., Napoli, S., La Russa, C., Gulotta, G., Averna, M.R., Montalto, G., Mansueto, S., and Notarbartolo, A. (2002). Comparison of anti-transglutaminase ELISAs and an anti-endomysial antibody assay in the diagnosis of celiac disease: a prospective study. *Clin. Chem. 48*, 1546–1550.

Cataldo, F., Marino, V., Bottaro, G., Greco, P., and Ventura, A. (1997). Celiac disease and selective immunoglobulin A deficiency. *J. Pediatr. 131*, 306–308.

Catassi, C., Fabiani, E., Corrao, G., Barbato, M., De Renzo, A., Carella, A.M., Gabrielli, A., Leoni, P., Carroccio, A., Baldassarre, M., Bertolani, P., Caramaschi, P., Sozzi, M., Guariso, G., Volta, U., and Corazza, G.R. (2002). Risk of non-Hodgkin lymphoma in celiac disease. *JAMA 287*, 1413–1419.

Cellier, C., Delabesse, E., Helmer, C., Patey, N., Matuchansky, C., Jabri, B., Macintyre, E., Cerf-Bensussan, N., and Brousse, N. (2000). Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. *Lancet 356*, 203–208.

Chatenoud, L. (2003). CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol. 3*, 123–132.

Chen, D., Ueda, R., Harding, F., Patil, N., Mao, Y., Kurahara, C., Platenburg, G., and Huang, M. (2003). Characterization of HLA DR3/DQ2 transgenic mice: a potential humanized animal model for autoimmune disease studies. *Eur. J. Immunol. 33*, 172–182.

Chen, Z., Dudek, N., Wijburg, O., Strugnell, R., Brown, L., Deliyannis, G., Jackson, D., Koentgen, F., Gordon, T., and McCluskey, J. (2002). A 320-kilobase artificial chromosome encoding the human HLA DR3-DQ2 MHC haplotype confers HLA restriction in transgenic mice. *J. Immunol. 168*, 3050–3056.

Ciacci, C., Cirillo, M., Sollazzo, R., Savino, G., Sabbatini, F., and Mazzacca, G. (1995). Gender and clinical presentation in adult celiac disease. *Scand. J. Gastroenterol. 30*, 1077–1081.

Ciacci, C., Cirillo, M., Auriemma, G., Di Dato, G., Sabbatini, F., and Mazzacca, G. (1996). Celiac disease and pregnancy outcome. *Am. J. Gastroenterol. 91*, 718–722.

Ciacci, C., Cirillo, M., Cavallaro, R., and Mazzacca, G. (2002). Long-term follow-up of celiac adults on gluten-free diet: prevalence and correlates of intestinal damage. *Digestion 66*, 178–185.

Clemente, M.G., Musu, M.P., Frau, F., Brusco, G., Sole, G., Corazza, G.R., and De Virgiliis, S. (2000). Immune reaction against the cytoskeleton in coeliac disease. *Gut 47*, 520–526.

Collin, P. (1999). New diagnostic findings in coeliac disease. *Ann. Med. 31*, 399–405.

Collin, P. and Reunala, T. (2003). Recognition and management of the cutaneous manifestations of celiac disease: a guide for dermatologists. *Am. J. Clin. Dermatol. 4*, 13–20.

Collin, P., Reunula, T., Pukkala, E., Laippala, P., Keyrilainen, O., and Pasternack, A. (1994). Coeliac disease-associated disorders and survival. *Gut 35*, 1215–1218.

Corrao, G., Corazza, G.R., Bagnardi, V., Brusco, G., Ciacci, C., Cottone, M., Sategna, G.C., Usai, P., Cesari, P., Pelli, M.A., Loperfido, S., Volta, U., Calabro, A., and Certo, M. (2001). Mortality in patients with coeliac disease and their relatives: a cohort study. *Lancet 358*, 356–361.

Counsell, C.E., Taha, A., and Ruddell, W.S. (1994). Coeliac disease and autoimmune thyroid disease. *Gut 35*, 844–846.

Cronin, C., Feighery, A., Ferriss, J.B., Liddy, C., Shanahan, F., and Feighery, C. (1997). High prevalence of celiac disease among patients with insulin-dependent (type I) diabetes mellitus. *Am. J. Gastroenterol. 92*, 2210–2212.

Dahele, A.V., Aldhous, M.C., Humphreys, K., and Ghosh, S. (2001). Serum IgA tissue transglutaminase antibodies in coeliac disease and other gastrointestinal diseases. *Q. J. Med. 94*, 195–205.

De Ritis, G., Auricchio, S., Jones, H.W., Lew, E.J., Bernardin, J.E., and Kasarda, D.D. (1988). *In vitro* (organ culture) studies of the toxicity of

specific A-gliadin peptides in celiac disease. *Gastroenterology 94*, 41–49.

Dicke, W.K. (1950). *Coeliac Disease. Investigations of the Harmful Effects of Certain Types of Cereal on Patients Suffering from Coeliac Disease*. Thesis, University of Utrecht.

Dieterich, W., Ehnis, T., Bauer, M., Donner, P., Volta, U., Riecken, E.O., and Schuppan, D. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat. Med. 3*, 797–801.

Dieterich, W., Laag, E., Schopper, H., Volta, U., Ferguson, A., Gillett, H., Riecken, E.O., and Schuppan, D. (1998). Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology 115*, 1317–1321.

Dieterich, W., Trapp, D., Esslinger, B., Leidenberger, M., Piper, J., Hahn, E., and Schuppan, D. (2003). Autoantibodies of patients with coeliac disease are insufficient to block tissue transglutaminase activity. *Gut 52*, 1562–1566.

Ek, J., Albrechtsen, D., Solheim, B.G., and Thorsby, E. (1978). Strong association between the HLA-Dw3-related B cell alloantigen-DRw3 and coeliac disease. *Scand. J. Gastroenterol. 13*, 229–233.

Ellis, A. (1981). Coeliac disease: previous family studies. In R.B. McConnell (ed.), *The Genetics of Coeliac Disease* (pp. 197–199). Lancaster: MTP Press.

Ellis, H.J., Pollock, E.L., Engel, W., Fraser, J.S., Rosen-Bronson, S., Wieser, H., and Ciclitira, P.J. (2003). Investigation of the putative immunodominant T cell epitopes in coeliac disease. *Gut 52*, 212–217.

Esposito, C., Paparo, F., Caputo, I., Rossi, M., Maglio, M., Sblattero, D., Not, T., Porta, R., Auricchio, S., Marzari, R., and Troncone, R. (2002). Anti-tissue transglutaminase antibodies from coeliac patients inhibit transglutaminase activity both *in vitro* and *in situ*. *Gut 51*, 177–181.

Falchuk, Z.M., Rogentine, G.N., and Strober, W. (1972). Predominance of histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. *J. Clin. Invest. 51*, 1602–1605.

Farrell, R.J. and Kelly, C.P. (2002). Celiac sprue. *N. Engl. J. Med. 346*, 180–188.

Fasano, A. and Catassi, C. (2001). Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology 120*, 636–651.

Fasano, A., Not, T., Wang, W., Uzzau, S., Berti, I., Tommasini, A., and Goldblum, S.E. (2000). Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. *Lancet 355*, 1518–1519.

Fasano, A., Berti, I., Gerarduzzi, T., Not, T., Colletti, R.B., Drago, S., Elitsur, Y., Green, P.H., Guandalini, S., Hill, I.D., Pietzak, M., Ventura, A., Thorpe, M., Kryszak, D., Fornaroli, F., Wasserman, S.S., Murray, J.A., and Horvath, K. (2003). Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch. Intern. Med. 163*, 286–292.

Ferguson, A., MacDonald, T.T., McClure, J.P., and Holden, R.J. (1975). Cell-mediated immunity to gliadin within the small intestinal mucosa in celiac disease. *Lancet 1,* 895–897.

Fleckenstein, B., Molberg, Ø., Qiao, S.W., Schmid, D.G., Von Der, M.F., Elgstoen, K., Jung, G., and Sollid, L.M. (2002). Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation process. *J. Biol. Chem. 277*, 34109–34116.

Fleckenstein, B., Qiao, S.W., Larsen, M.R., Jung, G., Roepstorff, P., and Sollid, L.M. (2004). Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. *J. Biol. Chem. 279*, 17607–17616.

Folk, J.E. (1983). Mechanism and basis for specificity of transglutaminase-catalyzed ε-(γ-glutamyl) lysine bond formation. *Adv. Enzymol. Relat. Areas. Mol. Biol. 54*, 1–56.

Forsberg, G., Hernell, O., Melgar, S., Israelsson, A., Hammarström, S., and Hammarström, M.L. (2002). Paradoxical coexpression of proinflammatory and down-regulatory cytokines in intestinal T cells in childhood celiac disease. *Gastroenterology 123*, 667–678.

Fraser, J.S., Engel, W., Ellis, H.J., Moodie, S.J., Pollock, E.L., Wieser, H., and Ciclitira, P.J. (2003). Coeliac disease: *in vivo* toxicity of the putative immunodominant epitope. *Gut 52*, 1698–1702.

Gee, S.J. (1888). On the coeliac affection. *St Bartholomew's Hosp. Res. 24*, 17–20.

Gjertsen, H.A., Sollid, L.M., Ek, J., Thorsby, E., and Lundin, K.E.A. (1994). T cells from the peripheral blood of coeliac disease patients recognize gluten antigens when presented by HLA-DR, -DQ, or -DP molecules. *Scand. J. Immunol. 39*, 567–574.

Gleimer, M. and Parham, P. (2003). Stress management: MHC class I and class I-like molecules as reporters of cellular stress. *Immunity 19*, 469–477.

Granito, A., Muratori, P., Cassani, F., Pappas, G., Muratori, L., Agostinelli, D., Veronesi, L., Bortolotti, R., Petrolini, N., Bianchi, F. B., and Volta, U. (2004). Anti-actin IgA antibodies in severe coeliac disease. *Clin. Exp. Immunol. 137*, 386–392.

Greco, L., Corazza, G., Babron, M.C., Clot, F., Fulchignoni-Lataud, M.C., Percopo, S., Zavattari, P., Bouguerra, F., Dib, C., Tosi, R., Troncone, R., Ventura, A., Mantavoni, W., Magazzu, G., Gatti, R., Lazzari, R., Giunta, A., Perri, F., Iacono, G., Cardi, E., De Virgiliis, S., Cataldo, F., De Angelis, G., Musumeci, S., Ferrari, R., Balli, F., Bardella, M.T., Volta, U., Catassi, C., Torre, G., Eliaou, J.F., Serre, J.L., and Clerget-Darpoux, F. (1998). Genome search in celiac disease. *Am. J. Hum. Genet. 62*, 669–675.

Greco, L., Babron, M.C., Corazza, G.R., Percopo, S., Sica, R., Clot, F., Fulchignoni-Lataud, M.C., Zavattari, P., Momigliano-Richiardi, P., Casari, G., Gasparini, P., Tosi, R., Mantovani, V., De Virgiliis, S., Iacono, G., D'Alfonso, A., Selinger-Leneman, H., Lemainque, A., Serre, J.L., and Clerget-Darpoux, F. (2001). Existence of a genetic risk factor on chromosome 5q in Italian coeliac disease families. *Ann. Hum. Genet. 65*, 35–41.

Greco, L., Romino, R., Coto, I., Di Cosmo, N., Percopo, S., Maglio, M., Paparo, F., Gasperi, V., Limongelli, M.G., Cotichini, R., D'Agate, C., Tinto, N., Sacchetti, L., Tosi, R., and Stazi, M.A. (2002). The first large population based twin study of coeliac disease. *Gut 50*, 624–628.

Green, P.A., Wollaeger, E.E., Sprague, R.G., and Brown, A.L. (1962). Diabetes mellitus associated with nontropical sprue. *Diabetes 11*, 388–392.

Green, P.H., Fleischauer, A.T., Bhagat, G., Goyal, R., Jabri, B., and Neugut, A.I. (2003). Risk of malignancy in patients with celiac disease. *Am. J. Med. 115*, 191–195.

Groh, V., Steinle, A., Bauer, S., and Spies, T. (1998). Recognition of stress-induced MHC molecules by intestinal epithelial γδ T cells. *Science 279*, 1737–1740.

Haas, S.V. (1932). Celiac disease, its specific treatment and cure without nutritional relapse. *JAMA 99*, 448–452.

Hadjivassiliou, M., Grunewald, R., Sharrack, B., Sanders, D., Lobo, A., Williamson, C., Woodroofe, N., Wood, N., and Davies-Jones, A. (2003). Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. *Brain 126*, 685–691.

Halstensen, T.S., Scott, H., and Brandtzaeg, P. (1989). Intraepithelial T cells of the TcRγδ+ CD8− and Vδ1/Jδ1+ phenotypes are increased in coeliac disease. *Scand. J. Immunol. 30*, 665–672.

Halstensen, T.S., Scott, H., Fausa, O., and Brandtzaeg, P. (1993). Gluten stimulation of coeliac mucosa *in vitro* induces activation (CD25) of lamina propria CD4+ T cells and macrophages but no crypt-cell hyperplasia. *Scand. J. Immunol. 38*, 581–590.

Halttunen, T. and Mäki, M. (1999). Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. *Gastroenterology 116*, 566–572.

Hansson, T., Dahlbom, I., Rogberg, S., Dannaeus, A., Hopfl, P., Gut, H., Kraaz, W., and Klareskog, L. (2002). Recombinant human tissue trans-

glutaminase for diagnosis and follow-up of childhood coeliac disease. *Pediatr. Res. 51*, 700–705.

Hausch, F., Shan, L., Santiago, N.A., Gray, G.M., and Khosla, C. (2002). Intestinal digestive resistance of immunodominant gliadin peptides. *Am. J. Physiol. Gastrointest. Liver Physiol. 283*, G996-G1003.

Hausch, F., Halttunen, T., Mäki, M., and Khosla, C. (2003). Design, synthesis, and evaluation of gluten peptide analogs as selective inhibitors of human tissue transglutaminase. *Chem. Biol. 10*, 225–231.

Heneghan, M.A., Stevens, F.M., Cryan, E.M., Warner, R.H., and McCarthy, C.F. (1997). Celiac sprue and immunodeficiency states: a 25-year review. *J. Clin. Gastroenterol. 2*, 421–425.

Hin, H., Bird, G., Fisher, P., Mahy, N., and Jewell, D. (1999). Coeliac disease in primary care: case finding study. *BMJ 318*, 164–167.

Hoffenberg, E.J., Haas, J., Drescher, A., Barnhurst, R., Osberg, I., Bao, F., and Eisenbarth, G. (2000). A trial of oats in children with newly diagnosed celiac disease. *J. Pediatr. 137*, 361–366.

Holopainen, P., Arvas, M., Sistonen, P., Mustalahti, K., Collin, P., Mäki, M., and Partanen, J. (1999). CD28/CTLA4 gene region on chromosome 2q33 confers genetic susceptibility to celiac disease. A linkage and family-based association study. *Tissue Antigens 53*, 470–475.

Holopainen, P., Mustalahti, K., Uimari, P., Collin, P., Mäki, M., and Partanen, J. (2001). Candidate gene regions and genetic heterogeneity in gluten sensitivity. *Gut 48*, 696–701.

Houlston, R.S., Tomlinson, I.P., Ford, D., Seal, S., Marossy, A.M., Ferguson, A., Holmes, G.K., Hosie, K.B., Howdle, P.D., Jewell, D.P., Godkin, A., Kerr, G.D., Kumar, P., Logan, R.F., Love, A.H., Johnston, S., Marsh, M.N., Mitton, S., O'Donoghue, D., Roberts, A., Walker-Smith, J.A., and Stratton, M.F. (1997). Linkage analysis of candidate regions for coeliac disease genes. *Hum. Mol. Genet. 6*, 1335–1339.

Ivarsson, A., Persson, L.A., Nystrom, L., Ascher, H., Cavell, B., Danielsson, L., Dannaeus, A., Lindberg, T., Lindquist, B., Stenhammar, L., and Hernell, O. (2000). Epidemic of coeliac disease in Swedish children. *Acta Paediatr. 89*, 165–171.

Ivarsson, A., Hernell, O., Stenlund, H., and Persson, L.A. (2002). Breast-feeding protects against celiac disease. *Am. J Clin. Nutr. 75*, 914–921.

Ivarsson, A., Hernell, O., Nyström, L., and Persson, L.A. (2003a). Children born in the summer have increased risk for coeliac disease. *J. Epidemiol. Community Health 57*, 36–39.

Ivarsson, A., Persson, L.A., Nyström, L., and Hernell, O. (2003b). The Swedish coeliac disease epidemic with a prevailing twofold higher risk in girls compared to boys may reflect gender specific risk factors. *Eur. J. Epidemiol. 18*, 677–684.

Jabri, B., de Serre, N.P., Cellier, C., Evans, K., Gache, C., Carvalho, C., Mougenot, J.F., Allez, M., Jian, R., Desreumaux, P., Colombel, J.F., Matuchansky, C., Cugnenc, H., Lopez-Botet, M., Vivier, E., Moretta, A., Roberts, A.I., Ebert, E.C., Guy-Grand, D., Brousse, N., Schmitz, J., and Cerf-Bensussan, N. (2000). Selective expansion of intraepithelial lymphocytes expressing the HLA-E-specific natural killer receptor CD94 in celiac disease. *Gastroenterology 118*, 867–879.

Janatuinen, E.K., Pikkarainen, P.H., Kemppainen, T.A., Kosma, V.M., Jarvinen, R.M., Uusitupa, M.I., and Julkunen, R.J. (1995). A comparison of diets with and without oats in adults with celiac disease. *N. Engl. J. Med. 333*, 1033–1037.

Janatuinen, E.K., Kemppainen, T.A., Julkunen, R.J., Kosma,V.M., Mäki, M., Heikkinen, M., and Uusitupa, M.I. (2002). No harm from five year ingestion of oats in coeliac disease. *Gut 50*, 332–335.

Jarvinen, T.T., Kaukinen, K., Laurila, K., Kyronpalo, S., Rasmussen, M., Mäki, M., Korhonen, H., Reunala, T., and Collin, P. (2003). Intraepithelial lymphocytes in celiac disease. *Am. J. Gastroenterol. 98*, 1332–1337.

Jensen, K., Sollid, L.M., Scott, H., Paulsen, G., Kett, K., Thorsby, E., and Lundin, K.E.A. (1995). Gliadin-specific T cell responses in peripheral blood of healthy individuals involve T cells restricted by the coeliac disease associated DQ2 heterodimer. *Scand. J. Immunol. 42*, 166–170.

Johansen, B.H., Vartdal, F., Eriksen, J.A., Thorsby, E., and Sollid, L.M. (1996). Identification of a putative motif for binding of peptides to HLA-DQ2. *Int. Immunol. 8*, 177–182.

Kagnoff, M.F., Austin, R.K., Hubert, J.J., Bernardin, J.E., and Kasarda, D.D. (1984). Possible role for a human adenovirus in the pathogenesis of celiac disease. *J. Exp. Med. 160*, 1544–1557.

Karell, K., Louka, A.S., Moodie, S.J., Ascher, H., Clot, F., Greco, L., Ciclitira, P.J., Sollid, L.M., and Partanen, J. (2003). HLA types in celiac disease patients not carrying the *DQA1*05-DQB1*02* (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Hum. Immunol. 64*, 469–477.

Kaukinen, K., Partanen, J., Mäki, M., and Collin, P. (2002a). HLA-DQ typing in the diagnosis of celiac disease. *Am. J. Gastroenterol. 97*, 695–699.

Kaukinen, K., Sulkanen, S., Mäki, M., and Collin, P. (2002b). IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. *Eur. J. Gastroenterol. Hepatol. 14*, 311–315.

Keuning, J.J., Peña, A.S., van Leeuwen, A., van Hooff, J.P., and van Rood, J.J. (1976). HLA-DW3 associated with coeliac disease. *Lancet 1*, 506–508.

Kim, C.Y., Quarsten, H., Bergseng, E., Khosla, C., and Sollid, L.M. (2004). Structural basis for HLA-DQ2 mediated presentation of gluten epitopes in celiac disease. *Proc. Natl. Acad. Sci. USA 101*, 4175–4179.

King, A.L., Yiannakou, J.Y., Brett, P.M., Curtis, D., Morris, M.A., Dearlove, A.M., Rhodes, M., Rosen-Bronson, S., Mathew, C., Ellis, H.J., and Ciclitira, P.J. (2000). A genome-wide family-based linkage study of coeliac disease. *Ann. Hum. Genet. 64*, 479–490.

King, A.L., Moodie, S.J., Fraser, J.S., Curtis, D., Reid, E., Dearlove, A.M., Ellis, H.J., and Ciclitira, P.J. (2002). CTLA-4/CD28 gene region is associated with genetic susceptibility to coeliac disease in UK families. *J. Med. Genet. 39*, 51–54.

Koletzko, S., Burgin-Wolff, A., Koletzko, B., Knapp, M., Burger, W., Gruneklee, D., Herz, G., Thon, A., and Wendel, U. (1988). Prevalence of coeliac disease in diabetic children and adolescents. A muliticentre study. *Eur. J. Pediatr. 148*, 113–117.

Larre, C., Chiarello, M., Blanloeil, Y., Chenu, M., and Gueguen, J. (1993). Gliadin modifications catalysed by guinea pig liver transglutaminase. *J. Food. Biochem. 17*, 267–282.

Liu, J., Juo, S.H., Holopainen, P., Terwilliger, J., Tong, X., Grunn, A., Brito, M., Green, P., Mustalahti, K., Mäki, M., Gilliam, T.C., and Partanen, J. (2002). Genomewide linkage analysis of celiac disease in Finnish families. *Am. J. Hum. Genet. 70*, 51–59.

Lorand, L. and Graham, R.M. (2003). Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat. Rev. Mol. Cell Biol. 4*, 140–156.

Lundin, K.E.A., Scott, H., Hansen, T., Paulsen, G., Halstensen, T.S., Fausa, O., Thorsby, E., and Sollid, L.M. (1993). Gliadin-specific, HLA-DQ(α1*0501,β1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J. Exp. Med. 178*, 187–196.

Lundin, K.E.A., Scott, H., Fausa, O., Thorsby, E., and Sollid, L.M. (1994). T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8. *Hum. Immunol. 41*, 285–291.

Lundin, K.E.A., Sollid, L.M., Norén, O., Anthonsen, D., Molberg, Ø., Thorsby, E., and Sjöström, H. (1997). Heterogenous reactivity patterns of HLA-DQ-restricted small intestinal T-cell clones from patients with celiac disease. *Gastroenterology 112*, 752–759.

Lundin, K.E.A., Nilsen, E.M., Scott, H.G., Løberg, E.M., Gjøen, A., Bratlie, J., Skar, V., Mendez, E., Løvik, A., and Kett, K. (2003). Oats induced villous atrophy in coeliac disease. *Gut 52*, 1149–1152.

MacDonald, W.C., Dobbins, I.W.O., and Rubin, C.E. (1965). Studies of the familial nature of celiac sprue using biopsy of the small intestine. *N. Engl. J. Med. 272*, 448–456.

Maiuri, L., Picarelli, A., Boirivant, M., Coletta, S., Mazzilli, M.C., De Vincenzi, M., Londei, M., and Auricchio, S. (1996). Definition of the initial immunologic modifications upon *in vitro* gliadin challenge in the small intestine of celiac patients. *Gastroenterology 110*, 1368–1378.

Maiuri, L., Ciacci, C., Auricchio, S., Brown, V., Quaratino, S., and Londei, M. (2000). Interleukin 15 mediates epithelial changes in celiac disease. *Gastroenterology 119*, 996–1006.

Maiuri, L., Ciacci, C., Raia, V., Vacca, L., Ricciardelli, I., Raimondi, F., Auricchio, S., Quaratino, S., and Londei, M. (2001). FAS engagement drives apoptosis of enterocytes of coeliac patients. *Gut 48*, 418–424.

Maiuri, L., Ciacci, C., Ricciardelli, I., Vacca, L., Raia, V., Auricchio, S., Picard, J., Osman, M., Quaratino, S., and Londei, M. (2003). Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet 362*, 30–37.

Mäki, M., Kallonen, K., Lahdeaho, M.L., and Visakorpi, J.K. (1988). Changing pattern of childhood coeliac disease in Finland. *Acta Paediatr. Scand. 77*, 408–412.

Mäki, M., Mustalahti, K., Kokkonen, J., Kulmala, P., Haapalahti, M., Karttunen, T., Ilonen, J., Laurila, K., Dahlbom, I., Hansson, T., Hopfl, P., and Knip, M. (2003). Prevalence of celiac disease among children in Finland. *N. Engl. J. Med. 348*, 2517–2524.

Marietta, E., Black, K., Camilleri, M., Rogers, R.S., 3rd, David, C., Pittelkow, M.R., and Murray, J.A. (2004). A new model for dermatitis herpeitiformis that uses HLA-DQ8 transgenic NOD mice. *J. Clin. Invest. 114*, 1090–1097.

Marley, N.J., Macartney, J.C., and Ciclitira, P.J. (1987). HLA-DR, DP and DQ expression in the small intestine of patients with coeliac disease. *Clin. Exp. Immunol. 70*, 386–393.

Marsh, M.N. (1992). Mucosal pathology in gluten sensitivity. In M. N. Marsh (ed) *Coeliac Disease* (pp. 136–191). Oxford: Blackwell Scientific Publications.

Marsh, M.N. and Crowe, P.T. (1995). Morphology of the mucosal lesion in gluten sensitivity. *Baillières Clin. Gastroenterol. 9*, 273–293.

Martucci, S., Fraser, J.S., Biagi, F., Corazza, G.R., Ciclitira, P.J., and Ellis, H.J. (2003). Characterizing one of the DQ2 candidate epitopes in coeliac disease: A-gliadin 51–70 toxicity assessed using an organ culture system. *Eur. J. Gastroenterol. Hepatol. 15*, 1293–1298.

Marzari, R., Sblattero, D., Florian, F., Tongiorgi, E., Not, T., Tommasini, A., Ventura, A., and Bradbury, A. (2001). Molecular dissection of the tissue transglutaminase autoantibody response in celiac disease. *J. Immunol. 166*, 4170–4176.

Mawhinney, H. and Tomkin, G.H. (1971). Gluten enteropathy associated with selective IgA deficiency. *Lancet 2*, 121–124.

Mazzarella, G., Maglio, M., Paparo, F., Nardone, G., Stefanile, R., Greco, L., van De,W.Y., Kooy, Y., Koning, F., Auricchio, S., and Troncone, R. (2003). An immunodominant DQ8 restricted gliadin peptide activates small intestinal immune response in *in vitro* cultured mucosa from HLA-DQ8 positive but not HLA-DQ8 negative coeliac patients. *Gut 52*, 57–62.

Mearin, M.L., Biemond, I., Pena, A.S., Polanco, I., Vazquez, C., Schreuder, G.T., de Vries, R.R., and van Rood, J.J. (1983). HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease. *Gut 24*, 532–537.

Mention, J.J., Ben Ahmed, M., Begue, B., Barbe, U., Verkarre, V., Asnafi, V., Colombel, J.F., Cugnenc, P.H., Ruemmele, F.M., McIntyre, E., Brousse, N., Cellier, C., and Cerf-Bensussan, N. (2003). Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. *Gastroenterology 125*, 730–745.

Molberg, Ø., Kett, K., Scott, H., Thorsby, E., Sollid, L.M., and Lundin, K.E.A. (1997). Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. *Scand. J. Immunol. 46*, 103–109.

Molberg, Ø., McAdam, S.N., Körner, R., Quarsten, H., Kristiansen, C., Madsen, L., Fugger, L., Scott, H., Roepstorff, P., Lundin, K.E.A., Sjöström, H., and Sollid, L.M. (1998). Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells. *Nat. Med. 4*, 713–717.

Molberg, Ø., McAdam, S., Lundin, K.E.A., Kristiansen, C., Arentz-Hansen, H., Kett, K., and Sollid, L.M. (2001). T cells from celiac disease lesions recognize gliadin epitopes deamidated *in situ* by endogenous tissue transglutaminase. *Eur. J. Immunol. 31*, 1317–1323.

Molberg, Ø., Solheim, F.N., Jensen, T., Lundin, K.E., Arentz-Hansen, H., Anderson, O.D., Kjersti, U.A., and Sollid, L.M. (2003). Intestinal T-cell responses to high-molecular-weight glutenins in celiac disease. *Gastroenterology 125*, 337–344.

Monk, N.J., Hargreaves, R.E., Marsh, J.E., Farrar, C.A., Sacks, S.H., Millrain, M., Simpson, E., Dyson, J., and Jurcevic, S. (2003). Fc-dependent depletion of activated T cells occurs through CD40L-specific antibody rather than costimulation blockade. *Nat. Med. 9*, 1275–1280.

Monteleone, G., Pender, S.L., Alstead, E., Hauer, A.C., Lionetti, P., McKenzie, C., and MacDonald, T.T. (2001a). Role of interferon α in promoting T helper cell type 1 responses in the small intestine in coeliac disease. *Gut 48*, 425–429.

Monteleone, G., Pender, S.L., Wathen, N.C., and MacDonald, T.T. (2001b). Interferon-α drives T cell-mediated immunopathology in the intestine. *Eur. J Immunol. 31*, 2247–2255.

Moss, S.F., Attia, L., Scholes, J.V., Walters, J.R., and Holt, P.R. (1996). Increased small intestinal apoptosis in coeliac disease. *Gut 39*, 811–817.

Murray, J.A. (1999). The widening spectrum of celiac disease. *Am. J. Clin. Nutr. 69*, 354–365.

Naluai, Å.T., Nilsson, S., Samuelsson, L., Gudjonsdottir, A.H., Ascher, H., Ek, J., Hallberg, B., Kristiansson, B., Martinsson, T., Nerman, O., Sollid, L.M., and Wahlström, J. (2000). The CTLA4/CD28 gene region on chromosome 2q33 confers susceptibility to celiac disease in a way possibly distinct from that of type 1 diabetes and other chronic inflammatory disorders. *Tissue Antigens 56*, 350–355.

Naluai, T.Å., Nilsson, S., Gudjonsdottir, A.H., Louka, A.S., Ascher, H., Ek, J., Hallberg, B., Samuelsson, L., Kristiansson, B., Martinsson, T., Nerman, O., Sollid, L.M., and Wahlström, J. (2001). Genome-wide linkage analysis of Scandinavian affected sib-pairs supports presence of susceptibility loci for celiac disease on chromosomes 5 and 11. *Eur. J. Hum. Genet. 9*, 938–944.

Newberry, R.D., Stenson, W.F., and Lorenz, R.G. (1999). Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. *Nat. Med. 5*, 900–906.

Nilsen, E.M., Jahnsen, F.L., Lundin, K.E.A., Johansen, F.E., Fausa, O., Sollid, L.M., Jahnsen, J., Scott, H., and Brandtzaeg, P. (1998). Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. *Gastroenterology 115*, 551–563.

Nunes, I., Gleizes, P. E., Metz, C.N., and Rifkin, D.B. (1997). Latent transforming growth factor-β binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-β. *J. Cell Biol. 136*, 1151–1163.

Oberhuber, G., Granditsch, G., and Vogelsang, H. (1999). The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur. J. Gastroenterol. Hepatol. 11*, 1185–1194.

Ohta, N., Hiroi, T., Kweon, M.N., Kinoshita, N., Jang, M.N., Mashimo, T., Miyazaki, J., and Kiyono, H. (2002). IL-15-dependent activation-induced cell death-resistant Th1 type CD8 $\alpha\beta^{+}$NK1.1^{+} T cells for the development of small intestinal inflammation. *J. Immunol. 169*, 460–468.

Olaussen, R.W., Johansen, F.E., Lundin, K.E.A., Jahnsen, J., Brandtzaeg, P., and Farstad, I.N. (2002). Interferon-gamma-secreting T cells localize to the epithelium in coeliac disease. *Scand. J. Immunol. 56*, 652–664.

Paulley, J.R. (1954). Observations on aetiology of idiopathic steatorrhoea. *BMJ ii*, 1318–1321.

Petronzelli, F., Bonamico, M., Ferrante, P., Grillo, R., Mora, B., Mariani, P., Apollonio, I., Gemme, G., and Mazzilli, M.C. (1997). Genetic contribution of the HLA region to the familial clustering of coeliac disease. *Ann. Hum. Genet. 61*, 307–317.

Piper, J.L., Gray, G.M., and Khosla, C. (2002). High selectivity of human tissue transglutaminase for immunoactive gliadin peptides: implications for celiac sprue 4. *Biochemistry 41*, 386–393.

Popat, S., Hearle, N., Wixey, J., Hogberg, L., Bevan, S., Lim, W., Stenhammar, L., and Houlston, R.S. (2002). Analysis of the CTLA4 gene in Swedish coeliac disease patients. *Scand. J. Gastroenterol. 37*, 28–31.

Risch, N. (1987). Assessing the role of HLA-linked and unlinked determinants of disease. *Am. J. Hum. Genet. 40*, 1–14.

Roberts, A.I., Lee, L., Schwarz, E., Groh, V., Spies, T., Ebert, E.C., and Jabri, B. (2001). NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. *J. Immunol. 167*, 5527–5530.

Rubin, C.E., Brandborg, L.L., Phelps, P.C., and Taylor, H.C. (1960). Studies of celiac disease. I. Apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. *Gastroenterology 38*, 28–49.

Ryan, B.M. and Kelleher, D. (2000). Refractory celiac disease. *Gastroenterology 119*, 243–251.

Salvati, V.M., Bajaj-Elliott, M., Poulsom, R., Mazzarella, G., Lundin, K.E., Nilsen, E.M., Troncone, R., and MacDonald, T.T. (2001). Keratinocyte growth factor and coeliac disease. *Gut 49*, 176–181.

Salvati, V.M., MacDonald, T.T., Bajaj-Elliott, M., Borrelli, M., Staiano, A., Auricchio, S., Troncone, R., and Monteleone, G. (2002). Interleukin 18 and associated markers of T helper cell type 1 activity in coeliac disease. *Gut 50*, 186–190.

Sanchez, D., Tuckova, L., Sebo, P., Michalak, M., Whelan, A., Sterzl, I., Jelinkova, L., Havrdova, E., Imramovska, M., Benes, Z., Krupickova, S., and Tlaskalova-Hogenova, H. (2000). Occurrence of IgA and IgG autoantibodies to calreticulin in coeliac disease and various autoimmune diseases. *J. Autoimmun. 15*, 441–449.

Sanders, D.S., Patel, D., Stephenson, T.J., Ward, A.M., McCloskey, E.V., Hadjivassiliou, M., and Lobo, A.J. (2003). A primary care cross-sectional study of undiagnosed adult coeliac disease. *Eur. J. Gastroenterol. Hepatol. 15*, 407–413.

Sardy, M., Karpati, S., Merkl, B., Paulsson, M., and Smyth, N. (2002). Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J. Exp. Med. 195*, 747–757.

Sategna, G.C., Solerio, E., Scaglione, N., Aimo, G., and Mengozzi, G. (2001). Duration of gluten exposure in adult coeliac disease does not correlate with the risk for autoimmune disorders. *Gut 49*, 502–505.

Sblattero, D., Berti, I., Trevisiol, C., Marzari, R., Tommasini, A., Bradbury, A., Fasano, A., Ventura, A., and Not, T. (2000). Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. *Am. J. Gastroenterol. 95*, 1253–1257.

Scott, H., Sollid, L.M., Fausa, O., Brandtzaeg, P., and Thorsby, E. (1987). Expression of major histocompatibility complex class II subregion products by jejunal epithelium in patients with coeliac disease. *Scand. J. Immunol. 26*, 563–571.

Seah, P.P., Fry, L., Rossiter, M.A., Hoffbrand, A.V., and Holborow, E.J. (1971). Anti-reticulin antibodies in childhood coeliac disease. *Lancet 2*, 681–682.

Shan, L., Molberg, Ø., Parrot, I., Hausch, F., Filiz, F., Gray, G.M., Sollid, L.M., and Khosla, C. (2002). Structural basis for gluten intolerance in celiac sprue. *Science 297*, 2275–2279.

Shewry, P.R., Halford, N., and Lafiandra, D. (2003). Genetics of wheat gluten proteins. *Adv. Genet. 49*, 111–184.

Sjöström, H., Lundin, K.E.A., Molberg, Ø., Körner, R., McAdam, S.N., Anthonsen, D., Quarsten, H., Norén, O., Roepstorff, P., Thorsby, E., and Sollid, L.M. (1998). Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand. J. Immunol. 48*, 111–115.

Sollid, L.M. (2000). Molecular basis of celiac disease. *Annu. Rev. Immunol. 18*, 53–81.

Sollid, L.M., Markussen, G., Ek, J., Gjerde, H., Vartdal, F., and Thorsby, E. (1989). Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. *J. Exp. Med. 169*, 345–350.

Sollid, L.M., Molberg, Ø., McAdam, S., and Lundin, K.E.A. (1997). Autoantibodies in coeliac disease: tissue transglutaminase—guilt by association? *Gut 41*, 851–852.

Srinivasan, U., Leonard, N., Jones, E., Kasarda, D.D., Weir, D.G., O'Farrelly, C., and Feighery, C. (1996). Absence of oats toxicity in adult coeliac disease. *BMJ 313*, 1300–1301.

Stokes, P.L., Asquith, P., Holmes, G.K., Mackintosh, P., and Cooke, W.T. (1972). Histocompatibility antigens associated with adult coeliac disease. *Lancet 2*, 162–164.

Størsrud, S., Olsson, M., Arvidsson, L.R., Nilsson, L.A., Nilsson, O., and Kilander, A. (2003). Adult coeliac patients do tolerate large amounts of oats. *Eur. J. Clin. Nutr. 57*, 163–169.

Sturgess, R.P., Macartney, J.C., Makgoba, M.W., Hung, C.H., Haskard, D.O., and Ciclitira, P.J. (1990). Differential upregulation of intercellular adhesion molecule–1 in coeliac disease. *Clin. Exp. Immunol. 82*, 489–492.

Sturgess, R., Day, P., Ellis, H.J., Lundin, K.E.A., Gjertsen, H.A., Kontakou, M., and Ciclitira, P.J. (1994). Wheat peptide challenge in coeliac disease. *Lancet 343*, 758–761.

Sulkanen, S., Halttunen, T., Laurila, K., Kolho, K.L., Korponay-Szabo, I.R., Sarnesto, A., Savilahti, E., Collin, P., and Mäki, M. (1998). Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology 115*, 1322–1328.

Tosi, R., Vismara, D., Tanigaki, N., Ferrara, G.B., Cicimarra, F., Buffolano, W., Follo, D., and Auricchio, S. (1983). Evidence that celiac disease is primarily associated with a DC locus allelic specificity. *Clin. Immunol. Immunopathol. 28*, 395–404.

Townley, R.R., Cornell, H.J., Bhathal, P.S., and Mitchell, J.D. (1973). Toxicity of wheat gliadin fractions in coeliac disease. *Lancet 1*, 1363–1364.

Trabace, S., Giunta, A., Rosso, M., Marzorati, D., Cascino, I., Tettamanti, A., Mazzilli, M.C., and Gandini, E. (1984). HLA-ABC and DR antigens in celiac disease. A study in a pediatric Italian population. *Vox Sang. 46*, 102–106.

Troncone, R., Gianfrani, C., Mazzarella, G., Greco, L., Guardiola, J., Auricchio, S., and De Berardinis, P. (1998). Majority of gliadin-specific T-cell clones from celiac small intestinal mucosa produce interferon-gamma and interleukin-4. *Dig. Dis. Sci. 43*, 156–161.

Vader, L.W., de Ru, A., van Der, W.Y., Kooy, Y.M., Benckhuijsen, W., Mearin, M.L., Drijfhout, J.W., van Veelen, P., and Koning, F. (2002a). Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J. Exp. Med. 195*, 643–649.

Vader, W., Kooy, Y., van Veelen, P., de Ru, A., Harris, D., Benckhuijsen, W., Peña, S., Mearin, L., Drijfhout, J.W., and Koning, F. (2002b). The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology 122*, 1729–1737.

Vader, L.W., Stepniak, D.T., Bunnik, E.M., Kooy, Y.M., de Haan, W., Drijfhout, J.W., van Veelen, P.A., and Koning, F. (2003). Characteriza-

tion of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology 125*, 1105–1113.

van Belzen, M.J., Meijer, J.W., Sandkuijl, L.A., Bardoel, A.F., Mulder, C.J., Pearson, P.L., Houwen, R.H., and Wijmenga, C. (2003). A major non-HLA locus in celiac disease maps to chromosome 19. *Gastroenterology 125*, 1032–1041.

van de Wal, Y., Kooy, Y.M.C., Drijfhout, J.W., Amons, R., and Koning, F. (1996). Peptide binding characteristics of the coeliac disease-associated DQ(α1*0501, β1*0201) molecule. *Immunogenetics 44*, 246–253.

van de Wal, Y., Kooy, Y.C., Drijfhout, J.W., Amons, R., Papadopoulos, G.K., and Koning, F. (1997). Unique peptide binding characteristics of the disease-associated DQ(α1*0501, β1*0201) vs the non-disease-associated DQ(α1*0201, β1*0202) molecule. *Immunogenetics 46*, 484–492.

van de Wal, Y., Kooy, Y., van Veelen, P., Peña, S., Mearin, L., Papadopoulos, G., and Koning, F. (1998a). Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. *J. Immunol. 161*, 1585–1588.

van de Wal, Y., Kooy, Y.M., van Veelen, P.A., Peña, S.A., Mearin, L.M., Molberg, Ø., Lundin, K.E.A., Sollid, L.M., Mutis, T., Benckhuijsen, W.E., Drijfhout, J.W., and Koning, F. (1998b). Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc. Natl. Acad. Sci. USA 95*, 10050–10054.

van de Wal, Y., Kooy, Y.M., van Veelen, P., Vader, W., August, S.A., Drijfhout, J.W., Pena, S.A., and Koning, F. (1999). Glutenin is involved in the gluten-driven mucosal T cell response. *Eur. J. Immunol. 29*, 3133–3139.

Vartdal, F., Johansen, B.H., Friede, T., Thorpe, C., Stevanovic, S., Eriksen, J.A., Sletten, K., Thorsby, E., Rammensee, H.G., and Sollid, L.M. (1996). The peptide binding motif of the disease associated HLA-DQ(α1*0501, β1*0201) molecule. *Eur. J. Immunol. 26*, 2764–2772.

Ventura, A., Magazzu, G., and Greco, L. (1999). Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. SIGEP Study Group for Autoimmune Disorders in Celiac Disease. *Gastroenterology 117*, 297–303.

Wang, W., Uzzau, S., Goldblum, S.E., and Fasano, A. (2000). Human zonulin, a potential modulator of intestinal tight junctions. *J. Cell Sci. 113*, 4435–4440.

Weijers, H.A., Lindquist, B., Anderson, C.H.M., Rey, J., Shmerling, D.H., Visakorpi, J.K., Hadorn, B., and Gruttner, R. (1970). Diagnostic criteria in coeliac disease. *Acta. Paediatr. Scand. 59*, 461–463.

West, J., Logan, R.F., Hill, P.G., Lloyd, A., Lewis, S., Hubbard, R., Reader, R., Holmes, G.K., and Khaw, K.T. (2003). Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. *Gut 52*, 960–965.

Wong, R.C., Wilson, R.J., Steele, R.H., Radford-Smith, G., and Adelstein, S. (2002). A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits. *J. Clin. Pathol. 55*, 488–494.

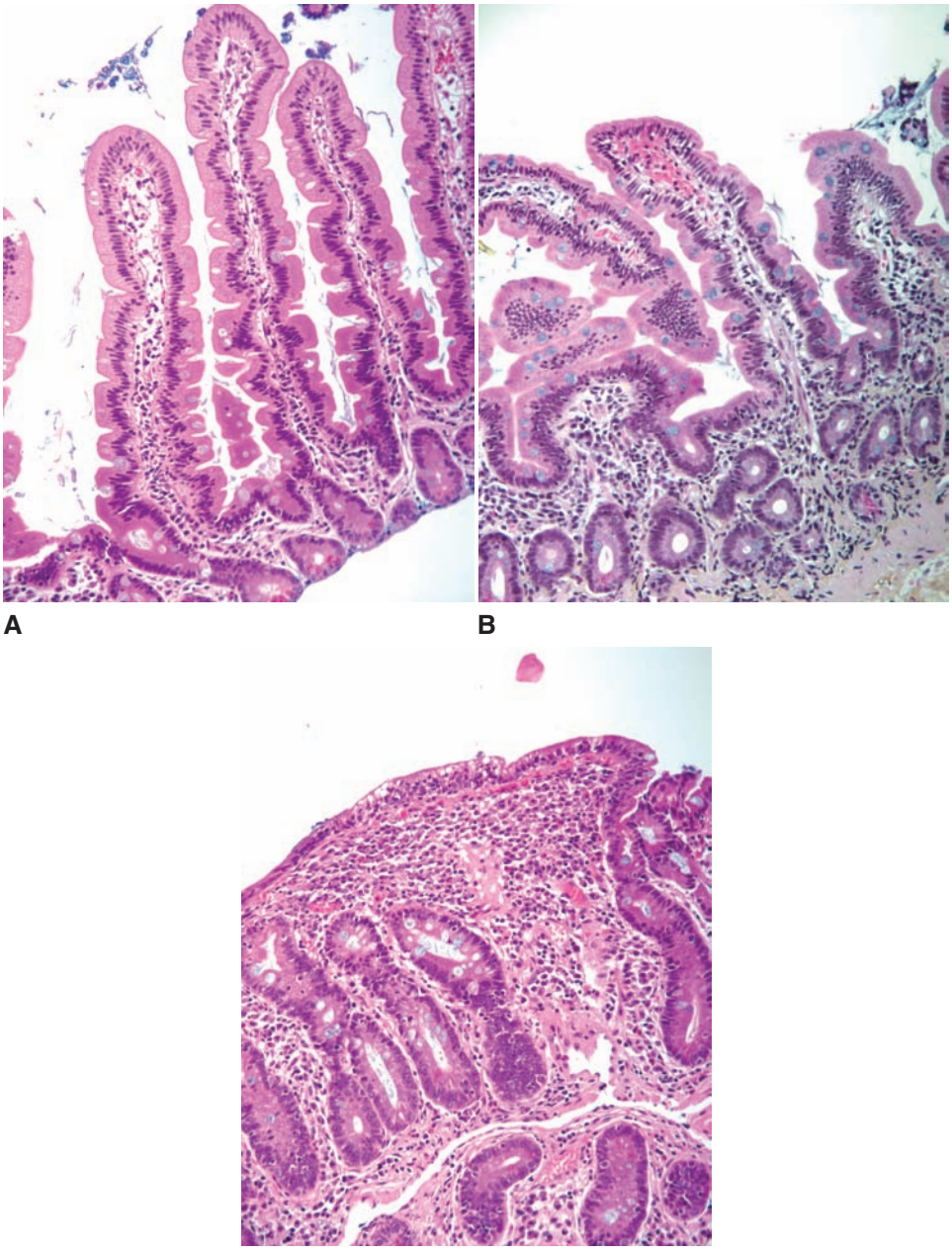
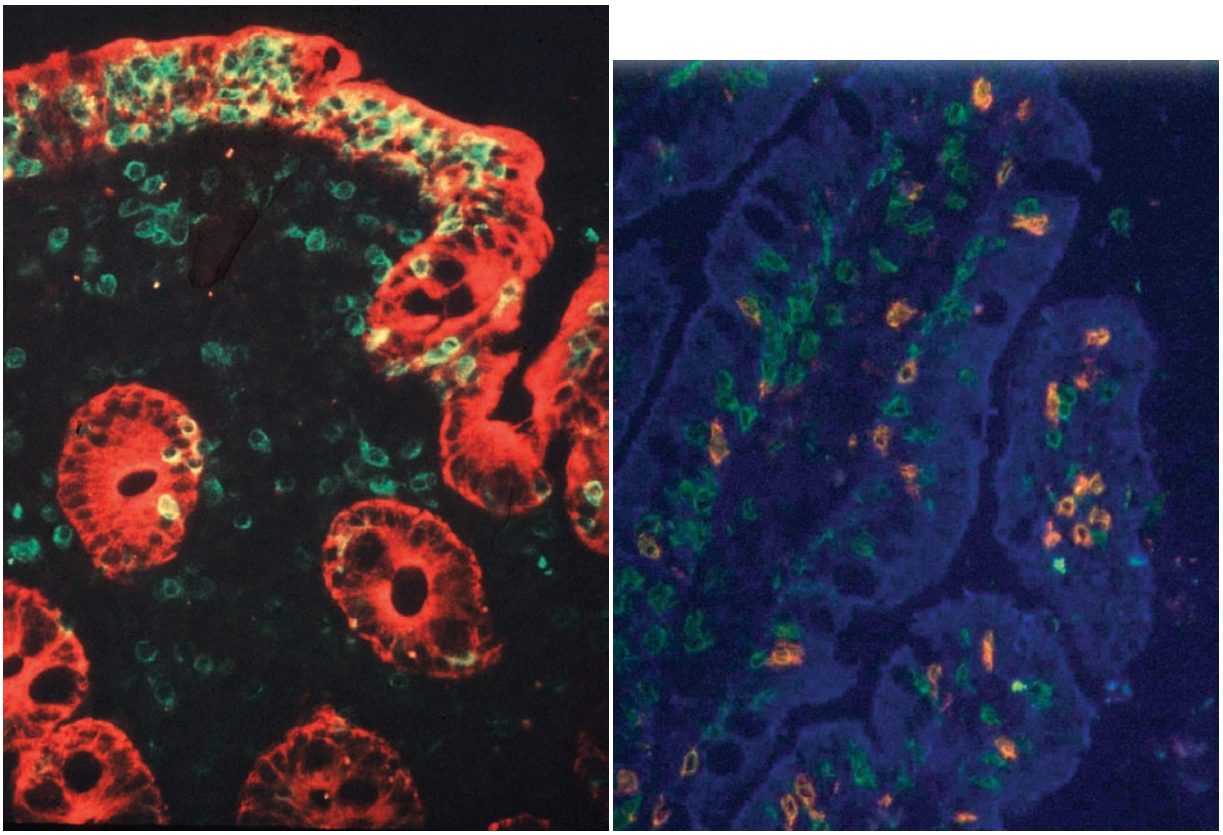


FIGURE 51.1 Histology of small intestinal biopsies. *A*, Normal intestine with long, slim villi and discrete crypts. *B*, Celiac disease Marsh 1 lesion (often referred to as an infiltrative lesion). The epithelium is seeded by intraepithelial lymphocytes but the villi are not significantly shortened and there is little crypt hyperplasia. The patient had a positive endomysium test and clinical celiac disease. *C*, Celiac disease Marsh 3C lesion. No villi are seen and there is predominant crypt hyperplasia. Note the marked increase of intraepithelial lymphocytes (IELs). The different stages of villous structure that define the Marsh 2, 3A (partial villous atrophy), and 3B (subtotal villous atrophy) lesions are not shown.

Courtesy of I. N. Farstad, Rikshospitalet.



A B

FIGURE 51.3 Typical features of celiac disease lesions as illustrated by immunohistochemistry. *A*, Marsh 3C lesion. $CD3^+$ T-cells are stained green and the epithelium is stained red. The striking and typical finding is the heavy infiltration of T-cells within the epithelium. *B*, Marsh 1 lesion. $CD3^+$ T-cells are stained green, T-cell receptor (TCR) $\gamma\delta^+$ cells are stained red and the epithelium is stained blue. There is high proportion of intraepithelial lymphocytes (IELs) expressing both CD3 and TCR $\gamma\delta$ (seen as yellow cells), but the majority of $CD3^+$ IELs are TCR $\gamma\delta^-$ (i.e., TCR $\alpha\beta^+$). The significance of the infiltration of T-cells in the lamina propria and the epithelium is discussed in the text.

Courtesy of I. N. Farstad and Helge Scott, Rikshospitalet.

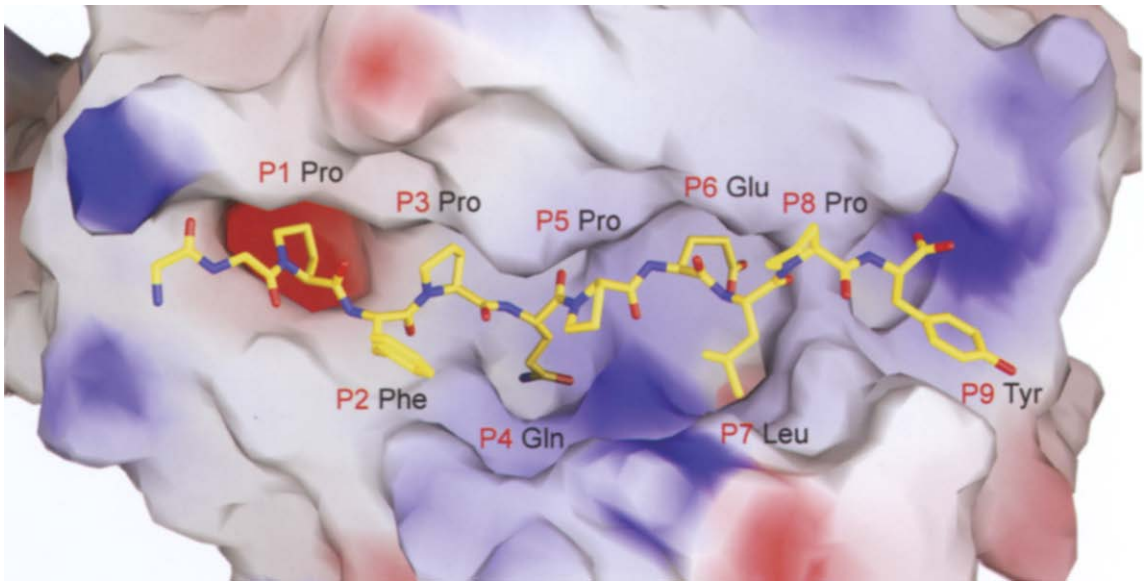
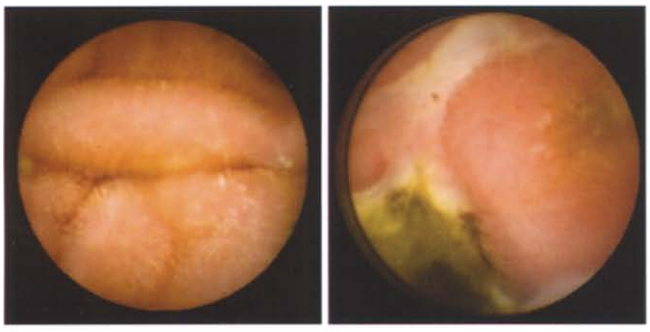


FIGURE 51.6 Three-dimensional structure of the binding site of HLA-DQ2 complexed with a deamidated gliadin peptide (DQ2-α-I epitope—carbon, white; nitrogen, blue; oxygen, red). The electrostatic potential surface of DQ2 is shown as red (negative) and blue (positive).

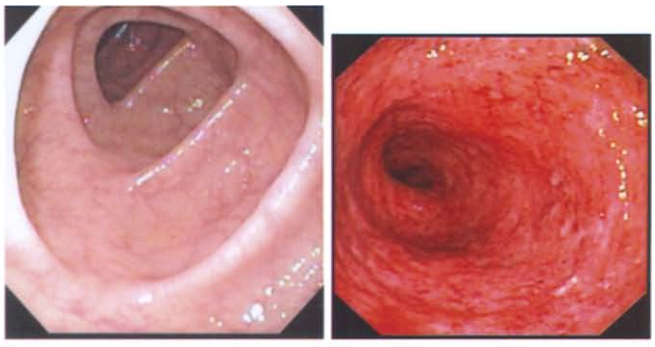
Courtesy of Dr Chu-Young Kim.



A B

FIGURE 52.1 Images from wireless capsule endoscopy. *A*, Normal small bowel. *B*, Deep linear ulcer in the small intestine of a patient with Crohn disease.

Courtesy of Dr Peter Legnani.



A B

FIGURE 52.2 Colonoscopic images. *A*, Normal colon. *B*, Sigmoid colon in severe ulcerative colitis, showing erythema, ulceration, and hemorrhage.

CHAPTER

52

Inflammatory Bowel Diseases: Ulcerative Colitis and Crohn's Disease

RON PALMON AND LLOYD F. MAYER
Mount Sinai School of Medicine, New York, NY

Inflammatory bowel disease (IBD) is divided into 2 distinct but related entities: Crohn's disease (CD) and ulcerative colitis (UC). While the two diseases share some features, there are important clinical differences between them, and so they will be presented separately for part of this discussion. It should also be noted that the etiology and pathophysiology of IBD is not yet clearly established; furthermore, the degree to which these diseases are truly autoimmune in nature is increasingly unapparent.

HISTORY

Numerous case reports exist from the 19th century describing inflammatory diseases of the small and large intestine that may have represented CD (Baron, 2000). Ileocecal inflammation as seen in CD is also characteristic of intestinal tuberculosis, which was the most commonly recognized disease of the small intestine at that time. As microscopy evolved, case series exist of patients with inflammation and strictures of the ileocecal region that were determined to be neither tuberculous nor malignant. These strongly suggest that CD, as we presently know it, existed at least as far back as the 19th century. In fact, the first real report of terminal ileitis (later termed CD) was made by a Scottish physician, Dalziel, in 1824.

The seminal work describing CD, however, was a paper from the Mount Sinai Hospital in New York by Burrill B. Crohn's, Leon Ginzburg, and Gordon D. Oppenheimer (1932) entitled *"Regional Ileitis: A Pathologic and Clinical Entity."* The authors described a series of 14 patients operated upon by Dr. A. A. Berg with granulomatous inflammation of the terminal ileum. The title of the paper reflected Crohn's belief that the disease affected only the ileum (Wells, 1952) despite evidence, presented by Ginzburg and Oppenheimer (1932) and others, of cases affecting the small and large intestine. Interestingly, the disease came to be known as Crohn's disease only because the authors agreed to list their names alphabetically and because the surgeon Berg humbly declined to be included as an author (Baron, 2000).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Early work on UC was also complicated by difficulty in distinguishing it from infectious colitides such as bacillary dysentery. Credit for the discovery of UC as a distinct entity has been given to Samuel Wilks (1859) who termed it "idiopathic colitis." The term "ulcerative colitis" was first used by Hale-White (1888), although in retrospect these patients may not have had UC but rather irritable bowel syndrome (Baron, 2000). Further work was facilitated by the introduction of the electric sigmoidoscope; studies by Sir William Hurst (1909) and also Lockhart Mummery (Mummery, 1907) helped to clarify the appearance of the colon in UC.

EPIDEMIOLOGY AND ENVIRONMENTAL FACTORS

The highest rates of IBD are found in northern Europe, the United Kingdom, and North America (Loftus, 2004). However, it is increasingly recognized that IBD can occur in persons from other areas such as Africa, Latin America, and Asia. Extrapolation of incidence and prevalence rates for North America as presented by Loftus (2004) and suggest the following: approximately 780,000 persons in the U.S. and Canada have UC and 630,000 have CD. Between 7,000 and 46,000 new diagnoses of UC are made yearly and between 10,000 and 47,000 new diagnoses of CD are made. Similar to multiple sclerosis, IBD does appear to be more frequent in the northern latitudes (Blanchard et al., 2001; Sonnenberg et al., 1991).

Inflammatory bowel disease may present at any age; however, it is most commonly diagnosed in the second or third decade of life. There does not seem to be a major gender preference in IBD. Other than genetic risk factors, which will be addressed in a separate section, several environmental risk factors have been hypothesized to predict the development or course of IBD. Cigarette smoking is an important risk factor with divergent effects on CD and UC. Numerous studies have confirmed that smoking is associated with a higher risk of developing CD (Calkins, 1989), an earlier need for surgery for CD (Sands et al., 2003), and earlier relapse after surgery (Timmer et al., 1998). Conversely, cigarette smokers are less likely to develop UC (Calkins, 1989) or to require colectomy for UC (Boyko et al., 1988). In numerous case–control studies as well as a meta-analysis by Koutroubakis et al. (2002), appendectomy has been shown to protect against the subsequent development of UC. The pathophysiologic and protective effects of smoking and appendectomy are as yet unclear. Numerous other environmental factors have been studied, including breastfeeding, diet, measles vaccination, oral contraceptives, and various infectious agents. However, none of these have been conclusively shown to affect the development or course of IBD.

CLINICAL AND PATHOLOGIC FEATURES

Disease Presentation

Crohn's Disease

A hallmark of CD is its ability to involve any part of the gastrointestinal (GI) tract from mouth to anus. Multiple sites in the intestine can be affected in the same individual. The inflammation in CD, as distinct from UC, is discontinuous; diseased bowel is separated by areas of healthy bowel (so-called "skip lesions"). The location of CD in the bowel usually falls into one of three patterns: isolated small bowel disease, combined small and large bowel disease, or isolated colitis. The most commonly affected segment of bowel is the terminal ileum, which is diseased in two-thirds of patients (Munkholm and Binder, 2004). Colonic involvement in CD typically does not involve the rectum, which is always involved in UC. A small percentage of patients have disease of the duodenum, jejunum, or esophagus only.

Crohn's disease can also be divided into 3 types of disease behavior: pure inflammatory, stricturing, or fistulizing. These behavior types are not mutually exclusive; however, the combination of disease behavior and location can nonetheless often dictate clinical presentation. For example, a common presentation of CD is inflammatory disease of the terminal ileum with symptoms of pain, diarrhea, and weight loss. When inflammatory disease involves the colon, a more UC-like syndrome ensues with diarrhea and occasional rectal bleeding. Stricturing disease can be more subtle in development, with a long time course of relatively normal bowel function before symptoms of pain, bloating, and eventually obstruction ensue.

The ability of CD to cause fistulae reflects its transmural nature. Ulcerative colitis involves only the mucosal layer of the GI tract while CD can involve all layers from mucosa to serosa. When inflammation is severe enough to extend from mucosa to serosa, fistulae result such that the contents of the intestinal tract spill out into an adjacent space. The perianal region is the most common site of fistula development in CD. Approximately 20% of patients with CD will have perianal fistulae (Schwartz et al., 2002) which present clinically with perianal abscesses that may spontaneously drain. Fistulas can also arise in the bowel and invade other parts of bowel, bladder, vagina, or psoas muscles causing abscesses or frank drainage of stool.

Another characteristic feature of CD is its capacity to recur following surgery. Resection of an affected segment of bowel followed by reanastomosis of the histologically uninflamed margins invariably (98% by endoscopy) leads to the development of recurrent disease at the anastomosis, generally on the proximal side, although other sites can develop disease. The predilection for this site of recurrence may relate to the presence of an overgrowth of luminal micro-

organisms or potentially to vascular factors. In contrast, surgery for UC is associated with a "cure." Since UC is a colonic disease, the surgery—total proctocolectomy and end ileostomy—completely removes the site where disease can exist. However, this concept has had to be reassessed in recent years. A new surgical approach, restorative proctocolectomy, eliminates the ileostomy and involves the creation of a reservoir, fashioned from the terminal ileum, which resides within a denuded rectal muscular tube. Up to 50% of UC patients develop a syndrome called pouchitis within the ileal reservoir that is clearly bacterially driven i.e. it responds to antibiotic therapy. Interestingly, patients with familial polyposis, a premalignant non-inflammatory disease of the colon, rarely if ever develop pouchitis following a similar procedure. This clinical observation provides evidence for a broader mucosal immune defect in UC patients that is only exposed following the "colonization" of the pouch flora.

Ulcerative Colitis

Ulcerative colitis affects only the colon. As a result, its presentation is more consistent than that of CD. It usually presents with symptoms of diarrhea and rectal bleeding. Abdominal pain and constitutional symptoms (fever, weight loss) are less frequent than in CD, except in severe UC flares. The amount of colon that is involved in UC can vary from the entire colon (pan-colitis) to just the most distal part (left-sided colitis or proctitis). As a rule, the more proximal colon will only be affected when the more distal area is as well. Thus the endoscopic findings of isolated right-sided colitis (in combination with other features such as rectal sparing) argue strongly for a diagnosis of CD as opposed to UC. Isolated proctitis can present as tenesmus or rectal bleeding in the absence of diarrhea given the normal proximal colon. Infrequently, pan-colitis can progress to toxic megacolon, a massively dilated colon with accompanying fever and tachycardia; immediate surgery is most often indicated for this condition.

Pathology

Microscopy of specimens from endoscopic mucosal biopsies or surgical resections can help ascertain the diagnosis of IBD and occasionally distinguish between UC and CD. Common features of IBD include ulceration, acute and chronic inflammatory infiltrate, crypt abscesses, and crypt distortion. Though suggestive of IBD, these changes can sometimes be seen in other conditions such as infectious colitis. The findings of transmural inflammation (usually discernible only in surgical specimens) or of non-caseating granulomas suggest a diagnosis of CD as opposed to UC. Unfortunately, granulomas may be identified in as few as 15% of endoscopic biopsies (Okada et al., 1991), and therefore the absence of granulomas does not rule out CD. The cellular infiltrate in the CD mucosa is one of activated lymphocytes (mostly T-cells) and macrophages. In UC, neutrophils and mast cells predominate although activated lymphocytes and macrophages are present as well.

There are characteristic gross features of CD and UC that can be seen by the naked eye at surgery or during endoscopy. The propensity to have normal areas interspersed with areas of active inflammation in CD ("skip lesions") favors the development of a classical gross feature called cobblestoning. Longitudinal fissures dissect normal mucosa, leading to this finding. Furthermore, the transmural nature of the inflammatory process in CD is seen as "creeping fat" with envelopment of the bowel with fat starting from the mesenteric border. Stricturing is also a common feature of CD with fixed fibrotic stenotic segments interspersed throughout the bowel. Areas of the bowel proximal to the stenotic segment are dilated and, therefore, subject to bacterial overgrowth, potentially worsening the malabsorption seen in this disease. In contrast, UC is characterized by an ulcerated, edematous, and friable mucosa with frank bleeding. The continuous nature of the inflammatory process allows a reasonable assessment of the degree of disease activity at all sites just by looking at the rectum (unless the patient is on topical medications).

Colorectal Cancer and Inflammatory Bowel Disease

The most feared complication of IBD is the development of colorectal cancer (CRC). Numerous studies have confirmed the increased risk of CRC in UC as well as Crohn's colitis. A meta-analysis by Eaden (2001) found the prevalence of CRC in patients with UC to be 3.7%. In anatomically extensive Crohn's colitis, the risk of colon cancer is similar (Sachar, 1994). The factors that increase risk of CRC in patients with IBD include duration and extent of disease, family history of CRC, and presence of primary sclerosing cholangitis (Itzkowitz and Harpaz, 2004); the most important of these is duration of disease. Colorectal cancer is rarely found in patients with less than 7 years of colitis. After 10 and 20 years of disease, the risk of CRC is 2% and 8%, respectively (Eaden, 2001).

The molecular mechanisms of CRC development in the sporadic population (those without IBD) have been extensively studied. In sporadic CRC, there is a sequence of mutations that leads to carcinogenesis. For example, a mutation in the *APC* gene is the initiating event, while mutations in p53 occur late in carcinogenesis. In colitis-associated CRC, the p53 mutations occur earlier and *APC* mutations occur late if at all (Itzkowitz, 2004). Clinically, mutations that appear earlier in the pathway are reflected in the development of dysplasia, precancerous changes that can be detected on mucosal biopsy. Colonoscopic surveillance

programs are recommended for patients with long-standing colitis to detect early evidence of malignancy and then perform colectomy before the development of CRC.

Extraintestinal Manifestations

Although IBD is considered a primary disease of the bowel, it is a systemic disease. The most commonly involved organs outside the gut are the skin, joints, eyes, and liver. Many of the extraintestinal manifestations of IBD are associated with other autoimmune and inflammatory disorders or can exist as primary disorders.

The most common skin manifestation of IBD is erythema nodosum (EN), which typically presents as painful red nodules on the pretibial region. It occurs in 5–10% of patients with IBD (Greenstein et al., 1976), is more frequent in women, and both flares and resolves along with the underlying bowel disease. Erythema nodosum also occurs with sarcoidosis, post-streptococcal infection, and oral contraceptive use. Pyoderma gangrenosum (PG) is less common but more troublesome than EN; it begins as a fluctuant nodule on the skin with eventual ulceration and central necrosis. It is most commonly observed on the trunk and extremities. Unlike EN, PG does not parallel the activity of underlying IBD. It can frequently occur in the absence of IBD or precede the development of IBD.

The joint manifestations of IBD include both axial and peripheral arthropathies. The axial arthropathies of IBD are ankylosing spondylitis (AS) and isolated sacroiliitis (see Chapter 33). Both present with back pain as a result of inflammation of the sacroiliac joint, but only in AS is there progressive involvement of the spinal column with severe limitation of mobility. Between 50% and 80% of patients with IBD-associated AS are HLA-B*27 positive (Orchard and Jewell, 2004); presumably, possession of this allele affects the presentation of an as yet unknown bacterial antigen. The course of AS is independent of the bowel disease. The most common peripheral arthropathy of IBD is the type I or pauciarticular arthritis, which can present as pain and swelling of the knees, wrists, ankles, or other joints. The arthritis is typically asymmetric, involves fewer than four joints, and does not cause destruction or erosion of the joints. This form of arthritis usually resolves with improvement of the underlying bowel disease.

Ocular involvement in IBD ranges from inflammation of the more superficial layers, such as the conjunctiva and sclera, to the inner layers such as the uvea. Superficial involvement can cause redness and irritation, but the development of pain or blurry vision suggests uveitis and warrants prompt ophthalmologic evaluation with a slit-lamp exam. Treatment with topical steroids is usually effective, and permanent loss of vision is rare.

The major extraintestinal manifestation of IBD in the liver is primary sclerosing cholangitis (see Chapter 55). This is a chronic cholestatic disease, which can present initially with abnormal liver chemistries, with the subsequent development of jaundice and then cirrhosis.

Differential Diagnosis

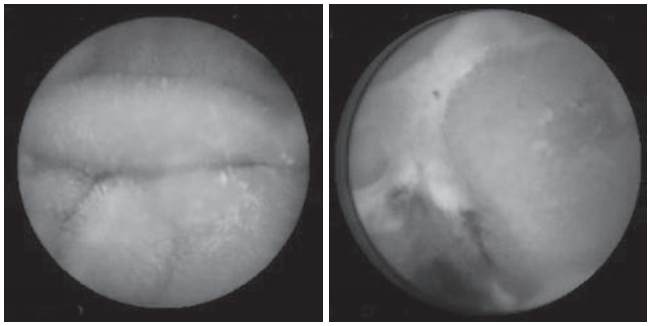
Common examples in the differential diagnosis of IBD are presented in Table 52.1. There is no pathognomonic clinical feature or test that allows one to make a diagnosis of CD or UC with absolute certainty. However, a careful examination of clinical, endoscopic, radiographic, laboratory, and pathologic data will usually allow the clinician to make a diagnosis (Figures 52.1 to 52.3). Features of the disease may change over time, occasionally even requiring a modification of the original diagnosis. In approximately 10% of patients with colitis, a distinction between UC and CD cannot be made; this is termed indeterminate colitis. Serologic tests may be helpful in this situation (see Serologic Markers).

Genetics

Several factors point to a genetic contribution to the etiology of IBD. Rates of IBD are considerably higher in certain ethnic groups such as Ashkenazi Jews compared with others living in the same geographic area. Relatives of patients with IBD also have a higher rate of developing

TABLE 52.1 Examples of the major categories in the differential diagnosis of inflammatory bowel disease

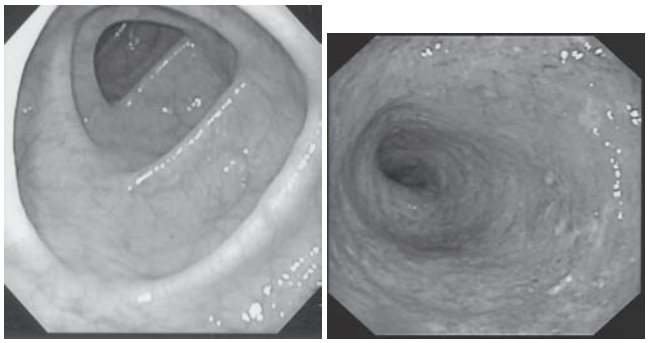
Category	Examples
Infectious	Bacterial (*Salmonella*, *Shigella*, *Escherichia coli*, *Yersinia*, *Clostridium difficile, Campylobacter*) Viral (Cytomegalovirus) Mycobacterial (*M. tuberculosis*, *M. avium-intracellulare*) Parasitic (*Entamoeba histiolytica*, *Strongyloides*, *Giardia*)
Vascular	Vasculitis (polyarteritis nodosa, Henoch-Schönlein purpura) Radiation-induced enteritis Ischemia
Oncologic	Lymphoma, adenocarcinoma, carcinoid, metastatic disease
Inflammatory	Microscopic colitis, diverticular colitis, diversion colitis, celiac disease
Functional	Irritable bowel syndrome
Drug-induced	Nonsteroidal anti-inflammatory drugs



A B

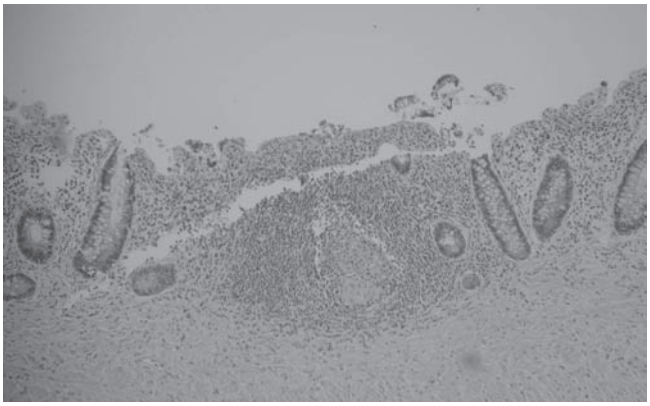
Figure 52.1 Images from wireless capsule endoscopy. *A,* Normal small bowel. *B,* Deep linear ulcer in the small intestine of a patient with Crohn's disease. (See color plate section.)

Courtesy of Dr Peter Legnani.

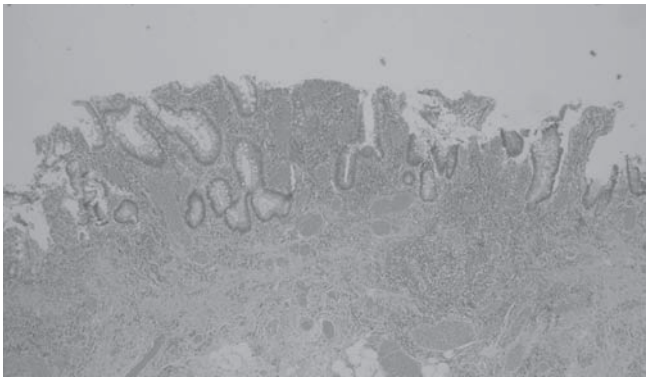


A B

Figure 52.2 Colonoscopic images. *A,* Normal colon. *B,* Sigmoid colon in severe ulcerative colitis, showing erythema, ulceration, and hemorrhage. (See color plate section.)



A



B

Figure 52.3 Histology of inflammatory bowel disease. *A,* Crohn's disease in the left colon showing a non-necrotizing granuloma, inflammatory cell infiltrate, and Paneth cell metaplasia. *B,* Ulcerative colitis showing crypt architecture distortion, hemorrhage, ulceration, and inflammatory cell infiltrate. (See color plate section.)

Courtesy of Dr Mikhail Tismenetsky.

the disease. For monozygotic twins, the concordance rate is approximately 40–60% for CD and 5–20% for UC (Halfvarson et al., 2003; Orholm et al., 2000; Subhani et al., 1998). This suggests that CD may have more of a genetic basis, but, clearly, since both rates are much less than 100% the environment must play a role as well. Between 5% and 10% of patients with IBD will have an affected first-degree relative, and the subtype (CD vs. UC) will be the same in 75–80% of cases (Binder, 1998). This corresponds to about a 15-fold increased risk of disease if one has a first-degree relative with IBD (Peeters et al., 1996; Satsangi et al., 1994). Lastly, certain rare genetic syndromes include IBD or an IBD-like component; these include Turner syndrome, Hermansky-Pudlak syndrome, and glycogen storage disease type Ib.

Much effort has been directed towards identifying the specific genes involved in IBD. Genome-wide screening using microsatellite markers have identified seven loci, named *IBD1–7*, that meet criteria for linkage to *IBD* (Ahmad et al., 2004). In 2001, the first CD susceptibility gene was identified within the *IBD1* locus on chromosome 16q12 (Hugot et al., 2001; Ogura et al., 2001a; Hampe et al., 2001). This gene, named *CARD15* (formerly called *NOD-2*), encodes an intracellular protein containing two C-terminal caspase recruitment domains (*CARDs*), a nuclear binding domain, and a leucine-rich repeat (LRR) region (Ogura et al., 2001b). All of the described CD mutations are in the LRR. The LRR appears to be involved in recognition of peptidoglycans (muramyl dipeptide) from gram-positive bacteria (Chamaillard et al., 2003). *In vitro* evidence suggests that mutations in the LRR region may affect innate immunity by decreasing the ability of intestinal epithelial cells and macrophages to clear luminal and intracellular bacteria (Hisamatsu et al., 2003). Normal activation of *CARD15* results in the activation of NFκB, which promotes an inflammatory response (Ogura et al., 2001b). *CARD15* mutations

appear to affect this pathway but the exact mechanisms are still unclear.

There are three common mutations within or near the LRR region on *CARD15* that are associated only with CD and not UC. Of patients with CD, 10–30% are heterozygotes for these mutations, and 10–15% are homozygotes or compound heterozygotes (Hugot et al., 2001; Ogura et al., 2001a; Hampe et al., 2001). In controls without CD, 8–15% are heterozygotes and 0–1% are homozygotes. Carrying one of these mutations increases the relative risk of CD significantly, although the absolute risk remains small (Table 52.2). Numerous studies have shown that *CARD15* variants are associated specifically with younger age of onset, ileal disease, and stricturing phenotype (Lesage et al., 2002; Ahmad et al., 2002). Of note, the contribution of *CARD15* to CD is strongest in whites; studies of African-Americans populations show weaker associations (Bonen et al., 2002), while *CARD15* mutations have not been found in Asians (Inoue et al., 2002).

The identification of *CARD15* as a CD susceptibility gene is an important development in IBD research. However, only a minority of cases of CD worldwide are associated with *CARD15* mutations. The relevant genes on other loci are beginning to be elucidated. *IBD3* on chromosome 6 contains the major histocompatibility complex (MHC) regions; numerous studies have shown various HLA associations with CD and UC (Stokkers et al., 1999). *IBD5* on chromosome 5 has recently been shown to contain the organic cation transporter (*OCTN*) gene cluster. Homozygosity for two linked single-nucleotide polymorphisms in the *OCTN* gene cluster confers a 3- to 5-fold increase in the risk of CD (Peltekova et al., 2004). Furthermore, possessing both the *OCTN* and *CARD15* mutations increases the risk of CD much more. The *OCTN* genes are expressed in the intestinal epithelium, but their function has not yet been explored. Variation in *DLG5*, a newly discovered gene on chromosome 10, appears to increase the risk of both UC and CD (Stoll et al., 2004). *DLG5* may be important in the pathogenesis of IBD as it encodes a protein involved in maintenance of epithelial integrity. Clearly, there is much that remains to be learned about the genetic basis of IBD.

TABLE 52.2 Relative and absolute risk of Crohn's disease based on *CARD15* genotype variants*

	Relative risk	Absolute risk
No variant	1	7×10^{-4}
Simple heterozygote	3	2×10^{-3}
Homozygous	38	3×10^{-2}
Compound homozygous	44	2×10^{-2}

*Data from Hugot et al. (2001).

ANIMAL MODELS OF INFLAMMATORY BOWEL DISEASE

Probably the greatest advance in the study of IBD has been in the identification of a number of animal models (Table 52.3). These models have enabled investigators to test hypotheses regarding the pathophysiology and treatment of IBD. Initial animal models were based on the induction of intestinal inflammation by a chemical agent. These chemically induced models are limited in that acute, rather than chronic, inflammation is usually the result. In addition, there does not appear to be a convincing role for T-lymphocytes in the majority of these models. Recently, genetically engineered mice and rats have been developed whereby chronic models of T-lymphocyte-mediated injury, more consistent with the human disease, can be tested.

Chemically-Induced Models

A representative example of a chemically-induced model of IBD is the dextran-sodium sulfate (DSS) model. Administration of 5% DSS in drinking water can induce an acute colitis in most mice with symptoms such as bloody diarrhea and weight loss (Cooper et al., 1993). Repeated administration of DSS can also induce chronic inflammation. Histologic changes ensue such as inflammatory cell infiltration and ulcerations (Sartor, 2004). However, the role of intestinal bacteria is questionable as germ-free mice are also susceptible to DSS-induced colitis (Kitajima et al., 2001). The effects of DSS appear to be directed at the intestinal epithelium (?direct toxic injury/effect). Furthermore, it does not appear that T-lymphocytes are important in this model as SCID mice will also develop colitis in response to DSS (Axelsson et al., 1996). Several other chemical agents have been used in mice and rats such as acetic acid, indomethacin, trinitrobenzene sulfonic acid (TBNS), and oxalazone (the latter 2 involving different sub-populations of T-cells, Th1 and Th2, respectively). These models appear to be more useful in studying epithelial response to injury rather than true chronic immune-mediated injury. However, they have been used extensively in drug discovery.

TABLE 52.3 Commonly used animal models of inflammatory bowel disease

Category	Examples
Chemically-induced	Dextran-sodium sulfate, acetic acid, indomethacin, trinitrobenzene sulfonic acid, oxalazone, peptidoglycan-polysaccharide
Genetic	IL-$2^{-/-}$, IL-$10^{-/-}$, TCR$\alpha^{-/-}$
Transfer	$CD45RB^{high}$ T-cell to SCID mice
Spontaneous	C3H/HeJ Bir, SAMP-1/Yit, cotton-top tamarin

An interesting variant of the induced model is the peptidoglycan-polysaccharide (PG-PS) enterocolitis model. Peptidoglycan-polysaccharide is a component of bacterial cell walls that stimulates inflammation via activation of NFκB. Injection of PG-PS directly into the wall of the ileum and colon induces a chronic enterocolitis, peripheral arthritis, and anemia in Lewis rats (McCall et al., 1994; Sartor et al., 1996). This chronic inflammation is T-lymphocyte mediated and reversed by corticosteroids (Herfarth et al., 1998).

Genetic Models

A common theme for the development of these models is the deletion of a gene encoding a cytokine or its receptor. Interleukin 2 (IL-2) is necessary for T-cell replication, regulatory T-cell activation, as well as programmed cell death. Mice deficient in IL-2 actually have increased numbers of activated T cells early in life, perhaps from defective apoptosis (Kneitz et al., 1995). The IL-$2^{-/-}$ mouse develops pancolitis, diarrhea, weight loss, and eventual death (Sadlack et al., 1993). Histologic changes resemble those of IBD. In this model there is also evidence of autoimmunity as they develop autoimmune hemolytic anemia at an early age. In fact many mice fail to survive long enough to develop the IBD due to the profound anemia seen at an early age. Evidence suggests that T cells secreting a Th1 cytokine profile are the mediators of colitis in this model (Ehrhardt et al., 1997). Germ-free mice develop only a mild colitis, if any (Schultz et al., 2001). The dominant T-cell nature of this and other models has been underscored by the finding that IBD is still seen in the $IL2^{-/-}$ mouse even in B-cell deficient mice. In fact, in some models the absence of B cells is associated with an even more aggressive course (i.e., $TCR\alpha^{-/-}$ mouse). Thus the animal models of IBD have helped to raise further doubt regarding the autoimmune nature of these diseases.

Interleukin 10 is an important inhibitory cytokine that can cause a decrease in T-cell activation. SPF IL-$10^{-/-}$ mice develop a progressive pan-colitis with weight loss (Kuhn et al., 1993). The ability of antibodies to interleukin 12 (anti-IL-12), interferon-γ (anti-IFNγ) and tumor necrosis factor (anti-TNF) to block colitis highlights the importance of the Th1 response, as in the IL-$2^{-/-}$ model (Davidson et al., 1998). As in the $IL2^{-/-}$ mouse, B-cells do not appear to play a positive role in the development of intestinal inflammation in the $IL10^{-/-}$ model. However, the role of commensal bacteria in pathogenesis of the colitis is suggested by observations that germ-free mice do not develop colitis, and that subsequent introduction of such bacteria will induce a rapid inflammatory response (Sellon et al., 1998). Furthermore, probiotics and antibiotics prevent or ameliorate colitis (Madsen et al., 2000). Interestingly, administration of nonsteroidal anti-inflammatory drugs (NSAIDs) to IL-$10^{-/-}$ mice results in a much more rapid development of colitis (Berg et al., 2002). Inhibition of prostaglandins by NSAIDs may hasten colitis by interfering with important immunomodulatory functions and by disrupting the intestinal epithelium, leading to an abnormal inflammatory response to intestinal bacteria.

The $TCR\alpha^{-/-}$ mouse also develops colitis spontaneously around 10–14 weeks of age but the immune response in the gut is more Th2 in nature (Mombaerts et al., 1993). These findings support the concept that an immune imbalance of any type (Th1 or Th2) is poorly tolerated by the mucosal immune system and that tight regulation is critical for normal mucosal homeostasis. T-cells are the major regulators of this mucosal homeostasis; B-cells and antibodies, while present in abundant quantities, play a regulatory rather than an inflammatory role in disease development.

Transfer Models

Adoptive transfer of $CD45RB^{high}$ $CD4^+$ T cells from normal mice to SCID mice induces non-bloody diarrhea, weight loss, and death within 4–8 weeks (Morrissey et al., 1993). $CD45RB^{high}$ is a marker of naïve T cells (as well as Th1 cells), and expression decreases once T cells are activated. Interestingly, co-transfer of $CD45RB^{low}$ $CD4^+$ T cells prevents colitis, suggesting that these cells have an important regulatory function. Evidence suggests that colitis in this model is induced by naïve T cells in the presence of commensal enteric bacteria (Sartor, 2004). This model was the first to truly document the role of regulatory cells in mucosal homeostasis. This has led to the concept that the defect in IBD is related to an overzealous immune response due to a defect in immune regulation.

Spontaneous Models

There are two inbred strains of mice, the C3H/HeJ Bir mouse (Sundberg et al., 1994) and the SAMP-1/Yit mouse (Matsumoto et al., 1998), that develop a Crohn's-like phenotype with ileocolonic and perianal disease. These spontaneous models are presumed to be genetic mutations but have yet to be fully elucidated. Some gene candidates have been identified and are similar to disease modifying genes in the IL-$10^{-/-}$ model. The cotton-top tamarin in captivity will spontaneously develop a chronic colitis with periodic flares of disease and ultimately colonic adenocarcinoma (Chalifoux and Bronson, 1981). This model is used less frequently due to limited access to the animals.

IMMUNOPATHOGENESIS

Since the mid-1990s there has been an explosion in our knowledge of IBD pathogenesis. Such advances have directly contributed to the growing number of specific

biologic therapies for IBD. These advances have come from the identification of a large number of animal models, genome-wide scans leading to the identification of a gene *NOD2/CARD15* (Hugot et al., 2001; Ogura et al., 2001a) associated with CD (as well as two other genes *OCTNI/II* and *DLG5*) (Peltekova et al., 2004; Stoll et al., 2004), and a renewed appreciation of the existence of regulatory cells. We have also learned from the successes and failures of available targeted biologic therapies in humans. The overall hypothesis guiding the current advances is that IBD occurs as a result of a dysregulated $CD4^+$ T-cell response to a number of bacterial antigens (normal flora) in a genetically susceptible host. The animal models have served to underscore the role of the luminal flora. In virtually every model identified to date (over 50 described), the animals fail to develop disease when reared in a germ-free environment (Podolsky, 1997). When normal flora are added, disease ensues rapidly. In some studies, addition of as few as one nonpathogen is sufficient to initiate the disease process. Furthermore, more recent studies from Sartor's group (Rath et al., 2001) suggest that specific bacteria trigger disease in different locations. Such a finding goes a long way towards explaining the segmental nature of CD. The models have also provided validation of the hypothesis regarding the concept that CD is a multigenic disorder and that the input of different genes provides the substrate for specific disease expression. For example, an $IL\text{-}10^{-/-}$ mouse on a C57BL/6 background develops disease early, but if the same defect in IL-10 is bred onto other backgrounds there is a modification of the disease expressed (ranging from more severe to protected from disease) (Powrie, 1995). Therefore, IBD is clearly a multigenic disorder providing a rationale for the variability in disease expression. We have also learned that the animal models are not really classical IBD. The overwhelming majority of models have colonic disease only and respond to therapies that have no effect on human disease (e.g. IL-10) (Lindsay and Hodgson, 2001). However, most importantly, we have learned that multiple distinct immune defects can result in overt clinical and histologic disease. Defects in innate immunity (targeted STAT3 knockout in macrophages) (Welte et al., 2003) as well as in Th1 and Th2 immune responses can lead to inflammation.

The take-home message is that too much of any type of immune response is poorly tolerated in the mucosal immune system. This makes immunologic sense since the mucosal immune system is continuously challenged by bacterial and dietary antigens, but a state of suppressed and controlled immunity is the norm. Disruption of this control in any way results in inflammation. Failure to regulate the inflammation results in its persistence. Oxazalone colitis occurs because of an overproduction of IL-13 by NK T cells (Heller et al., 2002). TNBS colitis is Th1 driven (Neurath et al., 2000). How these correlate to human CD or UC has yet to be defined.

The genetic defects described have a common feature in that the gene products are expressed in the epithelium. *NOD2/CARD15* is an intracellular pattern-recognition receptor recognizing muramyl dipeptide on gram-positive organisms (Girardin et al., 2003). The mutations result in a failure to signal and may be related to a loss of regulation of TLR2 signaling, or the persistence of intracellular bacteria in intestinal epithelial cells (IECs) or macrophages (Watanabe et al., 2004). Persistent intracellular infection is associated with granuloma formation similar to that seen in CD. *OCTNI* and II are cation transporters found in IECs (Peltekova et al., 2004) and in part may contribute to permeability or integrity of the mucosal barrier.

The identification of genes associated with IBD has served to clarify some of the pathogenetic issues, but the major focus of various laboratories studying these diseases has been on the role of the immune system. While there are several "camps" focused on a given cell type or component of the immune system responsible for development of disease (epithelial cell, T cell, innate immunity, adaptive immunity, macrophage/DC), the final outcome will likely be some mixture of the various models. Defects in permeability have been described for years, but a specific defect has not been identified (Meddings, 1997). Epithelial cells are at the interface between the outside world and the enormous lymphoid mass that is the Gut-associated lymphoid tissue (GALT). Epithelial cells can sample antigen, present it to T cells, form a tight barrier, transport IgA, and secrete pro- and anti-inflammatory cytokines and chemokines (Mayer and Shlien, 1987; Kaiserlian et al., 1989; Mostov, 1994; Jung et al., 1995; Allez et al., 2002). Due to the normal differentiation process of the IEC these properties may differ from the crypt to the surface. The IEC also expresses a large number of nonclassical class I molecules embedding its role as a bridge between innate and adaptive immunity (Campbell et al., 1999). Dendritic cells and macrophages also communicate with both arms of the immune system, and each has been implicated in human disease and murine models.

As alluded to above, there is increasingly less evidence that B cells and antibodies play a role in the disease process of IBD. This may be different in CD or UC: in CD, classic Th1-mediated immunopathology is seen (i.e. granulomas, T-cell, and macrophage activation); in UC, the pathology is more reminiscent of an Arthus reaction, where neutrophil emigration and mast cell degranulation are commonplace. Immune complexes might form in the tissues and activate the complement cascade. However, the initial description of anti-epithelial cell antibodies has yielded to the increasing evidence that many of these antibodies are cross-reactive with anti-bacterial antibodies. In addition, there is limited evidence to support the concept that these antibodies actually play an active role in the disease process other than potentially as a complement-activating immune complex as suggested above. The perinuclear antineutrophil cytoplas-

mic antibody (pANCA) associated with UC is a marker of the disease and persists even after colectomy (see below). Its titer does not correlate with disease activity and transfer of these antibodies from mouse models fails to transfer disease. Similarly the anti-40 kDa epithelial antibody described by Das that has been shown to recognize tropomyosin V does not vary with activity of disease. The TCR$\alpha^{-/-}$ mouse does develop these antibodies, and they have been shown to co-localize with terminal complement components, but no-one has been able to document that they can either trigger or foster the disease. The finding that colitis can develop in B cell deficient mice as well as in humans with hypogammaglobulinemia raises doubts as to whether these diseases are classically autoimmune. More persuasive are the findings that both T and B cells react inappropriately to the patient's own gut flora, contributing to the persistence of the inflammatory process.

However, the greatest focus relating to disease pathogenesis has been on the $CD4^+$ T cell. Studies from a large number of groups have documented the enhanced activation status of these cells in the lamina propria in IBD patients (Pallone et al., 1987). Isolated lamina propria mononuclear cells (LPMCs) from inflamed areas secrete high levels of cytokine (IFN-γ, IL-12, TNF-α in CD; IL-5, IL-13 in UC) (Colpaert et al., 2002; Monteleone et al., 1997). They also demonstrate a defect in apoptosis (Boirivant et al., 1999). Once activated, the cells persist. This is in direct contrast to normal lamina propria lymphocytes (LPLs) where there is easy induction of apoptosis after activation. This activation is inappropriate in an immune system that is highly regulated and tolerant of luminal antigens in the normal state. Duchmann et al. (1995) reported that patients with IBD fail to demonstrate tolerance to their own flora. Kraus et al. (2004) extended this to show that tolerance to a soluble protein antigen (oral tolerance) was defective in both UC and CD. Direct evidence for a defect in regulatory T cells (Tregs) in the gut of human IBD patients is lacking. It is becoming increasingly apparent that a large number of distinct Treg populations exist in the GI tract. In fact, all of the Tregs described to date have been identified in the gut (oral tolerance and Th3 cells) or as a component of an intestinal inflammatory disorder (Tr1 cells and graft-versus-host disease [GvHD], $CD4^+CD25^+foxp3^+$ Tregs and autoimmune gastritis in the mouse). If anything, there appears to be an increase in the amount of suppressive cytokines present in the lamina propria of IBD patients (increased TGF-β in CD, increased IL-10 in both UC and CD, and recent studies showing an increase in $CD4^+CD25^+$ Tregs in IBD tissues—areas of active disease) (Makita et al., 2004; Kelsen et al., 2005). Whether these are functional and/or appropriately placed within the mucosal immune milieu has not been addressed. Studies in mice strongly suggest that defects in Tregs provide the substrate for murine IBD. However, in all cases, the number of Tregs required to abrogate disease in these models (i.e. in a transfer model) is consistently greater than would be expected to exist *in vivo* in the normal state. The concept remains that it is the inability of the mucosal immune system to regulate an active mucosal immune response that allows for disease persistence.

Thus the current model provides a number of therapeutic insights. These have been exploited providing valuable therapies for the intractable patients.

SEROLOGIC MARKERS

A variety of serologic markers have been identified in patients with IBD. The best-described markers include pANCA, and anti-*Saccharomyces cerevisiae* antibodies (ASCA); pANCA is associated with ulcerative colitis and is distinguished from the ANCA associated with Wegener and other vasculitides in that the target antigen is located in the perinuclear region and is sensitive to degradation by DNase I (Targan and Karp, 2004). The exact target of pANCA in UC is thought to be the histone H1 protein. The ASCA is associated with CD and targets mannose sequences in the cell wall of the yeast *S. cerevisiae* (Sendid et al., 1996). The prevalence of pANCA and ASCA in patients with and without IBD is listed in Table 52.4. These prevalence data can be used to devise serologic strategies for diagnosing CD and UC. However, the utility of these strategies is debatable as no combination of serologies can make the diagnosis

TABLE 52.4 Prevalence, measured by three different groups, of perinuclear antineutrophil cytoplasmic antibody (pANCA) and anti-*Saccharomyces cerevisiae* antibody (ASCA) positivity in patients with Crohn's disease (CD), ulcerative colitis (UC), and controls

	UC		CD		Controls	
Reference	**pANCA$^+$ (%)**	**ASCA$^+$ (%)**	**pANCA$^+$ (%)**	**ASCA$^+$ (%)**	**pANCA$^+$ (%)**	**ASCA$^+$ (%)**
Peeters et al. (1996)	50	14	6	60	3–8	3–11
Quinton et al. (1998)	65	12	15	61	1	1
Ruemmele et al. (1998)	57*	6†	13*	55†	0[a]	5†

*Indirect immunoflourescence after DNase. †IgG or IgA ASCA.

of UC or CD with absolute certainty. Serology may be particularly helpful in the 10% of IBD patients with indeterminate colitis, in which the distinction between UC and CD cannot be made on clinical grounds (Joosens et al., 2002).

Serology has been used not only for diagnostic purposes but also for prediction of disease course. The subset of CD patients who are $pANCA^+$ uniformly have a more UC-like disease with left-sided colonic involvement, bleeding, mucus discharge, urgency, and the requirement for topical therapy (Vasiliauskas et al., 1996). These patients may be less likely to respond to infliximab therapy than the pANCA-CD patients (Taylor et al., 2001). This suggests that CD and UC may not be as much distinct diseases as they are two ends of a spectrum of disease. Patients with UC who have high titers of pANCA may be more likely to develop the complication of pouchitis after ileal pouch-anal anastomosis (Fleshner et al., 2001).

Several new antibodies have been discovered in CD patients. The first is the outer membrane porin C antibody (Omp-C). Anti-Omp-C is directed against a protein in the outer membrane of *Escherichia coli* and has been recently shown by Landers et al. (2002) to be present in 55% of patients with CD. The same group has identified another antibody, I2, which recognizes a bacterial protein associated with *Pseudomonas flourescens*. I2 antibodies were found in 50% of CD patients. The presence of anti-Omp-C and I2 in CD patients suggests that loss of tolerance to microbial antigens may in fact be important in the pathogenesis of IBD.

TREATMENT

The medical treatment of IBD involves a growing armamentarium of medications, as a fundamental knowledge of disease pathogenesis expands. Many of these drugs are used for both UC and CD. Clinical trials are hampered by a consistent finding of placebo response rates of about 30%. Some of the major classes of medication will be presented here, with specific mention of their use in CD and UC.

Aminosalicylates

The first aminosalicylate used in IBD was sulfasalazine, which consists of 5-aminosalicylic acid (5-ASA) bound to the antibiotic sulfapyridine via an azo-bond. This bond is split by enzymes present in colonic bacteria. It is the free 5-ASA that is released into the colon that exerts the therapeutic effect. 5-ASA has multiple anti-inflammatory properties including inhibition of production of IL-1 (Mahida et al., 1991), interference with binding of TNF (Shanahan et al., 1990), and as a free radical scavenger (Ahnfelt-Ronne et al., 1990). These effects appear to be mediated in part by inhibition of the activation of NFκB (Bantel et al., 2000). The most commonly used aminosalicylate in the treatment of IBD today is mesalamine. Various mesalamine preparations exist including coated drugs designed to delay release until the terminal ileum or colon, and topical enemas or suppositories for treatment of the distal colon and rectum.

Glucocorticoids

Corticosteroids are used extensively in the treatment of IBD. Glucocorticoids are steroid hormones that bind to cytosolic receptors and are then translocated to the nucleus where they affect transcription of various genes. This leads to inhibition of various proinflammatory mediators such as cytokines, leukotrienes, adhesion molecules, and nitric oxide synthase (Sands, 2002). Most patients with active UC or CD will respond to courses of oral glucocorticoids, although occasionally the intravenous route is required for severe disease. In many patients, however, tapering the drug will lead to a flare; this phenomenon is termed steroid-dependence. The well-known side effects of chronic steroid use are common and troublesome; these include diabetes, bone loss, acne, moon facies, and adrenal suppression. Attempts to limit these side effects have resulted in other formulations such as topical foam and enemas, which can be effective for left-sided colonic disease. Budesonide is an oral glucocorticoid that undergoes extensive first-pass metabolism in the liver. As a result, high topical levels are achieved in the GI tract with only about 10% systemic absorption and thus less steroid side effects (Edsbacker et al., 1999). Budesonide has been shown to have some efficacy as a treatment for mild-to-moderately active CD (Greenberg et al., 1994; Tremaine et al., 2002).

Thiopurines

The thiopurines include azathioprine and 6-mercaptopurine (6-MP). Azathioprine is metabolized to 6-MP, which can eventually be converted into a number of metabolites including 6-thioguanine nucleotides (6-TG). The therapeutic effect is thought to be due to induction of apoptosis of activated T-cells. The beneficial effect of thiopurines is generally not seen until after 3–6 months of use. The ideal use of thiopurines is as so-called "steroid-sparing" agents: to allow for the discontinuation of steroids in the patient who requires chronic or frequent use. Clinical studies have confirmed the utility of the thiopurines as steroid-sparing agents effective in inducing and maintaining remission in CD and UC (Present et al., 1980; George et al., 1996). The side effects of thiopurines are far less than those of glucocorticoids but include leukopenia, pancreatitis, and hepatitis. Interestingly, genetic variations in the enzyme thiopurine methyltransferase increase the risk of leukopenia by increasing 6-TG levels.

Infliximab

An exciting development in IBD is the approval of the first so-called "biological" agent. Infliximab is a chimeric monoclonal antibody against TNFα. The exact mechanism of action of infliximab is unknown. It appears that neutralization of soluble TNF is not the primary mechanism, since the soluble TNF-receptor etanercept is not effective in CD (Sandborn et al., 2001). Recent data suggest that binding of infliximab to membrane-bound TNF on inflammatory cells leads to apoptosis (Lugering et al., 2001; Van Den Brande et al., 2003) or reverse signaling resulting in the inhibition of cytokine synthesis by tissue macrophages.

For moderate-to-severe luminal (non-fistulizing) CD that is resistant to other therapies, an intravenous infusion of infliximab will lead to clinical response in more than half of patients within 2–4 weeks (Targan et al., 1997). Infliximab is also effective in the treatment of perianal fistulae due to CD (Present et al., 1999). After initial treatment with infliximab for luminal or fistulizing CD, a regimen of infliximab given every 2 months is effective in maintaining remission (Hanauer et al., 2002; Sands et al., 2004). Antibodies to infliximab (ATIs) are of concern in that they appear to increase the risk of infusion reactions and limit effectiveness (Baert et al., 2003); maintenance dosing and concomitant immunomodulator use will decrease the formation of these ATIs. The safety profile of infliximab is excellent. The major side effects are infusion reactions, which are generally mild and easily managed. Inhibition of TNF with infliximab can cause reactivation of latent intracellular infections (e.g. tuberculosis) and, therefore, all patients need to be screened for tuberculosis prior to treatment.

New Biologic Therapies

Inhibitors of Inflammatory Cytokines

As mentioned, the use of infliximab is limited by the formation of ATIs. Several other antibodies to TNF have been developed in an attempt to reduce immunogenicity. CDP571 is an IgG4 humanized monoclonal antibody to TNF. The largest placebo-controlled study of CDP571 in CD showed benefit only in the subgroup of patients with elevated baseline serum C-reactive protein (CRP) (Sandborn et al., 2004). The use of the marker CRP may be important in the future in identifying patients whose disease is truly inflammatory in nature and, therefore, would benefit from anti-TNF therapy. CDP870 is an anti-TNF Fab fragment conjugated to polyethylene glycol (PEG). The conjugation to PEG is used to prolong the half-life of the Fab fragment and reduce immunogenicity. However, an exploratory randomized study of CDP870 in CD failed to show a significant effect on clinical response (Winter et al., 2004). Adalimumab is a fully human IgG1 antibody to TNF that is already approved in the USA for the treatment of rheumatoid arthritis. Preliminary open-label studies suggest that adalimumab is safe and effective in the treatment of CD in patients who have had attenuated responses to infliximab (Papadakis et al., 2005). Further studies with adalimumab are underway.

Etanercept is a fully human fusion protein of the p75 TNF receptor with the Fc portion of IgG1. Although efficacious in rheumatoid arthritis, a placebo-controlled trial in CD failed to show any benefit (Sandborn et al., 2001). The lack of efficacy may be related to the fact that unlike infliximab, etanercept does not bind to membrane-bound TNF to induce apoptosis of inflammatory cells.

IL-12 has been shown in various animal models to be an important component of the Th1-mediated immune response in CD. A study of a weekly subcutaneous injections of human monoclonal antibody to IL-12 in patients with CD provides preliminary evidence for the efficacy of this new drug (Mannon et al., 2004).

Adhesion Molecule Inhibitors

Natalizumab is a humanized monoclonal antibody against α4 integrin. The integrins are a family of adhesion molecules on the cell surface of leukocytes that bind to endothelial cells. This binding is upregulated at sites of inflammation and leads to recruitment of leukocytes to these tissues. A large study of natalizumab in CD showed some improvement in clinical response (Ghosh et al., 2003); further studies are underway. Another antibody against the more specific target α4β7 integrin, LDP-02, has shown efficacy in induction of remission in UC. However, the recent description of 3 cases of progressive multifocal leukoencephalopathy (PML) in patients with multiple sclerosis or CD have resulted in the removal of this agent from the therapeutic armamentarium.

T-Cell Inhibitors

The ability to directly modulate the T-cell response could lead to benefit in the treatment of CD and UC. Monoclonal antibodies have been developed against the T-cell surface molecule, CD-3. CD-3 is associated with the T-cell receptor and is expressed on all T cells. Visiluzimab binds to CD-3 and is thought to induce apoptosis of T cells; it is presently being studied as a treatment for severe, steroid-resistant UC. Visiluzimab has been noted to cause a profound but temporary depletion in peripheral T cells limiting concerns regarding development of opportunistic infections.

Apheresis Procedures

Photochemotherapy involves *ex vivo* exposure of autologous peripheral blood mononuclear cells to 8-methoxypsoralen and long-wavelength ultraviolet A light. The cells are subsequently re-infused into the patient. Photoactivation leads to an immune response against pathogenic

T-cell clones. This has been used successfully against T-cell lymphomas, and appears to be promising in the treatment of steroid-dependent CD (Reinisch et al., 2001).

Another technique involves exposure of blood to an extracorporeal filter containing cellulose acetate beads or woven fiber filters, which provide selective adsorption of granulocytes and monocytes or granulocytes and monocytes and lymphocytes, respectively. This leads in turn to a decrease in the number of inflammatory cells in the blood and eventually to a decrease in such cells in the intestine. Japanese investigators have demonstrated that weekly treatments with this device has efficacy in the treatment of UC (Hanai et al., 2004; Yamamoto et al., 2004).

Hematologic Strategies

Several rare immunodeficiencies involving neutrophils, monocytes, or macrophages have CD-like intestinal inflammation, suggesting that defects in innate immunity are important. This has led to the use of growth factors such as granulocyte-monocyte colony-stimulating factor (GM-CSF) to treat CD. A recent open-label study of GM-CSF was very promising (Dieckgraefe and Korzenik, 2002).

In a different approach, a preliminary study of autologous hematopoietic stem cell transplant for the treatment of CD reported a sustained remission in 11 of 12 patients (Oyama et al., 2005). Although encouraging, it is as yet unclear if the improvement is secondary to the transplant itself or to the intense immune ablative pretransplant conditioning regimen.

SUMMARY

Inflammatory bowel disease is a complex, multifactorial disease with a wide range of clinical presentations and complications. Great strides have been made recently in understanding the immunopathology and genetics of the disease. The pathogenesis of IBD appears to represent an abnormal response by T-cells to normal gastrointestinal flora on a genetically susceptible background. Advances in our understanding of IBD have already led to an explosion of new targeted therapies. Further research will undoubtedly lead to better treatment and perhaps even cure or prevention of disease.

References

Ahmad, T., Armuzzi, A., Bunce, M., Mulcahy-Hawes, K., Marshall, S.E., Orchard, T.R., Crawshaw, J., Large, O., de Silva, A., Cook, J.T., Barnardo, M., Cullen, S., Welsh, K.I., and Jewell, D.P. (2002). The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology 122,* 854–866.

Ahmad, T., Tamboli, C.P., Jewell, D., and Colombel, J.F. (2004). Clinical relevance of advances in genetics and pharmacogenetics of IBD. *Gastroenterology 126,* 1533–1549.

Ahnfelt-Ronne, I., Nielsen, O.H., Christensen, A., Langholz, E., Binder, V., and Riis, P. (1990). Clinical evidence supporting the radical scavenger mechanism of 5-aminosalicylic acid. *Gastroenterology 98,* 1162–1169.

Allez, M., Brimnes, J., Dotan, I., and Mayer, L. (2002). Expansion of CD8+ T cells with regulatory function after interaction with intestinal epithelial cells. *Gastroenterology 123,* 1516–1526.

Axelsson, L.G., Landstrom, E., Goldschmidt, T.J., Gronberg, A., and Bylund-Fellenius, A.C. (1996). Dextran sulfate sodium (DSS) induced experimental colitis in immunodeficient mice: effects in CD4(+)-cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm. Res. 45,* 181–191.

Baert, F., Noman, M., Vermeire, S., Van Assche, G., D'Haens, G., Carbonez, A., and Rutgeerts, P. (2003). Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N. Engl. J. Med. 348,* 601–608.

Bantel, H., Berg, C., Vieth, M., Stolte, M., Kruis, W., and Schulze-Osthoff, K. (2000). Mesalazine inhibits activation of transcription factor NF-κB in inflamed mucosa of patients with ulcerative colitis. *Am. J. Gastroenterol. 95,* 3452–3457.

Baron, J. (2000). Inflammatory bowel disease up to 1932. *Mt. Sinai. J. Med. 67*, 174–189.

Berg, D.J., Zhang, J., Weinstock, J.V., Ismail, H.F., Earle, K.A., Alila, H., Pamukcu, R., Moore, S., and Lynch, R.G. (2002). Rapid development of colitis in NSAID-treated IL–10-deficient mice. *Gastroenterology 123,* 1527–1542.

Binder, V. (1998). Genetic epidemiology in inflammatory bowel disease. *Dig. Dis. 16,* 351–355.

Blanchard, J.F. Bernstein, C.N., Wajda, A., and Rawsthorne, P. (2001). Small-area variations and sociodemographic correlates for the incidence of Crohn's disease and ulcerative colitis. *Am. J. Epidemiol.154,* 328–335.

Boirivant, M., Marini, M., Di Felice, G., Pronio, A.M., Montesani, C., Tersigni, R., and Strober, W. (1999). Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology 116,* 557–565.

Bonen, D.K., Nicolae, D.L., Moran, T., Turkyilmaz, M.A., Ramos, R., Karaliukas, R., Brant, S.R., Duerr, R.H., Kirschner, B.S., Hanauer, S., and Cho, J.H. (2002). Racial differences in Nod2 variation: characterization of Nod2 in African-Americans with Crohn's disease. *Gastroenterology 122,* A29.

Boyko, E.J., Perera, D.R., Keopsell, T.D., Keane, E.M., and Inui, T.S. (1988). Effect of cigarette smoking on the clinical course of ulcerative colitis. *Scand. J. Gastroenterol. 23,* 1147–1152.

Calkins, B.M. (1989). A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig. Dis. Sci. 34,* 1841–1854.

Campbell, N.A., Kim, H.S., Blumberg, R.S., and Mayer, L. (1999). The nonclassical class I molecule CD1d associates with the novel CD8 ligand gp180 on intestinal epithelial cells. *J. Biol. Chem. 274,* 26259–26265.

Chalifoux, L.V. and Bronson, R.T. (1981). Colonic adenocarcinoma associated with chronic colitis in cotton top marmosets, Saguinus oedipus. *Gastroenterology 80,* 942–946.

Chamaillard, M., Girardin, S.E., Viala, J., and Philpott, D (2003). Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. *Cell Microbiol. 5,* 581–592.

Colpaert, S., Vastraelen, K., Liu, Z., Maerten, P., Shen, C., Penninckx, F., Geboes, K., Rutgeerts, P., and Ceuppens, J.L. (2002). *In vitro* analysis of interferon gamma (IFN-gamma) and interleukin-12 (IL-12) production and their effects in ileal Crohn's disease. *Eur. Cytokine Netw. 13,* 431–437.

Cooper, H.S., Murthy, S.N., Shah, R.S., and Sedergran, D.J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab. Invest. 69,* 238–249.

Davidson, N.J., Hudak, S.A., Lesley, R.E., Menon, S., Leach, M.W., and Rennick, D.M. (1998). IL-12, but not IFN-gamma, plays a major role in sustaining the chronic phase of colitis in IL-10-deficient mice. *J. Immunol. 161,* 3143–3149.

Dieckgraefe B.K. and Korzenik J.R. (2002). Treatment of active Crohn's disease with recombinant human granulocyte-macrophage colony-stimulating factor. *Lancet 360,* 1478–1480.

Duchmann, R., Kaiser, I., Hermann, E., Mayet, W., Ewe, K., and Meyer zum Buschenfelde, K.H. (1995). Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin. Exp. Immunol. 102,* 448–455.

Eaden, J., Abrams, K.R., and Mayberry, J.F. (2001). The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut 48,* 526–535.

Edsbacker, S., Nilsson, M., and Larsson, P. (1999). A cortisol suppression dose-response comparison of budesonide in controlled ileal release capsules with prednisolone. *Aliment Pharmacol Ther. 13*, 219–224.

Ehrhardt, R.O., Ludviksson, B.R., Gray, B., Neurath, M., and Strober, W. (1997). Induction and prevention of colonic inflammation in IL–2-deficient mice. *J. Immunol. 158,* 566–573.

Fleshner, P.R., Vasiliauskas, E.A., Kam, L.Y., Fleshner, N.E., Gaiennie, J., Abreu-Martin, M.T., and Targan, S.R. (2001). High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. *Gut 49,* 671–677.

George, J., Present, D.H., Pou, R., Bodian, C., and Rubin, P.H. (1996). The long-term outcome of ulcerative colitis treated with 6-mercaptopurine. *Am. J. Gastroenterol. 91,* 1711–1714.

Ghosh, S., Goldin, E., Gordon, F.H., Malchow, H.A., Rask-Madsen, J., Rutgeerts, P., Vyhnalek, P., Zadorova, Z., Palmer, T., and Donoghue, S.; Natalizumab Pan-European Study Group. (2003). Natalizumab for Active Crohn's Disease. *N. Engl. J. Med. 348,* 24–32.

Ginzburg, L. and Oppenheimer, G.D. (1932). Non-specific granulomata of the intestines (inflammatory tumors and strictures of the bowel). *Trans. Am. Gastro-Enterol. Assoc. 35,* 241–283.

Girardin, S.E., Boneca, I.G., Viala, J., Chamaillard, M., Labigne, A., Thomas, G., Philpott, D.J., and Sansonetti, P.J. (2003). Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J. Biol. Chem. 278,* 8869–8872.

Greenberg, G.R., Feagan, B.G., Martin, F., Sutherland, L.R., Thomson, A.B., Williams, C.N., Nilsson, L.G., and Persson, T. (1994). Oral budesonide for active Crohn's disease. Canadian Inflammatory Bowel Disease Study Group. *N. Engl. J. Med. 331,* 836–841.

Greenstein, A., Janowitz, H.D., and Sachar, D.B. (1976). The extra-intestinal complications of Crohn's disease and ulcerative colitis. *Medicine 55,* 401–412.

Hale-White, W. (1888). On simple ulcerative colitis and other rare intestinal ulcers. *Guys Hosp. Rep. 3rd series 30,* 131–162.

Halfvarson, J., Bodin, L., Tysk, C., Lindberg, E., and Jarnerot, G. (2003). Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology 124,* 1767–1773.

Hampe, J., Cuthbert, A., Croucher, P.J., Mirza, M.M., Mascheretti, S., Fisher, S., Frenzel, H., King, K., Hasselmeyer, A., MacPherson, A.J., Bridger, S., van Deventer, S., Forbes, A., Nikolaus, S., Lennard-Jones, J.E., Foelsch, U.R., Krawczak, M., Lewis, C., Schreiber, S., and Mathew, C.G. (2001). Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet 357,* 1925–1928.

Hanai, H., Watanabe, F., Yamada, M., Sato, Y., Takeuchi, K., Iida, T., Tozawa, K., Tanaka, T., Maruyama, Y., Matsushita, I., Iwaoka, Y., Kikuch, K., and Saniabadi, A.R. (2004). Adsorptive granulocyte and monocyte apheresis versus prednisolone in patients with corticosteroid-dependent moderately severe ulcerative colitis. *Digestion 70,* 36–44.

Hanauer, S.B., Feagan, B.G., Lichtenstein, G.R., Mayer, L.F., Schreiber, S., Colombel, J.F., Rachmilewitz, D., Wolf, D.C., Olson, A., Bao, W., and Rutgeerts, P. (2002). Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet 359,* 1541–1549.

Heller, F., Fuss, I.J., Nieuwenhuis, E.E., Blumberg, R.S., and Strober, W. (2002). Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity 17,* 629–638.

Herfarth, H.H., Mohanty, S.P., Rath, H.C., Tonkonogy, S., and Sartor, R.B. (1996). Interleukin 10 suppresses experimental chronic, granulomatous inflammation induced by bacterial cell wall polymers. *Gut 96*, 836–845.

Hisamatsu, T., Suzuki, M., Reinecker, H.C., Nadeau, W.J., McCormick, B.A., and Podolsky, D.K. (2003). CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology 124,* 993–1000.

Hugot, J.P., Chamaillard, M., Zouali, H., Lesage, S., Cezard, J.P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C.A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J.F., Sahbatou, M., and Thomas, G. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature 411,* 599–603

Hurst, A.F. (1909). Ulcerative colitis. *Guy's Hosp Rep 71,* 26.

Inoue, N., Tamura, K., Kinouchi, Y., Fukuda, Y., Takahashi, S., Ogura, Y., Inohara, N., Nunez, G., Kishi, Y., Koike, Y., Shimosegawa, T., Shimoyama, T., and Hibi, T. (2002). Lack of common Nod2 variants in Japanese patients with Crohn's disease. *Gastroenterology 123,* 86–91.

Itzkowitz, S.H. (2004). Colorectal cancer in inflammatory bowel disease: molecular considerations. In R.B. Sartor and W.J. Sandborn (eds.), *Kirsner's Inflammatory Bowel Diseases*, (pp. 230–242). New York: Saunders.

Itzkowitz, S.H. and Harpaz, N. (2004). Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. *Gastroenterology 126,* 1634–1648.

Joossens, S., Reinisch, W., Vermeire, S., Sendid, B., Poulain, D., Peeters, M., Geboes, K., Bossuyt, X., Vandewalle, P., Oberhuber, G., Vogelsang, H., Rutgeerts, P., and Colombel, J.F. (2002). The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology 122,* 1242–1247.

Jung, H.C., Eckmann, L., Yang, S.K., Panja, A., Fierer, J., Morzycka-Wroblewska, E., and Kagnoff M.F. (1995). A distinct array of pro-inflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *J. Clin. Invest. 95,* 55–65.

Kaiserlian, D., Vidal, K., and Revillard, J.P. Murine enterocytes can present soluble antigen to specific class II-restricted CD4+ T cells. (1989). *Eur. J. Immunol. 19,* 1513–1516.

Kelsen, J., Agnholt, J., Hoffmann, H.J., Romer, J.L., Hvas, C.L., and Dahlerup, J.F. (2005). $FoxP3^{+}CD4^{+}CD25^{+}$ T cells with regulatory properties can be cultured from colonic mucosa of patients with Crohn's disease. *Clin Exp Immunol. 141*, 549–557.

Kitajima, S., Morimoto, M., Sagara, E., Shimizu, C., and Ikeda, Y. (2001). Dextran sodium sulfate-induced colitis in germ-free IQI/Jic mice. *Exp. Anim. 50,* 387–395.

Kneitz, B., Herrmann, T., Yonehara, S., and Schimpl, A. (1995). Normal clonal expansion but impaired Fas-mediated cell death and anergy induction in interleukin-2-deficient mice. *Eur. J. Immunol. 25,* 2572–2577.

Koutroubakis, I.E., Vlachonikolis, I.G., and Kouroumalis, E.A. (2002). Role of appendicitis and appendectomy in the pathogenesis of ulcerative colitis: a critical review. *Inflamm. Bowel Dis. 8,* 277–286.

Kraus, T.A., Toy, L., Chan, L., Childs, J., and Mayer L. (2004). Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. *Gastroenterology 126,* 1771–1778.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Muller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. *Cell 75,* 263–274.

Landers, C.J., Cohavy, O., Misra, R., Yang, H., Lin, Y.C., Braun, J., and Targan, S.R. (2002). Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology 123,* 689–699.

Lesage, S., Zouali, H., Cezard, J.P., Colombel, J.F., Belaiche, J., Almer, S., Tysk, C., O'Morain, C., Gassull, M., Binder, V., Finkel, Y., Modigliani, R., Gower-Rousseau, C., Macry, J., Merlin, F., Chamaillard, M., Jannot, A.S., Thomas, G., and Hugot, J.P. (2002). CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am. J. Hum. Genet. 70,* 845–857.

Lindsay, J.O. and Hodgson, H.J. (2001). Review article: the immunoregulatory cytokine interleukin–10—a therapy for Crohn's disease? *Aliment. Pharmacol. Ther. 15,* 1709–1716.

Loftus, E.V. (2004). Clinical epidemiology of inflammatory bowel disease. *Gastroenterology 126,* 1504–1517.

Lugering, A., Schmidt, M., Lugering, N., Pauels, H.G., Domschke, W., and Kucharzik, T. (2001). Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. *Gastroenterology 121,* 1145–1157.

McCall, R.D., Haskill, S., Zimmermann, E.M., Lund, P.K., Thompson, R.C., and Sartor, R.B. (1994). Tissue interleukin 1 and interleukin-1 receptor antagonist expression in enterocolitis in resistant and susceptible rats. *Gastroenterology 106*, 960–972.

Madsen, K.L., Doyle, J.S., Tavernini, M.M., Jewell, L.D., Rennie, R.P., and Fedorak, R.N. (2000). Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. *Gastroenterology 118,* 1094–1105.

Mahida, Y.R., Lamming, C.E., Gallagher, A., Hawthorne, A.B., and Hawkey, C.J. (1991). 5-Aminosalicylic acid is a potent inhibitor of interleukin 1 beta production in organ culture of colonic biopsy specimens from patients with inflammatory bowel disease. *Gut 32,* 50–54.

Makita, S., Kanai, T., Oshima, S., Uraushihara, K., Totsuka, T., Sawada, T., Nakamura, T., Koganei, K., Fukushima, T., and Watanabe, M. (2004). $CD4^{+}CD25^{bright}$ T cells in human intestinal lamina propria as regulatory cells. *J Immunol. 173*, 3119–3130.

Mannon, P.J., Fuss, I.J., Mayer, L., Elson, C.O., Sandborn, W.J., Present, D., Dolin, B., Goodman, N., Groden, C., Hornung, R.L., Quezado, M., Neurath, M.F., Salfeld, J., Veldman, G.M., Schwertschlag, U., and Strober, W. (2004) Anti-IL-12 Crohn's Disease Study Group. Anti-interleukin-12 antibody for active Crohn's disease. *N. Engl. J. Med. 351,* 2069–2079.

Matsumoto, S., Okabe, Y., Setoyama, H., Takayama, K., Ohtsuka, J., Funahashi, H., Imaoka, A., Okada, Y., and Umesaki, Y. (1998). Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. *Gut 43,* 71–78.

Mayer, L. and Shlien, R. (1987). Evidence for function of Ia molecules on gut epithelial cells in man. *J. Exp. Med. 166,* 1471–1483.

Meddings, J.B. (1997). Review article: Intestinal permeability in Crohn's disease. *Aliment. Pharmacol. Ther. 11* (Suppl 3), 47–53.

Mombaerts, P., Mizoguchi, E., Grusby, M.J., Glimcher, L.H., Bhan, A.K., and Tonegawa, S. (1993). Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell 75,* 274–282.

Monteleone, G., Biancone, L., Marasco, R., Morrone, G., Marasco, O., Luzza, F., and Pallone, F. (1997). Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology 112,* 1169–1178.

Morrissey, P.J., Charrier, K., Braddy, S., Liggitt, D., and Watson, J.D. (1993) CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4+ T cells. *J. Exp. Med. 178,* 237–244.

Mostov, K.E. (1994). Transepithelial transport of immunoglobulins. *Ann. Rev. Immunol. 12,* 63–84.

Mummery, L.P. (1907). The causes of colitis with special reference to its surgical treatment. With an account of 36 cases. *Lancet 1,* 1638–1643

Munkholm, P. and Binder V. (2004). Clinical features and natural history of Crohn's disease. In R.B. Sartor and W.J. Sandborn (eds.), *Kirsner's Inflammatory Bowel Diseases,* (pp. 289–300). New York: Saunders.

Neurath, M., Fuss, I., and Strober, W. (2000). TNBS-colitis. *Int. Rev. Immunol. 19,* 51–62.

Ogura, Y., Bonen, D.K., Inohara, N., Nicolae, D.L., Chen, F.F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R.H., Achkar, J.P., Brant, S.R., Bayless, T.M., Kirschner, B.S., Hanauer, S.B., Nunez, G., and Cho, J.H. (2001a). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature 411,* 603–606

Ogura, Y., Inohara, N., Benito, A., Chen, F.F., Yamaoka, S., and Nunez, G. (2001b). Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB. *J. Biol. Chem. 276,* 4812–4818

Okada, M., Maeda, K., Yao, T., Iwashita, A., Nomiyama, Y., and Kitahara, K. (1991). Minute lesions of the rectum and sigmoid colon in patients with Crohn's disease. *Gastrointest. Endosc. 37,* 319–324

Orchard, T.R. and Jewell, D.P. (2004). Extraintestinal manifestations: skin, joints, and mucocutaneous manifestations. In R.B. Sartor and W.J. Sandborn (eds.), *Kirsner's Inflammatory Bowel Diseases,* (pp. 658–672). New York: Saunders.

Orholm, M., Binder, V., Sorensen, T.I., Rasmussen, L.P., Kyvik, K.O. (2000). Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand. J. Gastroenterol. 35,* 1075–1081

Oyama, Y., Craig, R.M., Traynor, A.E., Quigley, K., Statkute, L., Halverson, A., Brush, M., Verda, L., Kowalska, B., Krosnjar, N., Kletzel, M., Whitington, P.F., and Burt, R.K. (2005). Autologous hematopoietic stem cell transplantation in patients with refractory Crohn's disease. *Gastroenterology 128,* 552–563.

Pallone, F., Fais, S., Squarcia, O., Biancone, L., Pozzilli, P., and Boirivant, M. (1987). Activation of peripheral blood and intestinal lamina propria lymphocytes in Crohn's disease. *In vivo* state of activation and *in vitro* response to stimulation as defined by the expression of early activation antigens. *Gut 28,* 745–753.

Papadakis, K.A., Shaye, O.A., Vasiliauskas, E.A., Ippoliti, A., Dubinsky, M.C., Birt, J., Paavola, J., Lee, S.K., Price, J., Targan, S.R., and Abreu M.T. (2005). Safety and efficacy of adalimumab (D2E7) in Crohn's disease patients with an attenuated response to infliximab. *Am. J. Gastroenterol. 100,* 75–79.

Peeters, M., Nevens, H., Baert, F., Hiele, M., de Meyer, A.M., Vlietinck, R., and Rutgeerts, P. (1996). Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. *Gastroenterology 111,* 597–603.

Peltekova, V.D., Wintle, R.F., Rubin, L.A., Amos, C.I., Huang, Q., Gu, X., Newman, B., Van Oene, M., Cescon, D., Greenberg, G., Griffiths, A.M., St George-Hyslop, P.H., and Siminovitch, K.A. (2004). Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat. Genet. 36,* 471–475.

Podolsky, D.K. (1997). Lessons from genetic models of inflammatory bowel disease. *Acta Gastroenterol. Bel*g. 60, 163–165.

Powrie, F. (1995). T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity 3,* 171–174.

Present, D.H., Korelitz, B.I., Wisch, N., Glass, J.L., Sachar, D.B., and Pasternack, B.S. (1980). Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. *N. Engl. J. Med. 302,* 981–987.

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.

Quinton, J.F., Sendid, B., Reumaux, D., Duthilleul, P., Cortot, A., Grandbastien, B., Charrier, G., Targan, S.R., Colombel, J.F., and Poulain, D. (1998). Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut 42,* 788–791.

Rath, H.C., Schultz, M., Freitag, R., Dieleman, L.A., Li, F., Linde, H.J., Scholmerich, J., and Sartor, R.B. (2001). Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. *Infect. Immun. 69,* 2277–2285.

Reinisch, W., Nahavandi, H., Santella, R., Zhang, Y., Gasche, C., Moser, G., Waldhor, T., Gangl, A., Vogelsang, H., and Knobler, R. (2001). Extracorporeal photochemotherapy in patients with steroid-dependent Crohn's disease: a prospective pilot study. *Aliment. Pharmacol. Ther. 15,* 1313–1322.

Ruemmele, F.M., Targan, S.R., Levy, G., Dubinsky, M., Braun, J., and Seidman, E.G. (1998). Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. *Gastroenterology 115,* 822–829.

Sachar, D.B. (1994). Cancer in Crohn's disease: dispelling the myths. *Gut 35,* 1507–1508.

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,* 253–261.

Sandborn, W.J., Hanauer, S.B., Katz, S., Safdi, M., Wolf, D.G., Baerg, R.D., Tremaine, W.J., Johnson, T., Diehl, N.N., and Zinsmeister, A.R. (2001). Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology. 121,* 1088–1094.

Sandborn, W.J., Feagan, B.G., Radford-Smith, G., Kovacs, A., Enns, R., Innes, A., and Patel, J. (2004). CDP571, a humanised monoclonal antibody to tumour necrosis factor alpha, for moderate to severe Crohn's disease: a randomised, double blind, placebo controlled trial. *Gut 53,* 1485–1493.

Sands, B. (2002). Crohn's disease. In M. Feldman, L.S. Friedman, W.O. Tschumy, M.H. Sleisenger (eds.), *Sleisenger and Fordtran's Principles of Gastrointestinal Disease,* (pp. 2005–2038). New York: Saunders.

Sands, B.E., Arsenault, J.E., Rosen, M.J., Alsahli, M., Bailen, L., Banks, P., Bensen, S., Bousvaros, A., Cave, D., Cooley, J.S., Cooper, H.L., Edwards, S.T., Farrell, R.J., Griffin, M.J., Hay, D.W., John, A., Lidofsky, S., Olans, L.B., Peppercorn, M.A., Rothstein, R.I., Roy, M.A., Saletta, M.J., Shah, S.A., Warner, A.S., Wolf, J.L., Vecchio, J., Winter, H.S., and Zawacki, J.K. (2003). Risk of early surgery for Crohn's disease: implications for early treatment strategies. *Am. J. Gastro. 98,* 2712–2718.

Sands, B.E., Anderson, F.H., Bernstein, C.N., Chey, W.Y., Feagan, B.G., Fedorak, R.N., Kamm, M.A., Korzenik, J.R., Lashner, B.A., Onken, J.E., Rachmilewitz, D., Rutgeerts, P., Wild, G., Wolf, D.C., Marsters, P.A., Travers, S.B., Blank, M.A., and van Deventer S.J. (2004). Infliximab maintenance therapy for fistulizing Crohn's disease. *N. Engl. J. Med. 350,* 876–885.

Sartor, R.B., DeLa Cadena, R.A., Green, K.D., Stadnicki, A., Davis, S.W., Schwab, J.H., Adam, A.A., Raymond, P., and Colman, R.W. (1996). Selective kallikrein-kinin system activation in inbred rats differentially susceptible to granulomatous enterocolitis. *Gastroenterology 110*, 1467–1481.

Sartor, R.B. (2004). Animal models of intestinal inflammation. In R.B. Sartor and W.J. Sandborn (eds.), *Kirsner's Inflammatory Bowel Diseases,* (pp. 120–137). New York: Saunders.

Satsangi, J., Jewell, D.P., Rosenberg, W.M., and Bell, J.I.. (1994). Genetics of inflammatory bowel disease. *Gut 35,* 696–700.

Schultz, M., Clarke, S.H., Arnold, L.W., Sartor, R.B., and Tonkonogy, S.L. (2001). Disrupted B-lymphocyte development and survival in interleukin-2-deficient mice. *Immunology 104,* 127–134.

Schwartz D.A., Loftus E.V. Jr., Tremaine W.J. Panaccione R., Harmsen W.S., Zinsmeister A.R., and Sandborn W.J. (2002). The natural history of fistulizing in Crohn's Disease in Olmstead County, Minnesota. *Gastroenterology 122,* 875–880

Sellon, R.K., Tonkonogy, S., Schultz, M., Dieleman, L.A., Grenther, W., Balish, E., Rennick, D.M., and Sartor, R.B. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin–10-deficient mice. *Infect. Immun. 66,* 5224–5231.

Sendid, B., Colombel, J.F., Jacquinot, P.M., Faille, C., Fruit, J., Cortot, A., Lucidarme, D., Camus, D., and Poulain, D. (1996). Specific antibody response to oligomannosidic epitopes in Crohn's disease *Clin. Diag. Lab. Immunol. 3,* 219–226.

Shanahan, F., Niederlehner, A., Carramanzana, N., and Anton, P. (1990). Sulfasalazine inhibits the binding of TNF alpha to its receptor. *Immunopharmacology 20,* 217–224.

Sonnenberg A., McCarty D.J., and Jacobsen S.J. (1991). Geographic variation of inflammatory bowel disease within the United States. *Gastroenterology 100,* 143–149.

Stokkers, P.C., Reitsma, P.H., Tytgat, G.N., and van Deventer, S.J. (1999). Inflammatory bowel disease and the genes for the natural resistance-associated macrophage protein-1 and the interferon-gamma receptor 1. *Int. J. Colorectal Dis. 14,* 13–17.

Stoll, M., Corneliussen, B., Costello, C.M., Waetzig, G.H., Mellgard, B., Koch, W.A., Rosenstiel, P., Albrecht, M., Croucher, P.J., Seegert, D., Nikolaus, S., Hampe, J., Lengauer, T., Pierrou, S., Foelsch, U.R., Mathew, C.G., Lagerstrom-Fermer, M., and Schreiber, S. (2004). Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat. Genet. 36,* 476–480.

Subhani,, J., Montgomery, S., Pounder, R., and Wakefield, A. (1998). Concordance rates of twins and siblings in inflammatory bowel diseases. *Gut 42,* A40.

Sundberg, J.P., Elson, C.O., Bedigian, H., and Birkenmeier, E.H. (1994). Spontaneous, heritable colitis in a new substrain of C3H/HeJ mice. *Gastroenterology 107,* 1726–1735.

Targan, S.R., Hanauer, S.B., van Deventer, S.J., Mayer, L., Present, D.H., Braakman, T., DeWoody, K.L., Schaible, T.F., and Rutgeerts, P.J. (1997). A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N. Engl. J. Med. 337,* 1029–1035.

Targan, S.R. and Karp, L.C. (2004). Serology and laboratory markers of disease activity. In R.B. Sartor and W.J. Sandborn (eds.), *Kirsner's Inflammatory Bowel Diseases,* (pp. 442–450). New York: Saunders.

Taylor, K.D., Plevy, S.E., Yang, H., Landers, C.J., Barry, M.J., Rotter, J.I., and Targan, S.R. (2001). ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. *Gastroenterology 120,* 1347–1355.

Tremaine, W.J., Hanauer, S.B., Katz, S., Winston, B.D., Levine, J.G., Persson, T., and Persson, A. (2002). Budesonide CIR capsules (once or twice daily divided-dose) in active Crohn's disease: a randomized placebo-controlled study in the United States. *Am. J. Gastroenterol. 97,* 1748–1754.

Timmer, A., Sutherland, L.R., and Martin, F. (1998). Oral contraceptive use and smoking are risk factors for relapse in Crohn's disease. *Gastroenterology 114*(6), 1143–1150

Van den Brande, J.M., Braat, H., van den Brink, G.R., Versteeg, H.H., Bauer, C.A., Hoedemaeker, I., van Montfrans, C., Hommes, D.W., Peppelenbosch, M.P., and van Deventer, S.J. (2003). Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology 124,* 1774–1785.

Vasiliauskas, E.A., Plevy, S.E., Landers, C.J., Binder, S.W., Ferguson, D.M., Yang, H., Rotter, J.I., Vidrich, A., and Targan, S.R. (1996). Perinuclear anti-neutrophil cytoplasmic antibodies in patients with

Crohn's disease define a clinical subgroup. *Gastroenterology 110,* 1810–1819.

Watanabe, T., Kitani, A., Murray, P.J., and Strober, W. (2004). NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat. Immunol. 5,* 800–808.

Wells, C. (1952). Ulcerative colitis and Crohn's disease. *Ann. R. Surg. Engl. 11,* 105–120

Welte, T., Zhang, S.S., Wang, T., Zhang, Z., Hesslein, D.G., Yin, Z., Kano, A., Iwamoto, Y., Li, E., Craft, J.E., Bothwell, A.L., Fikrig, E., Koni, P.A., Flavell, R.A., and Fu, X.Y. (2003). STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. *Proc. Natl. Acad. Sci. U.S.A. 100,* 1879–1884.

Wilks, S. (1859). *Lectures on Pathological Anatomy,* 3rd ed. London: Longmans.

Winter, T.A., Wright, J., Ghosh, S., Jahnsen, J., Innes, A., and Round, P. (2004). Intravenous CDP870, a PEGylated Fab′ fragment of a humanized antitumour necrosis factor antibody, in patients with moderate-to-severe Crohn's disease: an exploratory study. *Aliment. Pharmacol. Ther. 20,* 1337–1346.

Yamamoto, T., Umegae, S., Kitagawa, T., Yasuda, Y., Yamada, Y., Takahashi, D., Mukumoto, M., Nishimura, N., Yasue, K., and Matsumoto, K. (2004). Granulocyte and monocyte adsorptive apheresis in the treatment of active distal ulcerative colitis: a prospective, pilot study. *Aliment. Pharmacol. Ther. 20,* 783–792.

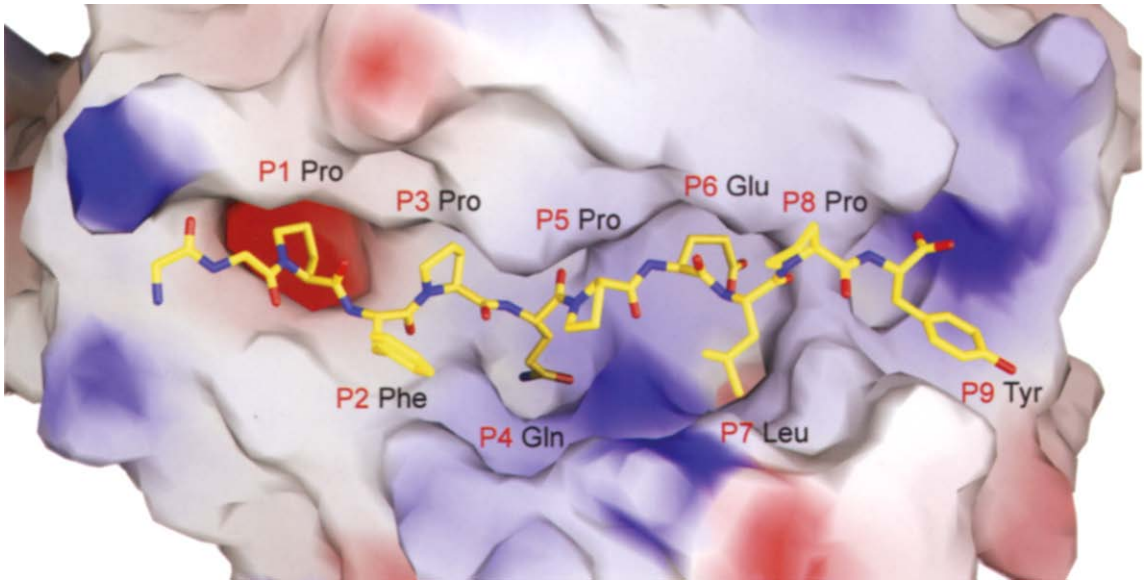


FIGURE 51.6 Three-dimensional structure of the binding site of HLA-DQ2 complexed with a deamidated gliadin peptide (DQ2-α-I epitope—carbon, white; nitrogen, blue; oxygen, red). The electrostatic potential surface of DQ2 is shown as red (negative) and blue (positive).

Courtesy of Dr Chu-Young Kim.

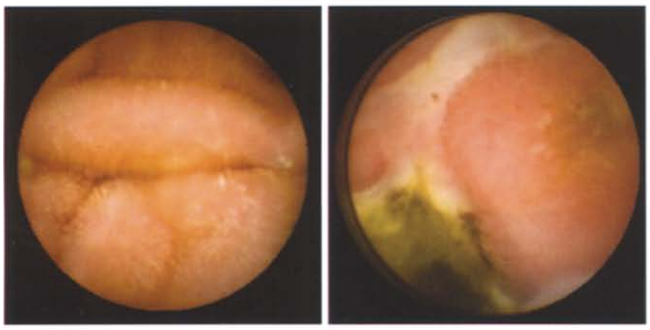


FIGURE 52.1 Images from wireless capsule endoscopy. *A*, Normal small bowel. *B*, Deep linear ulcer in the small intestine of a patient with Crohn disease.

Courtesy of Dr Peter Legnani.

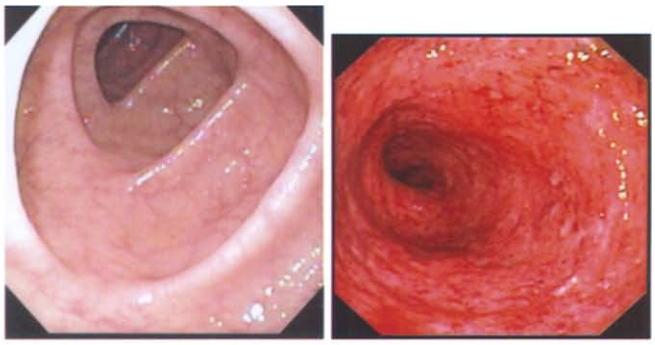
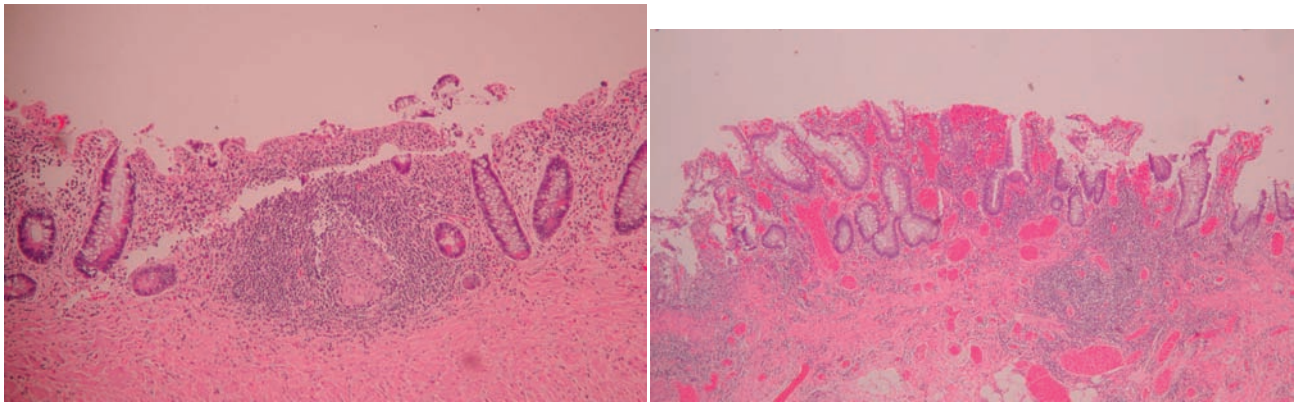


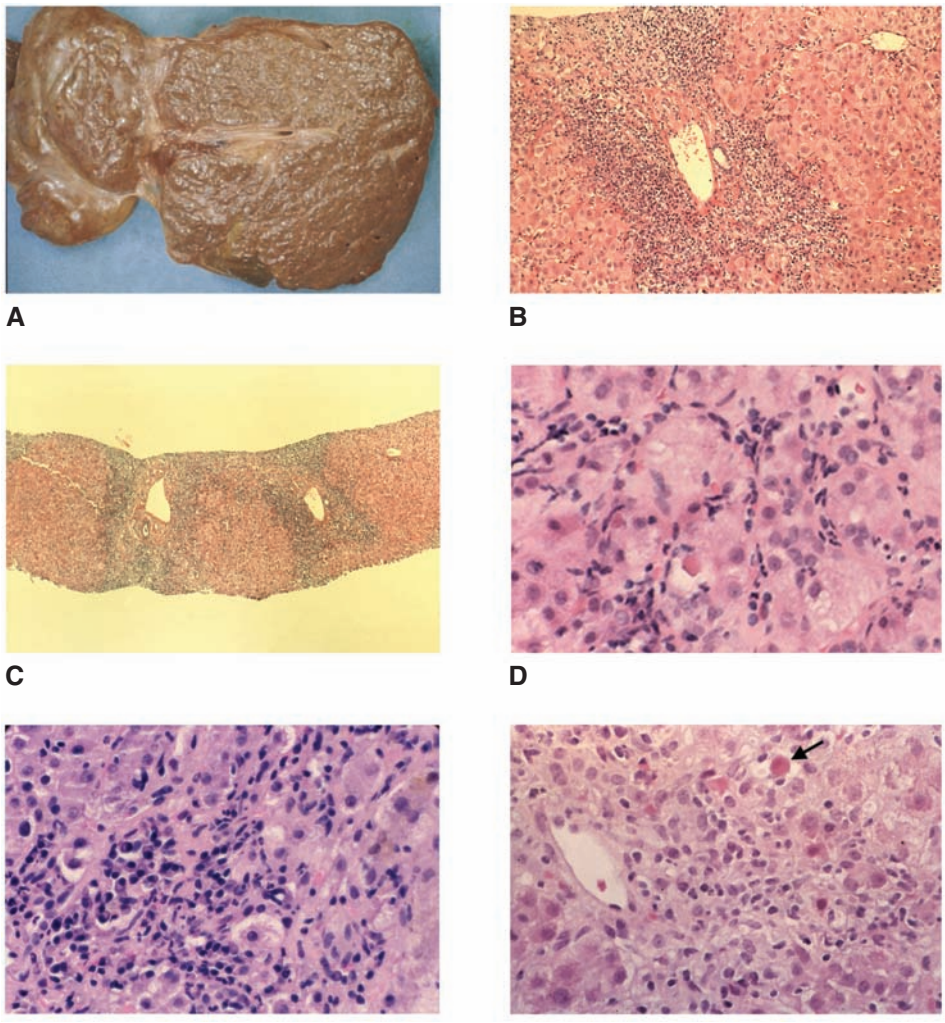
FIGURE 52.2 Colonoscopic images. *A*, Normal colon. *B*, Sigmoid colon in severe ulcerative colitis, showing erythema, ulceration, and hemorrhage.



A B

FIGURE 52.3 Histology of irritable bowel disease. *A,* Crohn disease in the left colon showing a non-necrotizing granuloma, inflammatory cell infiltrate, and Paneth cell metaplasia. *B,* Ulcerative colitis showing crypt architecture distortion, hemorrhage, ulceration, and inflammatory cell infiltrate.

Courtesy of Dr Mikhail Tismenetsky.



A B C D E F

FIGURE 53.1 Pathological features of autoimmune hepatitis (AIH) *A,* An autopsy specimen of a liver in advanced AIH illustrating coarse nodular cirrhosis and shrunken left lobe. *B–F,* photomicrographs from cases of AIH type 2. *B, Interface hepatitis* illustrating lymphocytic infiltrate in portal trail of liver abutting an adjacent liver lobules. *C, Bridging necrosis* illustrating necroinflammatory infiltrates connecting portal tracts and a likely forerunner to fibrosis and cirrhosis. *D, Lobular hepatitis* illustrating extra lobular lymphocytic infiltrates, damaged hepatocytes and prominence of (spindle-shaped) Kupffer cells. *E, Lobular hepatitis* illustrating severely degraded liver cells surrounded by lymphocytes, and prominence plasma cells. *F. Periportal hepatitis* illustrating *(arrow)* a shrunken eosinophilic remnant of an hepatocytic that has undergone apoptosis (Councilman body).

A, from Joske and King, (1955).

CHAPTER

53

Chronic Hepatitis

IAN R. MACKAY*
*Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia

ALBERT J. CZAJA†
†Division of Gastroenterology, Mayo Clinic, Rochester MN, USA

IAN G. McFARLANE§
§Institute of Liver Studies, King's College Hospital, London, UK

MICHAEL P. MANNS‡
‡Department of Gastroenterology, Hepatology and Endocrinology, Medizinische Hochschule, Hannover, Hannover, Germany

In the 1940s, the first reference to "chronic active hepatitis" was in relation to protracted viral hepatitis in soldiers (Barker et al., 1945). In the 1950s, there was recognized from Walderström in Sweden, Kunkel in the USA, and Wood and colleagues in Australia, a "malignant" chronic hepatitis affecting mainly young women, marked by systemic features and pronounced hyperglobulinemia (Mackay and Tait, 1994). Persisting high aminotransferase activities in blood and inflammation in the liver justified its designation as chronic active hepatitis (CAH). An immunologic basis for CAH was proposed, based on antinuclear antibodies (ANA) first detected by the test for lupus erythematosus (LE) cells, and a complement fixation (CF) reaction for anticytoplasmic antibodies (Mackay and Gajdusek, 1958). In the 1960s, ANA were detected more readily by indirect immunofluorescence (IIF) (Holbrow et al., 1963), and a further serologic reactivity was described by IIF as smooth muscle antibody (SMA) (Johnson et al., 1965; Whittingham et al., 1966). Also, efficacy of treatment with corticosteroid drugs alone, or with 6-mercaptopurine/azathioprine, became established (Mackay, 1968). These discoveries prompted replacement of the initially coined term *lupoid hepatitis* (Mackay et al., 1956) by autoimmune hepatitis (AIH) (Mackay et al., 1965). In the 1970s, efficacy of immunosuppressive treatment was substantiated by placebo-controlled trials (Cook et al., 1971; Soloway et al., 1972; Murray-Lyon et al., 1973; Kirk et al., 1980) that indicated greatly enhanced survival. Another autoantigenic reactivity was recognized by IIF to liver and kidney microsomes (LKM1) (Rizzetto et al., 1973). In the 1980s, anti-LKM1 was seen to distinguish two serologically distinct but otherwise similar forms of AIH (Homberg et al., 1987), type 1 (ANA and SMA) and type 2 (anti-LKM1). The identity of the LKM1 autoantigen as a cytochrome P450 isoform (2D6) was established biochemically and by gene cloning and, hereby, the relatively infrequent AIH type 2 differs strikingly from AIH type 1 for which a definitive disease-specific autoantigen has not yet been identified.

Until the late 1960s, CAH had been used generically for all types of chronic hepatitis, with cases mostly segregating into *autoimmune* and *cryptogenic* groups. However,

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

identification of viruses responsible for acute and chronic hepatitis, viruses A, B and later C (HAV, HBV, HCV), led to the recognition, in the 1970s, of chronic viral hepatitis (due to viruses B and C). The realization that "CAH" comprised multiple entities (Mackay, 1972) led to disuse of the term CAH, and a need was seen for a clearer identification of AIH. Consensus reports in 1993 and 1998 were prepared by a representative expert panel of hepatologists, the International Autoimmune Hepatitis Group (IAIHG), with diagnostic criteria and a scoring system that weighted the net strength of the diagnosis for research purposes (Johnson and McFarlane, 1993; Desmet et al., 1994; Alvarez et al., 1999).

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Clinical Features

Autoimmune hepatitis is a female-predominant disease with reported female-to-male sex ratios ranging from 4–6:1. Although AIH has a bimodal age distribution with peak occurrences between 10 and 30 years and 40 and 50 years (Mackay, 1975), current experience suggests that AIH can occur across all age ranges (Parker and Kingham, 1997; Schramm et al., 2001). Nevertheless, among the Japanese an elderly presentation is more common (Toda et al., 1997). In sharp contrast, AIH type 2 occurs mostly in children between the ages of 2 and 14 years, with severe inflammatory activity (Homberg et al., 1987), but some 5% of cases in Southern Europe are in older men, often in association with HCV infection (Lunel et al., 1992; Michel et al., 1992; Vergani et al., 2004). Elsewhere than in central Europe, AIH type 2 seems infrequent (Mackay, 2004). Additionally, outside of Europe, the presence of anti-LKM1 in chronic hepatitis C is likewise infrequent (Reddy et al., 1995). And further, epitope reactivities of anti-LKM1 in Europeans with chronic hepatitis C differ from those of anti-LKM1 in spontaneous AIH type 2.

Autoimmune hepatitis presents in 50% of cases as an indolent non-resolving hepatitis, in 25% as acute or even fulminant hepatitis (Crapper et al., 1986; Desmet et al., 1994; Porta et al., 1990, Nikias et al., 1994), and in 25% as an asymptomatic illness with incidentally discovered abnormal laboratory indices (Hay et al., 1989). The symptoms and signs are those for any form of hepatitis, and include anorexia, fatigue, jaundice, right upper abdominal discomfort, and enlargement of the liver and spleen. A former requirement for a 6-month observation period to define chronicity is now dispensable (Johnson et al., 1993; Alvarez et al., 1999). Approximately one-third of those affected are asymptomatic, but most become symptomatic during follow-up (Kogan et al., 2002). As the disease advances, features that are generic to cirrhosis of the liver supervene. Various extrahepatic expressions pointing to an autoimmune background include coexisting autoimmune thyroiditis, ulcerative colitis, thrombocytopenias, rheumatoid arthritis, or systemic lupus erythematosus (SLE). Considerable discussion is generated by the coexistence of features of AIH with those of other autoimmune liver diseases (see Chapters 54 and 55), called overlap syndromes (Mackay 2000; Woodward and Neuberger, 2001). Perhaps the greater proportion of cases of the usual overlap, that of AIH with primary biliary cirrhosis (PBC), actually represents the latter disease (Lohse et al., 1999; Kinoshita et al., 1999).

Pathologic Features

The characteristic histologic lesion, first called *piecemeal cirrhosis* (Popper et al., 1965) and later *interface hepatitis*, consists of lymphoid accumulation and hepatocellular destruction at junctions of liver lobules and portal tracts (Figure 53.1). In acute phases, mononuclear cells are seen invading liver lobules, in close contiguity with damaged hepatocytes, described as *lobular (panacinar) hepatitis* (Wilkinson et al., 1978). Kupffer cells are prominent in liver sinusoids. Zones of necrosis between portal tracts tend to coalesce, called *bridging necrosis*, a pattern of injury regarded as a forerunner of the scarring that initiates macronodular cirrhosis (Schalm et al., 1977). Hepatocytes appear ballooned or necrotic or as shrunken eosinophilic remnants, formerly known as Councilman bodies, and now as apoptotic cells (Searle et al., 1987). The lymphoid infiltrates show prominence of plasma cells, consistent with an earlier disease designation as plasma cell hepatitis (Page and Good, 1960). Notably, these florid necro-inflammatory changes are "wiped clean" with use of prednisolone (Czaja et al., 1984). Other manifestations of AIH include 1) perivenular (zone 3) necrosis with or without inflammation of the portal tracts (Pratt et al., 1997; Te et al., 1997; Singh et al., 2002; Misdraji et al., 2004); 2) giant syncytial multinucleated hepatocytes (Phillips et al., 1991; Devaney et al., 1992); and 3) coincidental bile duct injury (Ludwig et al., 1984). More detailed descriptions are available elsewhere (International Group of Pathologists, 1977; Dienes, 1989).

Epidemiology

Autoimmune hepatitis is cited as more prevalent among populations of Northern European extraction, and may share the polar–equatorial gradient characteristic of many autoimmune diseases. However, actual prevalences have been ascertained only for populations of Norway, Iceland, Alaska, and Japan, with these being rather lower than for many other autoimmune diseases (Mackay and Toh, 2002). Even so, AIH has been described in African Americans, Brazilians,

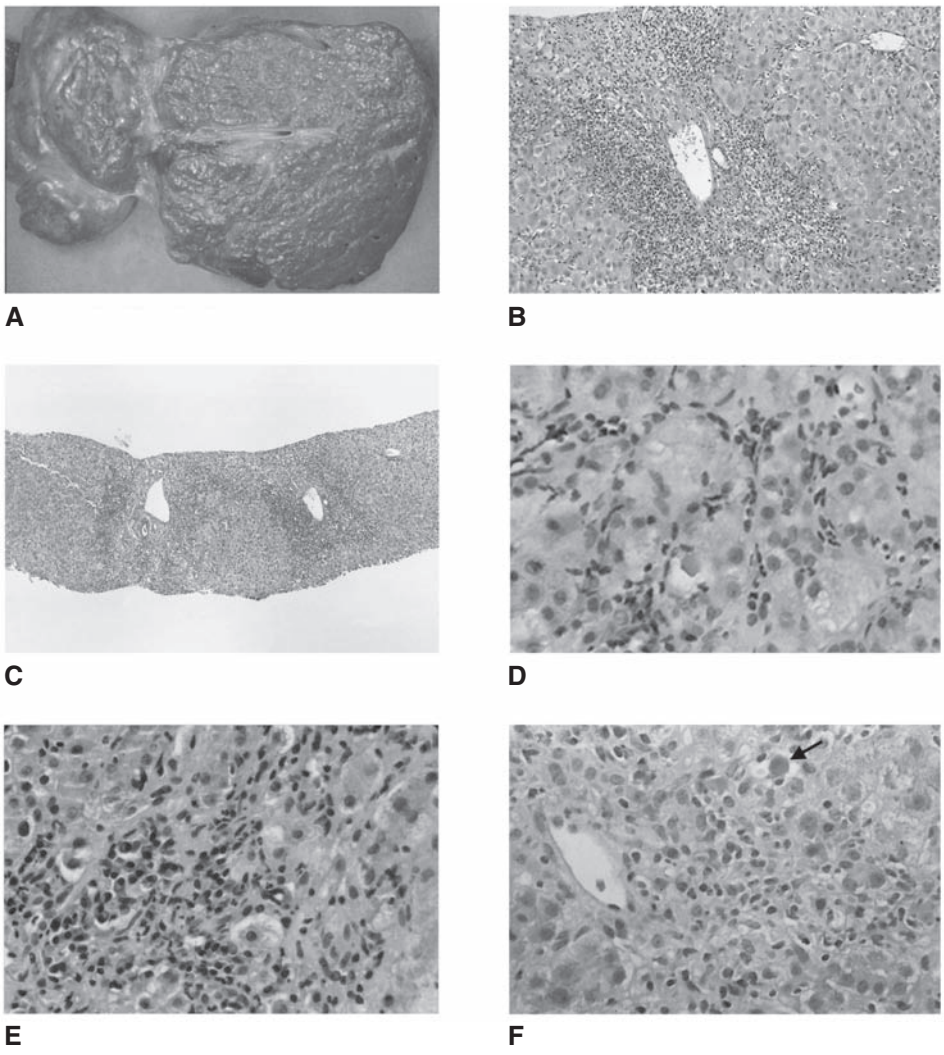


Figure 53.1 Pathological features of autoimmune hepatitis (AIH) *A,* An autopsy specimen of a liver in advanced AIH illustrating coarse nodular cirrhosis and shrunken left lobe (Case reported by Joske and King, 1955). *B–F,* photomicrographs from cases of AIH type 2. *B, Interface hepatitis* illustrating lymphocytic infiltrate in portal trail of liver abutting an adjacent liver lobule. *C, Bridging necrosis* illustrating necroinflammatory infiltrates connecting portal tracts and a likely forerunner to fibrosis and cirrhosis. *D, Lobular hepatitis* illustrating extra lobular lymphocytic infiltrates, damaged hepatocytes and prominence of (spindle-shaped) Kupffer cells. *E, Lobular hepatitis* illustrating severely degraded liver cells surrounded by lymphocytes, and prominence of plasma cells. *F, Periportal hepatitis* illustrating *(arrow)* a shrunken eosinophilic remnant of a hepatocytic that has undergone apoptosis (Councilman body). (See color plate section.)

Argentinians, Arabs, and subcontinental Indians. The incidence of AIH among Norwegians was 1.9 in 100,000 persons per year with a point prevalence of 16.9 in 100,000 (Boberg et al., 1998). A higher prevalence was reported among Alaskan natives, 43 in per 100,000 population (Hurlburt et al., 2002). In the USA, AIH may effect some 100,000 to 200,000 persons (Jacobson et al., 1997), although precise incidence and prevalence data are not available.

Genetic and indigenous risk factors probably affect disease occurrence in different regions and ethnic populations; the prevalence of AIH among Northern European white groups accords with a high frequency of HLA-DR3 and -DR4 and, expectedly, AIH occurs with a similar frequency in the derivative populations of North America and Australia (Donaldson et al., 1991). HLA-DR3 is rare in Japan, and there AIH is associated with HLA-DR4 (Seki et al., 1992). Overall, the prevalence of AIH type 1 appears greatly to exceed that of AIH type 2. Accepting some degree of case selection bias in earlier reports, the contribution of AIH to all cases of CAH in the 1960s was high—e.g., 60% in Melbourne, Australia, and 30% in Mainz, Germany (Mackay, 1985). Subsequently, this contribution has been

strikingly reduced by waves of chronic hepatitis due to HBV and HCV. There is a perception that the incidence of AIH may be decreasing, although one of us (IMcF) believes that in the UK it may be increasing, perhaps because of greater awareness. Whatever the case, AIH was not represented among causes of mortality from all types of liver disease in the USA (Vong and Bell, 2004), either because of its actual rarity, certification errors, or efficacy of contemporary therapy.

AUTOIMMUNE FEATURES

Autoimmune hepatitis belongs to that puzzling group of autoimmune disorders in which there is a disease-specific target organ yet non-organ-specific serologic markers, and it remains one of the less well understood in regard to both inductive and effector processes. This section provides an analysis of the range of antigenic reactants in the context of the defined serologic types, 1 and 2.

Autoimmune Hepatitis Type 1

In AIH, hypergammaglobulinemia reaches levels up to 10 g/L, which is seen in few other autoimmune diseases. The high serum levels of immunoglobulin G (IgG) are not explicable by a response to any single antigen or autoantigen and may reflect polyclonal B-cell activation as one of the pathogenetic components of the disease. Patterns of serologic reactants in AIH by IIF are shown in Figure 53.2. Fluctuations in level reflect the degree and duration of inflammatory activity, and a decrease marks efficacy of treatment.

Nuclear Antigens

In AIH type 1, there are multiple nuclear reactants. Antinuclear antibodies in untreated cases give homogeneous labeling by IIF, but this tends to fade during remission, revealing other coexisting patterns and reactants. The sensitivity of ANA for diagnosis of AIH type 1 (allowing for technical variations) would be 70%, but its specificity is low.

Nucleosome

As much by analogy with SLE as by direct observations, the reactant for ANA in AIH is taken to be the nucleosome (Mackay, 2001), an octamer with two coils of DNA (each of 146 bp), wrapped around the small nuclear proteins, the histones of which there are five main classes, H1, H2A, H2B, H3, and H4. A linear array of nucleosomes forms the primary chromatin fiber, which condenses into a quaternary protein structure and could generate an autoepitope for both T and B lymphocytes (Czaja et al., 2003). There is accompanying reactivity to histones, occasionally to native double-stranded DNA, and also to a variety of characterized and uncharacterized non-nucleosomal nuclear proteins (Czaja et al., 1994; 1995; 1997a) that may result from epitope spreading. On a "wheat and chaff" analogy, certain antibodies (wheat) seem pathogenetically relevant, while others (chaff) seem an incidental and irrelevant consequence of inflammatory activity and cellular degradation.

Double-stranded (ds) DNA

Studies on the frequency of anti-dsDNA in AIH have given divergent results. In two representative studies, employing either radioimmunoprecipitation (RIP) (Smeenk et al., 1982), or RIP together with IIF on *Crithidia luciliae* (Leggett et al., 1987); positivity rates were 10% and 16% (transient 8%, persisting 8%), respectively. Technical factors, case selection, and disease activity would materially influence results.

Others

The variety of ANA reactivities in AIH type 1 (Czaja and Norman, 2003; Mackay, 2004) resembles that in other multisystem autoimmune diseases, and does not provide additional diagnostic assistance nor pathogenetic clarity.

Cytoplasmic Antigens

Various anti-cytoplasmic reactivities in AIH have been characterized at the molecular level.

Soluble Liver Antigen/Liver Pancreas Antigen

A *liver-pancreas antigen* (LP) was identified by complement fixation (Berg et al., 1981) and reactivity to it in AIH reported by Stechemesser et al. (1993). Independently, a *soluble liver antigen* (SLA) was detected by inhibition enzyme-linked immunosorbent assay (ELISA) (Manns et al., 1987), which later became co-identified with LP. The SLA was successively nominated as cytokeratins 8,18 (Wächter et al., 1990) and glutathione S-transferase (Wesierska-Gadek et al., 1998), but cloning from cDNA expression libraries identified SLA/LP as the UGA-serine transfer RNA-protein complex ($tRNP^{(Ser)Sec}$) (Wies et al., 2000; Costa et al., 2000; Volkmann et al., 2001; Torres-Collado et al., 2004), and a member of the family of serine hydroxymethyl transferases (Gelpi et al., 1992; Kevenbeck et al., 2001). Anti-SLA/LP initially appeared to define a novel (third) serologic type of AIH, because it occurs in some cases that are otherwise seronegative (Baeres et al., 2002). However anti-SLA/LP coexists with other autoantibodies, in AIH type 1 particularly (Kanzler et al., 1999), but also occasionally in AIH type 2 and autoimmune sclerosing cholangitis (Vergani et al., 2004). Anti-SLA/LP is closely associated with HLA DR3, with relapse after corticosteroid withdrawal (Czaja et al., 2002), and with greater severity (Ma et al., 2002). However, all of the autoantibodies, and also serum IgG, are

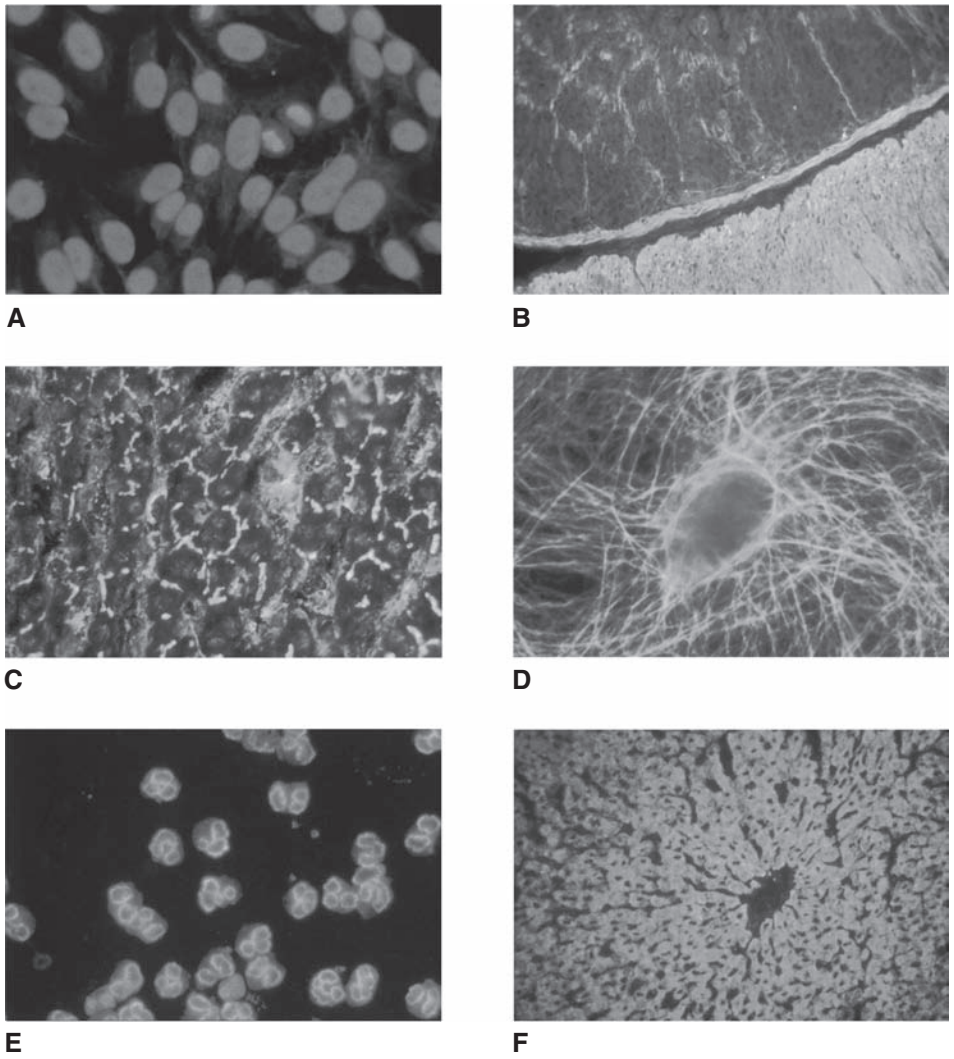


Figure 53.2 Serologic expressions of autoimmune hepatitis (AIH) by indirect immunofluorescence. *A,* Antinuclear antibody (ANA) from an AIH type 1 serum on cells of HEp2 cell line illustrating typical homogeneous pattern of labeling. *B,* Smooth muscle antibody (SMA) on mouse from an AIH type 1 serum illustrating labeling of submucosal smooth muscle layer, muscularis mucosal (thin band), and thin vertical strands in mucosa suggestive of anti-actin reactivity. *C,* Antiactin reactivity on mouse liver by an AIH type 1 serum illustrating polygonal pattern due to labeling of submembranous actin of hepatocytes. *D,* Anti-intermediate filament reactivity on cultured fibroblast of an SMA-positive but anti-actin-negative serum; this reactivity is not specific for AIH. *E,* Antineutrophil cytoplasmic antibodies (ANCA) reactivity on granutocytes exposed to an ANCA-positive serum, as seen in vasculitic and other diseases (see Chapter 65); note that the perinuclear ANCA seen in AIH may be a true antineutrophil nuclear autoantibody (see text). *F,* Anti-LKM1 reactivity on mouse liver by an AIH type 2 serum illustrating evenly granular labeling of cytoplasm of hepatocytes throughout the lobule. (See color plate section.)

present at higher levels in more severe types of AIH, and so anti-SLA/LP is not unique in this respect.

Ribosomal P Proteins

Antibodies to ribosomal P proteins (RPP) occur mostly in the setting of SLE (16% of cases), but were earlier described in "SLE and liver dysfunction" (Koren et al., 1993).

Ribosomal P proteins are present in the cytoplasm of all cells and comprise a set of three phosphoproteins, P0, P1, and P2, of molecular weight 38, 19, and 17 kDa, respectively. Natural RPP exists as a pentamer of P0 and two copies of P1 and P2 (Zampieri et al., 2003) with each polypeptide sharing a C-terminal 22-residue (C22) immunodominant epitope that is detectable on the surface of cultured hepatoma cells. Anti-RPP are cited as a particular example

of autoantibodies that penetrate living cells, with possible adverse intracellular effects (Koscec et al., 1997). There has been no systematic analysis of the frequency or epitope specificity of anti-RPP in AIH, and this is needed.

Neutrophil-specific Cytoplasmic Proteins

High titers of "granulocyte-specific" ANA in AIH were described in the 1960s (Smalley et al., 1968). They were later identified with antineutrophil cytoplasmic antibodies (ANCA), the cytoplasmic reactants for which clump with a perinuclear distribution and so simulate ANA. The two well-characterized species of ANCA, cytoplasmic (c) and perinuclear (p) that have particular disease associations and molecular identity (see Chapters 59 and 65), but the ANCA of AIH are not directed at either of the two main ANCA antigens, proteinase 3 or myeloperoxidase (Targan et al., 1995). The reactant in AIH has been ascribed to lactoferrin, elastase, a 50 kDa nuclear envelope protein restricted to neutrophil-myeloid lineages, or a high mobility group (HMG) protein (Mackay and Toh, 2002). Currently, the AIH-associated pANCA are cited as "atypical," and appear to react with nuclear lamina proteins, thereby justifying their earlier designation as "granulocyte-specific ANA," and current designation as antineutrophil nuclear antibodies (ANNA) (Terjung et al., 2001).

Hepalaminin

A recently described novel protein specific for the cytoplasm of hepatocytes and cholangiocytes, and called hepalaminin, consists of laminin p-2 fused to an unknown polypeptide (Fukuda et al., 2004). Responses to hepalaminin were postulated to exacerbate hepatic inflammation in chronic viral hepatitis.

Cytoskeletal Elements (Enriched in Smooth Muscle)

Cytoskeletal autoantibody was initially detected on gastric mucosal smooth muscle (SMA) (see Figure 53.2) and was validated as a marker for AIH by Toh (1979) and Kurki and Virtanen (1984). The reactant is cytoskeleton comprising abundant filamentous proteins that support cellular organization, contractility, locomotion, and pseudopod extrusion. Cytoskeletal proteins include 6 nm microfilaments (actin), 15 nm intermediate filaments (vimentin, desmin, and others), and 30 nm microtubules (tubulin) (Toh, 1979). Testing for SMA on different tissues by IIF showed particular patterns with vessels (SMA-V), glomerular mesangium (SMA-G), and renal tubular brush borders (SMA-T) (Bottazzo et al., 1976). SMA-T signifies actin microfilaments, as seen by testing sera on cultured cells on which SMA-positive sera label actin "cables" spanning the long axis of the cell (Toh, 1979; Muratori et al., 2002). Non-actin SMA are attributable to incidental antifilament reactivity after infections, particularly viral (Toh, 1979). Whether actin is the exclusive cytoskeletal autoantigen in AIH is debated (Vergani et al., 2004). The specificity and sensitivity of anti-actin for the diagnosis of AIH is high (~80%); however, some 16% of patients with AIH and SMA lack anti-actin (Czaja et al., 1996a). Since anti-actin positivity is associated with a worse overall outcome, it has prognostic as well as diagnostic utility.

Actin as an Autoantigen

Actin is a fascinating autoantigen. It is a globular protein found as one of the most abundant proteins, only in eukaryotic cells. The extreme conservation of actin structure in eukaryote evolution is attributed to the large number, some 70, of actin binding proteins including myosin that would exert high selective pressure (Galkin et al., 2002; Dos Remedios et al., 2002). These are three isoforms α, β, and γ, with minor differences in sequence. Monomeric G actin protein (mw 46 kDa) readily polymerizes to form helical filaments, so providing force-generating effects for cellular movement. Various actin-binding proteins regulate the multiple-step polymerization–depolymerization cycle, whereas others cross-link polymeric (filamentous, F) actin into bundles or networks. Autoantigenicity resides in the polymerized F-actin state. Surprisingly, given the advanced structural knowledge of actin and its binding partners (Dos Remedios et al., 2003), there has been rather meager dialog between cell biology and immunopathology in the context of actin autoimmunity. All cells contain actin, and hepatocytes are richly endowed, evident by the "polygonal" IIF labeling pattern when liver is used as substrate for anti-actin antisera (Mackay and Toh, 2002). Two populations of anti-actin autoantibodies (AAA) were described in AIH sera, natural (N)AAA and disease-associated (D)AAA. The latter were shown to have undergone isotype and subclass switching, were likely antigen-driven, and may recognize a different and disease-specific epitope (Zamanou et al., 2003).

Liver Membrane Antigens

Non-identified Membrane Components

The liver cell membrane has been long-suspected as a site of an autoantigenic reactant (Meyer zum Büschenfelde et al., 1980). Analysis by immunoblot of AIH sera on partially purified liver membrane preparations shows multiple reactants of widely differing molecular weight. However, none appears promising, since the intensity of immunoblot signal fades when sera are diluted beyond 1:1000 or when remission stage antisera are tested, and weakly reactive counterparts of many of the components are seen with sera from other inflammatory liver diseases (Frazer et al., 1987; Swanson et al., 1990; Matsuo et al., 2000). Hence, these multiple reactants may be consequential to hepatocellular degradation.

Anti-190 kDa Molecule

A human IgM monoclonal autoantibody generated from blood lymphocytes of a patient with AIH type 1 reacted specifically with the hepatocyte surface membrane (190 kDa molecule) and had complement-dependent cytotoxicity for hepatocyte-derived cell lines (Yamauchi et al., 2004). This observation redirects attention to humoral cytotoxicity as an immunopathogenic element in AIH.

Asialoglycoprotein Receptor

The asialoglycoprotein receptor (ASGPR) was identified in 1984 as a component of liver membrane protein preparations used as antigenic substrates for studies with AIH sera (McFarlane et al., 1984a; McFarlane, 1996). It is a highly conserved hepatocyte-specific glycoprotein involved in the binding and endocytosis of serum glycoproteins for disposal by the liver. A multinational study on anti-ASGPR reactivity by radioimmunoassay and ELISA, using the purified receptor as antigen, showed a 76% positivity rate in AIH, but positive results in 7.6–11% of cases with other liver diseases, indicating limited specificity (Treichel et al., 1994). However, anti-ASGPR was strongly and quantitatively associated with severity of interface hepatitis in these conditions (McFarlane et al., 1984b; Czaja et al., 1996b), and may become useful in defining end points of therapy. The structure and function of the ASGPR, its antibody and T-cell responses to it in disease, and potential autoepitopes are described by Treichel and Meyer zum Buschenfelde (1998).

Autoimmune Hepatitis Type 2

Microsomal Antigens

According to different pathogenetic backgrounds, several liver and kidney microsomal (LKM) antigens have been described and designated as LKM1, LKM2, LKM3 and LM. Studies by immunoblot (Waxman et al., 1988; Zanger et al., 1988; Guegen et al., 1988) revealed that most were isoforms of the cytochrome P450 oxidase (CYP) enzyme family, and function as autoantigens in various autoimmune liver disorders (Bogdanos and McFarlane, 2003).

Cytochrome P450 2D6—LKM1 Antigen

Anti-LKM1 react by IIF with liver cells and the proximal P3 segment of renal tubules (Rizzetto et al., 1973) (see Figure 53.2). Definitive identification of the target as CYP2D6 was achieved by molecular cloning from a gene expression library (Manns et al., 1989; Guegen et al., 1989). Anti-KLM1 antibodies inhibit CYP 2D6 enzyme function *in vitro* (Zanger et al., 1988). Epitope analysis using candidate synthetic peptides and ELISA identified five CYP2D6 antigenic regions recognized by anti-LKM1, located at the amino acid (aa) ranges 193–212, 257–269, 321–351, 373–389, and 410–429, with other minor regions as well (Obermeyer-Straub et al., 2000) (Figure 53.3). Mostly the antibodies reacted either with a main antigenic site, aa257–269 (DPAQPPRD) (Manns et al., 1991), or a second major site, aa321–351 (Yamamaoto et al., 1993a; Kerkar et al., 2003), whereas in chronic hepatitis C epitopes for anti-LKM1 were located at aa208–273 (Yamamoto et al., 1993b; Ma et al., 1994; Dalekos et al., 1999; Bogdanos and McFarlane, 2003). However, a differing antigenic site, aa181–245, was described by Kitazawa et al. (2001). According to the latest data, antibody-binding sites on a three-dimensional structure of CYP450 2D6 are shown in Figure 53.3 (Sigimura et al., 2002).

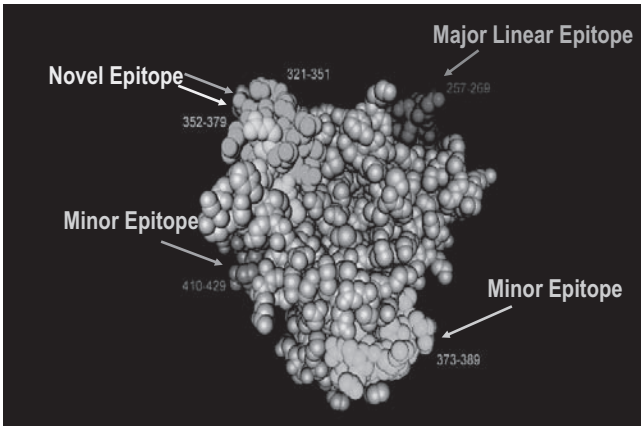


Figure 53.3 A three dimensional representation of cytochrome P450 2D6 showing various epitope regions. A major epitope region *(red)* at amino acids 257–269 is a reactant for autoantibodies in autoimmune hepatitis (AIH) type 2, and it has sequence homology with the intermediate-early protein IE 175 of herpes simplex virus type 1 (Manns et al., 1991). An epitope region *(green/yellow)* is a reactant for autoantibodies in AIH type 2 and chronic hepatitis C. Minor epitopes at amino acids 373–389 and amino acids 410–429 are shown in light blue and dark blue, respectively. (See color plate section.)

From Sigimura et al. (2002), and reproduced with permission from *Autoimmunity*.

Cytochrome P450 2C9. 2E1, 3A—LKM2 Antigen

Anti-LKM2 reactivity was recognized by Beaune et al. (1987) in acute and chronic hepatitis induced by exposure to tienilic acid, a drug which is no longer marketed, and called LKM2. The autoantigenic reactant was identified as the CYP450 isoform, 2C9. There are various other drugs or toxins that can induce an immune-mediated hepatitis with LKM2-type reactivities, and remarkably each is associated with the particular CYP isoform that hydroxylates the drug, as described in detail by Manns and Obermeyer-Straub (1997), and shown in Figure 53.4. Examples include reactivity to CYP450 2E1 in halothane hepatitis or occasionally alcohol-induced hepatitis, or to CYP450 3A in anticonvulsant hepatitis; in general, for drug-induced types of

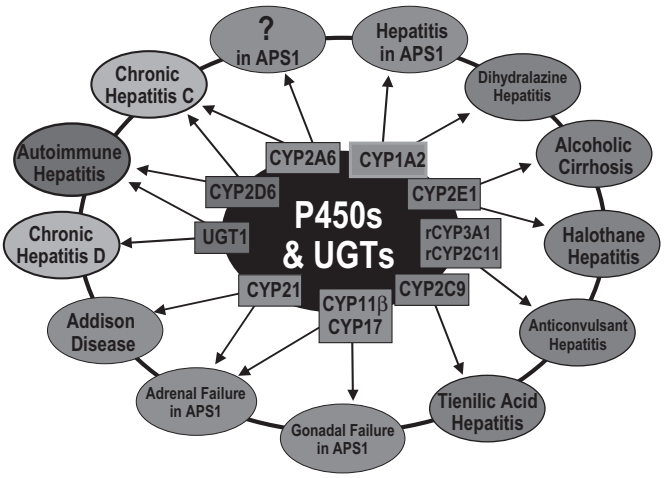


Figure 53.4 Cytochrome P450 isoforms (CYPs) and uridine-5′-diphosphate glucuronosyl transferase 1 (UGT1) as autoantigenic targets in various types of hepatitis, occurring in spontaneous autoimmune hepatitis (AIH) type 2 *(red)*, or associated with drug reactions *(blue)*, or hepatitis virus infection *(green)*, or other spontaneous autoimmune conditions *(orange)*. (APS1, autoimmune polyendocrine syndrome type 1.) (See color plate section.)

From Manns and Obermeyer-Straub (1997) and reproduced with permisstion from *Hepatology.*

hepatitis, disease and serologic expressions fade when the provocative agent is withdrawn.

Cytochrome P450 1A2—LM Antigen

In drug-induced hepatitis provoked by the anti-hypertensive medication dihydralazine, serologic reactivity by IIF is organ-restricted to the CYP450 1A2 isoform in liver microsomes (LM) (Bourdi et al., 1990). These also occur in children with AIH as part of the autoimmune polyendocrine syndrome type 1 (APS1).

Cytochrome P450 2A6 Antigen

In the APECED/APS1 syndrome antibodies to another liver-expressed cytochrome P450, CYP2A6 were described, but in contrast to anti-CYP1A2, these were not exclusively associated with liver disease (Obermayer-Straub et al., 2001). Interestingly, molecular mimicry involving T-cells between CYP2A6 and epitopes of the hepatitis C core protein was described by Kammer et al. (1999) but, in regard to B cells, anti-CYP2A6 occur only rarely in chronic hepatitis C (Dalekos et al., 2003).

UDP glucuronosyl transferase—LKM3 Antigen

Anti-LKM-like reactivity was recognized in 1983 in hepatitis delta (D) virus infection (Crivelli et al., 1983). The reactant, identified as the bilirubin-conjugating enzyme uridine-5′-diphosphate glucuronosyl transferase (UGT) rather than a CYP450 isoform (Philipp et al., 1994), was called LKM3. A B-cell epitope is common to several members of family 1 UDP-glucuronosyl transferases. Sera from 3 of 18 cases of AIH type 2 reacted mainly against UGT 1A1, the principal isoform required for the glucuronidation and disposal of bilirubin (Strassburg et al., 1996).

Liver Cytosol Antigen Type 1 (LC1)

Autoantibodies were recognized by Martini et al. (1988) to a soluble, organ-specific *liver cytosolic* antigen LC1 in cases of spontaneous AIH type 2. Anti-LC1 was said to be more specific for AIH type 2 than anti-LKM1, but it has since been demonstrated in chronic HCV infection (Lenzi et al., 1995), and occasionally in AIH type 1 (Vergani et al., 2004). Anti-LC1 elutes from liver cytosol as a protein of 240–290 kDa but, since there is a 62 kDa signal by denaturing immunoblot, the protein likely exists as a tetramer (Abauf et al., 1992). LC1 can be detected by IIF on liver by a progressively decreasing homogeneous labeling of hepatocytes towards the central vein of the lobule, by immunoblot, and by counter-immunoelectrophoresis (Muratori et al., 1995). Molecular cloning revealed LC1 to be the enzyme forminotransferase cyclodeaminase (FTCD) (Lapierre et al., 1999), which has two domains—a globular FT domain containing conformational epitopes joined by a short linker to the CD domain (Muratori et al., 2001).

T-Lymphocyte Reactivity to Hepatic Autoantigens

Details on the participation of T-cells in the pathogenesis of AIH are scanty because of the lack of experimental models, the non-availability (for AIH type 1) of identified or recombinant autoantigens, and, perhaps, the relatively "lower profile" of AIH among the autoimmune disorders. Immunohistochemical studies indicated that T lymphocytes predominated among cells in the intrahepatic infiltrates (Frazer et al., 1985), but their antigenic specificity is undefined. Various earlier studies on cytotoxic activities of blood T cells on hepatocellular targets might not withstand contemporary scrutiny, but might bear repeating with modern technologies, given that they pointed to the possibility in AIH of antibody-dependent cellular cytotoxicity (ADCC) as a mechanism of liver cell damage (Mackay, 1985). The cellular immune responses to ASGPR described by Treichel and Meyer zum Buschenfelde (1998) could be relevant here.

GENETIC FEATURES

Evolving methods for identification of the multiple loci for genetic risk for autoimmunity promise much (see Chapters 20 and 21). It will be interesting to know, when

data from genome-wide screening for AIH types 1 and 2 do become available, which loci are AIH-specific, and which are generic for autoimmunity.

Human Leukocyte Antigen (Major Histocompatibility Complex) Alleles

Risk Alleles

Associations were recognized between AIH type 1 and HLA alleles A1 and B8 by Mackay and Morris (1972), and then DR3. Family studies revealed that HLA A1-B8-DR3 was inherited as a haplotype, with DR3 as the likely primary risk allele (Mackay and Tait, 1980); the estimated relative risk conferred by DR3 was 6–7. Included in the DR3 haplotype were null alleles (gene deletion) for complement (C4A) (Tait et al., 1989), implicating C4 deficiency as an additional risk factor. A weaker association with HLA-DR4 was recognized by Donaldson et al. (1991). Two immunogenetic subsets of AIH type 1 include the DR3 subset (younger age of onset, more severe disease, and higher requirement for liver transplantation), and the DR4 subset (older age more benign disease) (Czaja et al., 1997b). Molecular genotyping has revealed a high representation (52% vs. 19% in controls) of the *DRB1*0301-DRB3*0101-DQA1*0501-DQB1*0201* haplotype, and an association in *DRB3*0101*-negative subjects with the DR4 allele, *DRB1*0401* (54% vs. 23% in controls). *DRB1*0301* and *DRB1*0401* each encode a shared motif, amino acids LLEQKR, at positions 67–72 in the DRβ polypeptide chain of the HLA DR, with the presence of lysine (K) at DRβ 71 proposed as a determinant of susceptibility (Strettel et al., 1997).

The genetic determinants of AIH type 2 are uncertain. HLA DR7 and the allele *DRB1*0701* were implicated in cases from Germany by Czaja et al., (1997b) and South America by Bittencourt et al. (1998), but with insufficient case numbers for a confident association.

A Disease Susceptibility Motif

Explanations for HLA-disease associations (see Chapter 5) include the presence, within the antigen-binding groove of MHC class II molecules, of a *disease susceptibility motif* (DSM) that efficiently presents an autoantigenic peptide to responder T cells. The observations in AIH type 1 are that *DRB1*1501*, which encodes ILEQAR at DRβ 67–72, is protective in white Northern European and North American patients (Doherty et al., 1994), and this motif differs from the presumed DSM, LLEQKR, at two positions, isoleucine (I) at DRβ67 and alanine (A) at DRβ71. The substitution of I for L at DRβ67 is neutral whereas A for K at DRβ71 replaces a highly charged polar amino acid with a non-polar amino acid, so likely influencing antigen binding and immunocyte recognition, and in turn susceptibility to disease (Czaja and Donaldson, 2000). Converse to this argument, the DSM could function by inefficient presentation of self antigens by dendritic cells during negative selection in the thymus, and so impair deletional tolerance.

Ethnic Differences

Different ethnic groups have different susceptibility alleles. Those for AIH type 1 are *DRB1*0405* for Japanese (Seki et al., 1992), *DRB1*0405* in Argentine adults (Pando et al., 1999), *DRB1*1301* in Argentine children (Fainboim et al., 1994), *DRB1*13* and *DRB1*03* for Brazilians (Goldberg et al., 2001), and *DRB1*0404* for Mestizo Mexicans (Vazquez-Garcia et al., 1998). This multiplicity of different MHC-encoded risk alleles suggests that different alleles can encode one or more common determinants (shared motifs) which are critical for disease expression or that, in different geographic regions, the genes which promote AIH are selected by region-specific etiologic factors. Protracted infection with HAV has been associated with *DRB1*1301*, which is implicated in the occurrence of AIH type 1 (Fainboim et al., 2001). Perhaps children with *DRB1*1301* in South America are naturally selected to have prolonged exposure to virus and liver antigens during HAV infection, so conferring increased risk of developing AIH. In this fashion, the susceptibility allele for the disease within a geographical region may be a clue to an etiologic agent.

Non-MHC Alleles

TNF-A

Various non-MHC alleles have been identified, or suspected, as risk elements for AIH type 1. The tumor necrosis factor-α (TNF-α) gene promoter *TNF-A* results in high constitutive and induced levels of TNF-α (Cookson et al., 1999; Czaja et al., 1999) and has a weak effect on risk.

CTLA-4

The cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a molecule that normally downregulates T-cell responses. The CLTA-4 G allele, representing a polymorphism in exon 1 (position 49 A/G), is at higher frequency in AIH type 1 than in controls (Agarwal et al., 2000a; Djilali-Saiah et al., 2001).

SLE1

The *sle1* gene region identified in murine models of SLE has a likely ortholog in humans at 1q22-22, and includes multiple genes that contribute to production of antibodies to nuclear (chromatin) antigen, and hence is a suspect locus for AIH type 1.

Fas

The Fas gene (*tumor necrosis factor receptor super family-6* or *TNFRSF6*) encodes the apoptosis-related molecule CD95 (Fas), noting that apoptosis is critically involved in immune regulation (see Chapter 70). There are high constitutive levels of CD95 expression on hepatocytes, Kupffer cells, and sinusoidal endothelial cells, and hence the liver may be an important site for apoptosis-mediated immunocyte destruction (Galle et al., 1995). CD95 is abundantly expressed on activated lymphocytes, and the ligand CD178 (FasL) can be expressed on liver cells after oxidative stress. If enhanced apoptosis were to contribute to collateral damage in AIH type 1, this could lead to abnormal display of autoantigen (see Chapter 15); conversely, impaired apoptosis may extend the effects of activated immunocytes and so perpetuate disease (see Chapter 70). Patients with AIH with the *TNFRSF6*G,G* or **A,G* genotype have higher serum levels of aspartate aminotransferase and IgG at presentation than do patients with the TNFRSF6*A,A genotype (Agarwal et al., 2000b).

MHC Class I Chain-Related A Gene

The MHC class I chain-related A gene (MICA) is highly polymorphic, and encodes a stress-inducible protein that interacts with the NKG2D receptor of various immune effector cells, including cytotoxic T cells and natural killer (NK) cells (Molinero et al., 2002; Vivier et al., 2002). As sensors of cell stress, the MICα molecules identify cells that can be targeted and destroyed by cytotoxic immunocytes including $\gamma\delta$ T lymphocytes. Furthermore, they act as restriction elements for NK and NK T-cells, which constitute a large resident population within the liver. Homozygosity for the *MICA*008* allele confers an increased risk of AIH (OR = 2.2), but the association may be simply due to linkage with the ancestral 8.1 haplotype and *DRB1*0301* (McKiernan et al., 2002). Homozygosity for the *MICA*008* allele may lead to the activation of $CD4^+$ and $CD8^+$ T lymphocytes that are not readily targeted by cytotoxic immunocytes bearing the NKG2D receptor; reduced clearance of these activated T-lymphocytes may in turn increase the risk and severity of hepatocyte injury.

Vitamin D Receptor

The polymorphic gene encoding the vitamin D receptor (VDR) can modulate immune responses (Adorini, 2002). Vitamin D receptors are expressed on blood monocytes and activated T-lymphocytes, and 1,25 hydroxyvitamin D can inhibit lymphocyte activation, prevent dendritic cells from differentiating into antigen presenting cells, decrease production of type 1 cytokines, and increase expression of interleukin 4 (IL-4) and transforming growth factor-β (TGF-β). Polymorphisms of the VDR gene occur more commonly among patients with AIH type 1 than in controls, and Fok polymorphisms of the VDR distinguish patients with AIH from those with PBC who have mainly BsmI polymorphisms (Vogel et al., 2002).

AIRE

An AIH type 2-like disease with anti-LKM antibodies is inherited as an autosomal recessive disease, APS1, marked by autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy (APECED) (Manns et al., 1990; Sacker et al., 1980; Clemente et al., 1997; Gebre-Medhir et al., 1997; Obermeyer-Straub et al., 2001). This disease is associated with a recessively inherited mutation on chromosome 21q22.3 (see Chapter 38). The nonfunctional gene product is the autoimmune regulator (AIRE), a transcriptional factor. Deficiency of AIRE causes impaired intrathymic negative selection/deletional tolerance to various tissue-specific autoantigens. CYP1A2 and CYP2A6 are the autoantigens described in associated with AIRE-deficiency liver disease (Clemente et al., 1998). Curiously, these particular autoantigenic reactants differ from the CYP2D6 isoform characteristic of classical AIH type 2 (Obermeyer-Straub et al., 2001).

Analysis of Genetic Risk for Autoimmunity

We suggest that predisposition to autoimmunity in general is attributable to the inheritance, together with MHC risk alleles, of a large "package" of immune response genes of low potency or penetrance acting through any one of four pathways. *First*, by influencing overall immunologic responsiveness and/or tolerance, as judged by clustering of multiple autoimmune expressions in patients and/or their families, and exemplified by genes particular to female sex, or genes influencing apoptosis. *Second*, by directing immune responses to particular tissues or autoantigens, exemplified by effects of class II HLA (MHC) alleles, or perhaps genes involved in the assembly and structure of T- and B-cell antigen receptors. *Third*, by influencing events during and after immune activation, including expression of costimulatory molecules such as CTLA-4, pro-inflammatory cytokines, or chemokines or their receptors. *Fourth*, by conferring vulnerability on a particular target tissue, as so-called "end-organ" genes.

ENVIRONMENTAL INFLUENCES

It is dogma that environment contributes substantially to autoimmunity, but the nature of the agents, and the way these operate, remain cryptic (see Chapters 22 and 23). Virus infection ranks highly, and candidates have included measles virus (now dismissed), Epstein–Barr virus, hepati-

tis viruses, and others. Interestingly, the surge of case reports on autoimmune-type CAH in the 1960s coincided in time with the last major endemic wave of HAV infection. But even if virus infections were to be implicated, what might be their mode of action? One explanation is simply "spillage" of autoantigenic cellular fragments in a genetically predisposed host, perhaps abetted by the cytokine-rich milieu of the antiviral response in which potentially self-reactive T cells can, as "bystanders," become coincidentally activated. A popular explanation is molecular (epitope) mimicry, with the evidence strongest in the case of initiation of AIH type 2 by HCV, although most childhood cases of AIH type 2 occur "spontaneously" in the absence of infection with HCV, and the mimicry sequences between CYP2D6 and the HCV polyprotein (Manns et al., 1991) are not particularly impressive.

Medicinal drugs induce acute or chronic hepatitis occasionally with serologic expressions that may simulate those of AIH type 1 or type 2, but these syndromes typically recede after the offending drug is withdrawn. Such drugs include α-methyl dopa and minocycline that provoke AIH type 1-like disease, but act in an undefined way, perhaps by causing interference with peripheral tolerogenesis. Drugs that provoke AIH type 2-like disease (anti-LKM2 or other cytochrome P450 enzymes) appear to act by generation of drug-associated neoepitopes in the course of enzymatic disposal, thereby inducing autoimmune reactions (Liu and Kaplowitz, 2002).

ANIMAL MODELS

Close animal models of AIH have proven difficult to generate by conventional procedures (Mackay and Toh, 2002). Those that have been developed are reviewed by Vierling (2003). Acute and, under certain conditions, chronic hepatitis can be induced by injection of the plant lectin, concanavalin A (Tiegs et al., 1992), and illustrate effector pathways for hepatocellular damage dependent on pro-inflammatory cytokines (Howell, 2002); however, since autoimmune features do not supervene, the model does not address the inductive phases of AIH.

There are well-exploited models of autoimmunity dependent on transgenically introduced proteins (see Chapter 26), wherein the encoded proteins elicit natural self-tolerance but, on suitable provocation, are induced to function like autoantigens. One example is the introduction of the transgene under a promoter that can be activated in postnatal life, illustrated by the metallothionine (Met) promoter that is activated by exposure to heavy metals. Mice with a transgene construct comprising Met-ovine growth hormone (Met-oGH) could be induced by exposure to a heavy metal (zinc) to express oGH extracellularly and secrete it as a foreign protein; the outcome was a giant mouse, which developed a characteristic interface and lobular hepatitis with oval cell proliferations and, terminally, hepatocellular carcinoma (Orian et al., 1990; Hardy et al., 1997). Another illustrative system in mice was based on transgenic allo-MHC class I molecules (H-2K^b), which, after activation of the transgene (Met-K^b), caused a severe CD8 T-cell driven hepatitis. The inflammatory process, however, dissipated in time as a result of intrahepatic tolerogenesis and/or activation-induced cell death of lymphocytes (Bertolini et al., 1995). In a further development of similar transgenic technology, in which the transgene was expressed in both liver and lymph nodes, Bowen et al. (2004) found that when naïve CD8$^+$ T cells were activated in lymph nodes hepatitis ensued, but not so when naïve T cells were activated in the liver.

Various other genetic manipulations are cited by Lapierre et al. (2004). Moreover, these authors developed a model of AIH type 2 by DNA immunization in mice with a construct comprising coding sequences for the antigenic regions of CYP2D6 and FTCD, leading to development of a T-cell predominant hepatitis with autoantibodies to CYP2D6 (LKM1) and FTCD (LC 1).

PATHOGENIC MECHANISMS

Autoimmune hepatitis belongs to a family of autoimmune diseases in which there is organ-directed pathology to the liver, but multisystem-type autoantibodies. The added complexity is that the liver is a lymphoid organ with an abundant resident population of cells of the innate and adaptive immune systems (Kita et al., 2001), and an intrahepatic milieu tilted towards tolerogenesis. Notwithstanding, intrahepatic immune responses can be long sustained, as illustrated by the persistently destructive pathologies seen in chronic viral and autoimmune hepatitis. Autoimmune hepatitis could be regarded as a genetically biased immune response to products of apoptotic or necrotic hepatocellular breakdown with ensuing self-perpetuating damage after the initial injury. Such products would undergo endocytosis by intrahepatic dendritic or other antigen-presenting cells that express MHC risk alleles, and would be transported to regional lymph nodes where there is presentation of self peptide-MHC and induction of reactive T cells. However, the actual peptide that is presented is still a mystery, perhaps an epitope sequence derived from F-actin in AIH type 1, or an epitope sequence derived from CYP2D6 in AIH type 2. Effector processes are just as poorly defined as are inductive processes, with both T-cell or antibody-dependent cellular cytotoxicity seen as credible mechanisms.

Finally, there is occurrence of *de novo* AIH in transplanted human liver allografts for diseases other than AIH (Mieli-Vergani and Vergani, 2004; Miyagawa-Hayashino et al., 2004). Sounding first a cautionary note on whether these cases are actual AIH or merely rejection, the possible

occurrence of AIH *de novo* merits serious investigation for clues to the pathogenesis of the naturally occurring disease.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

The specification of, and agreement on, criteria for diagnosis by the IAIHG was a distinct advance (see earlier). These criteria include results for autoimmune serologic assays, which, unfortunately, are not always ideally performed. A recent report from a Serology Subcommittee of IAIHG regarded traditional IIF as the mainstay for diagnosis and discrimination of the two major types of AIH (Vergani et al., 2004) (Table 53.1). The initial screening should be performed on frozen unfixed sections from a composite tissue block that includes kidney, liver, and stomach, with a secondary screening if needed on a cell-line (HEp2) for patterns of ANA reactivity. The distinction of SMA and anti-actin reactivities can require special tissue substrates (Mackay and Toh, 2002), and sera should be preheated (56°C, 30 min) to eliminate interfering serum factors (Cancado et al., 2001). Arbitrary cut-off titers for IIF positivity were set at 1:40, but should be lower for children (Vergani et al., 2004). Practicalities may dictate the use of commercially available sections, for which mild fixation is usually used to improve shelf-life, but with attendant loss of clarity of IIF patterns. In commercial laboratories, there will be a preference for semiautomated assays, usually an ELISA, using recombinant or purified native antigens. Those currently available include anti-SLA-LP, SMA, and anti LKM1, but so far these have been validated only "in-house."

The lack of well-standardized assays has led to a poorly founded perception of unreliability (low specificity) of routine serologic procedures for the diagnosis of AIH, even to the degree that these were seen as dispensable for diagnosis (Pratt and Kaplan, 2000). The hope is that, following the lead of the diabetes community (see Chapter 74), standard reference sera can become available, that international serum exchange workshops can be inaugurated to validate existing assays, and that evaluation of new substrates can be undertaken as these come on stream, based on the autoantigens described earlier.

TREATMENT AND OUTCOME

The treatment of choice for AIH is immunosuppression, comprehensively reviewed by Heneghan and McFarlane (2002). The preference is for prednisolone monotherapy in children and young females, in combination with azathioprine in adults. This standard regimen has been in use worldwide for many years since its introduction in the 1960s (Mackay, 1968), and evidence-based treatment guidelines have been promulgated by the American Association for the Study of Liver Diseases (Czaja and Freese, 2002). Immunosuppression markedly improves the clinical status, bio-

TABLE 53.1 Methods, associations, and reactants for autoantibodies in liver diseases

Autoantibody	Conventional method of detection*	Molecularly based assays	Disease association	Molecular target(s)
ANA	IIF	N/A	AIH-1; overlap syndromes	Multiple targets, particularly chromatin
SMA	IIF	N/A	AIH-1; overlap syndromes	Microfilaments (actin?); intermediate filaments (vimentin, etc.)
Anti-LKM-1	IIF†	ELISA, IB, RIA	AIH-2; HCV infection (5%)	Cytochrome P450 2D6
Anti-LC1	IIF, DID, CIE	ELISA, RIA	AIH-2	Formiminotransferase cyclodeaminase
SLA/LP	Inhibition ELISA	ELISA, IB, RIA	AIH-1; AIH-2 & AIH-negative for other reactivities	tRNP(Ser)Sec (see text)
Atypical pANCA (pANNA)	IIF	N/A	AIH-1; sclerosing cholangitis	Unidentified antigen(s) at nuclear periphery
AMA	IIF	ELISA, IB, RIA	primary biliary cirrhosis	E2 subunits of 2-oxo-acid dehydrogenase complexes, particularly PDC-E2

AIH, autoimmune hepatitis; AMA, anti-mitochondrial antibody; ANA, anti-nuclear antibody; CIE, counter-immuno-electrophoresis; DID, double dimension immunodiffusion; ELISA, enzyme linked immunosorbent assay; IB, immunoblot; IIF, indirect immunofluorescence; LC1, anti-liver cytosol type 1 antibody; LKM-1, anti-liver/kidney microsomal antibody type 1; pANCA, perinuclear anti-neutrophil cytoplasmic antibody; pANNA, perinuclear anti-neutrophil antibody; SLA/LP, anti-soluble liver antigen/liver pancreas antibody; RIA, radio-immunoprecipitation assay; SMA, anti-smooth muscle antibody.

*IIF—recommended cut-off titer for positivity is 1:40 except in children (see text). †Anti-LKM-1 and AMA both stain renal tubules and are frequently confused (see text).

From Vergani et al. (2004), reproduced with permission from *Journal of Hepatology*

chemical indices of liver dysfunction, and histologic abnormalities, and greatly influences survival (Soloway et al., 1972; Roberts et al., 1996). Corticosteroids may also decrease hepatic fibrosis or prevent its progression by reducing inflammatory activity (Czaja and Carpenter, 2004). More than 90% of patients with AIH experience an initial clinical, biochemical, and histologic remission, and only 2% fail to show some response. Response is usually evident within days or weeks at the most, and the induction doses of prednisolone of 40–60 mg daily can be tapered after 2–4 weeks to maintenance daily doses of 10 mg or even less. Liver functional indices and serum IgG levels should be assessed monthly for 3 months and thereafter at 3-month intervals. Autoantibody titers decrease during remission, often to negative, but are not useful markers of disease course (Czaja, 1999). Repetitive liver biopsies are unnecessary.

Treatment should be continued for at least 2 years. Thereafter some 80% of treatment responders will relapse whereas, if maintenance treatment is continued for four years, fewer (only 33%) will relapse (Kanzler et al., 2001; Heneghan and McFarlane, 2002). Post-treatment relapse requires reintroduction of immunosuppression and maintenance for an indefinite period on either low dose (5–15 mg/day) of prednisolone (Czaja, 1990) or azathioprine monotherapy at 2 mg/kg body weight (Johnson et al., 1995); azathioprine as monotherapy does not induce, but can sustain, remission. Recent data have shown that long-term azathioprine confers less risk for oncogenic and teratogenic complications than previously suspected. Relapse and retreatment do not preclude the possibility of ultimately sustaining a long-term remission without therapy. Patients with HLA DR3 less frequently experience full remission, and relapse rates are higher than for others, so that HLA DR3 is more prevalent among transplanted patients with AIH (González-Koch et al., 2001).

There are certain situations where the decision to treat AIH must be debatable. The first is AIH that appears biochemically, serologically and histologically "definite" yet the patient is asymptomatic, the diagnosis having been made incidentally and functional indices are only mildly deranged: does one commit such patients to a 2-year course of prednisolone or simply maintain surveillance. Evidence-based medicine does not help here but, considering the risk of slow evolution to "cryptogenic" cirrhosis, we lean to therapy. The second is "treatment-resistant" AIH, a relative term since it includes patients that require drug doses above accepted safety ranges, and also patients (of uncertain number) who are non-compliant with prescribed regimens. Small trials have been made of numerous alternative immunosuppressive agents, cyclosporine A, FK506, mycophenolate mofetil, cyclophosphamide, and budesonide, but outcomes have not been encouraging enough to warrant formal evaluations. Hence, a modified standard regimen is recommended, with a watch for effects of drug overdose, and liver transplantation a medium-term option. The serologic type of AIH, 1 or 2, does not influence treatment except for the some 5% of cases of type 2 that carry markers for HCV infection, wherein antiviral regimens may be preferable.

Notwithstanding the efficacy of "standard therapy," some cases of AIH undergo slow evolution to cirrhosis and liver insufficiency. Autoimmune hepatitis is among the best indications for transplantation with long-term (5-year) survival rates over 90%. Recurrence of disease after liver transplantation is managed by post-transplant immunosuppression, and does not prejudice long-term outcome (Manns and Bahr, 2000).

CONCLUDING REMARKS, FUTURE PROSPECTS

Hepatologists who are keen for a (much needed) better understanding of etiopathogenesis of AIH are, in a sense, victims of their successes! The disease is infrequent relative to the 0.1–1% prevalence for various other autoimmune disorders but, added to this, contemporary protocols for diagnosis and therapy are so well accepted and effective that research into the basic nature of AIH lacks the profile that pertains for the more prevalent and less successfully treatable autoimmune diseases. Increasingly, it is being ascertained, based particularly on autoantibody prevalences in long-archived serum samples, that autoimmune diseases have a prolonged subclinical phase (incubation period) before disease becomes overt, and AIH is likely no exception. If so, an initiating event to pathogenesis would be undetected. The problems relating to AIH remain abundant. What initiates the disease? What (for type 1) is the true autoantigen? Why (for type 1) is anti-actin such a striking marker? How (for type 2) does reactivity to CYP2D6 participate in pathogenesis? How does the special "lymphoid milieu" of the liver influence the expression of intrahepatic autoimmunity? What role do T cells play in either type of AIH? How earnestly should we seek to develop models based on transgenic systems? Will Treg cells intrude into AIH as for so many other autoimmune conditions? And so the list goes on. Supporters of research in hepatoimmunology should be sustained by the thought that lessons learned from the liver could have implications extending well beyond the capsule of this large, multifunctional, and immunologically interesting organ.

References

Abuaf, 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.

Adorini, L. (2002). Immunomodulatory effects of vitamin D receptor ligands in autoimmune diseases. *Internat. Immunopharmacol. 2,* 1017–1028.

Agarwal, K., Czaja, A.J., Jones, D.E.J., and Donaldson P.T. (2000a). CTLA-4 gene polymorphism and susceptibility to type 1 autoimmune hepatitis. *Hepatology 31,* 49–53.

Agarwal, K., Czaja, A.J., and Donaldson, P.T. (2000b). Fas (CD95/APO-1) promoter and CCR5 polymorphisms in type 1 autoimmune hepatitis (abstract). *Hepatology 32* (No.4, Pt.2), 166A.

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.

Baeres, M., Herkel, J., Czaja, A.J., Wies, I., Kanzler, S., Cancado, E.L.R., Porta, G., Nishioka, M., Simon, T., Daehnrich, C., Schlumberger, W., Galle, P.R., and Lohse, A.W. (2002). Establishment of standardized SLA/LP immunoassays: specificity for autoimmune hepatitis, worldwide occurrence, and clinical characteristics. *Gut 51,* 259–264.

Barker, M.H., Capps, R.B., and Allen, F.W. (1945). Chronic hepatitis in the Mediterranean theatre. *JAMA 129,* 653–659.

Beaune, P., Dansette, P.M., Mansuy, D., Kiffel, L., Finck, M., Amar, C., Letoux, J.P., and Heinberg, 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.

Berg, P.A., Stechemesser, E., and Strienz, J. (1981). Hypergammaglobulinämische chronische aktive Hepatitis mit Nachweis von leber-pankreas-spezifischen komplemtbindenden Autoantiköpern. *Verh. Deutsch Ges. Inn. Med. 87,* 921–927.

Bertolino, P., Heath, W.R., Hardy, C.L., Monahan, G. and Miller, J.F. (1995). Peripheral deletion of autoreactive CD8+ T cells in transgenic mice expressing H-2Kb in the liver. *Eur. J. Immunol. 25,* 1932–1942.

Bittencourt, P.L., Goldberg, A.C., Cancado, E.L.R., Porta, G., Laudanna, A.A., and Kalil, J. (1998). Different HLA profiles confer susceptibility to autoimmune hepatitis type 1 and 2. *Am. J. Gastroenterol. 93,* 1394–1395.

Boberg, K.M., Aadland, E., Jahnsen, J., Raknerud, N., Stiris, M., and Bell, H. (1998) Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand. J. Gastroenterol. 33,* 99–103.

Bogdanos, D.P. and McFarlane, I.G., (2003). Cytochromic P450 2A6 meets P450 2D6: an enigma of viral infections and autoimmunity. *J. Hepatol. 39,* 860–863.

Bottazzo, G.F., Florin-Christensen, A., Fairfax A., Swana, G., Doniach, D., and Groeschel-Stewart, U. (1976) Classification of smooth muscle autoantibodies detected by immunofluorescence. *J. Clin. Pathol. 29,* 403–410.

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.

Bowen, D.G., Zen, M., Holz, L., Davis, T., McCaughan, G.W., and Bertolino, P. (2004). The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *J Clin Invest. 114,* 701–712.

Cancado, E.L., 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.

Clemente, M.G., Obermayer-Straub, P., Meloni, A., Strassburg, C.P., Arangino V., Tukey, R.H., de Virgiliis, S., and Manns, M.P. (1997). Cytochrome P450 1A2 is a hepatic autoantigen in autoimmune polyglandular syndrome type l. *J. Clin. Endocrin. Metab. 82,*1853–1861.

Clemente, M.G., Meloni, A., Obermayer-Staub, P., Frau, F., Manns, M.P., and De Virgiliis, S. (1998) Two cytochromes P450 are major hepatocellular autoantigens in autoimmune polyglandular syndrome type 1. *Gastroenterology 114,* 324–328.

Cook, G.C., Mulligan, R., and Sherlock, S. (1971). Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Quart. J. Med. 40,* 159–185.

Cookson, S., Constantini, P.K., Clare, M., Underhill, J.A., Bernal, W., Czaja, A.J., and Donaldson, P.T. (1999). Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. *Hepatology 30,* 851–856.

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.

Crapper, R.M., Bhathal, P.S., and Mackay, I.R. (1986). Acute autoimmune hepatitis. *Digestion 34,* 216–225.

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.

Czaja, A.J. (1990). Low dose corticosteroid therapy after multiple relapses of severe HBsAg-negative chronic active hepatitis. *Hepatology 11,* 1044–1049.

Czaja, A.J. (1999). Behavior and significance of autoantibodies in type 1 autoimmune hepatitis. *J. Hepatol. 30,* 394–401.

Czaja, A.J. and Carpenter, H.A. (2004). Progressive fibrosis during corticosteroid therapy of autoimmune hepatitis. *Hepatology 39,* 1631–1638.

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.

Czaja, A.J. and Freese, D.K. (2002). Diagnosis and treatment of autoimmune hepatitis. *Hepatology 36,* 479–497.

Czaja, A.J. and Norman, G.L. (2003). Autoantibodies in the diagnosis and management of liver disease. *J. Clin. Gastroenterol. 37,* 315–329.

Czaja, A.J., Davis, G.L., Ludwig, J., and Taswell, H.F. (1984) Complete resolution of inflammatory activity following corticosteroid treatment of HBsAg-negative chronic active hepatitis. *Hepatology 4,* 622–627.

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.

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.

Czaja, A.J., Cassani, F., Cataleta, M., Valentini, P., and Bianchi, F.B. (1996a). Frequency and significance of antibodies to actin in type 1 autoimmune hepatitis. *Hepatology 24,* 1068–1073.

Czaja, A.J., Pfeifer, K.D., Decker, R.H., and Vallari, A.S. (1996b). Frequency and significance of antibodies to asialoglycoprotein receptor in type 1 autoimmune hepatitis. *Dig. Dis. Sci. 41,* 1733–1740.

Czaja, A.J., Morshed, S.A., Parveen, S., and Nishioka, M. (1997a). Antibodies to single-stranded and double-stranded DNA in antinuclear antibody-positive type 1-autoimmune hepatitis. *Hepatology 26,* 567–572.

Czaja, A.J., Kruger, M., Santrach, P.J., Moore, S.B., and Manns, M.P. (1997b). Genetic distinctions between types 1 and 2 autoimmune hepatitis. *Am. J. Gastroenterol. 92,* 2197–2200.

Czaja, A.J., Cookson, S., Constantini, P.K., Clare, M., Underhill, J.A., and Donaldson, PT. (1999). Cytokine polymorphisms associated with clinical features and treatment outcome in type 1 autoimmune hepatitis. *Gastroenterology 117,* 645–652.

Czaja, A.J., Donaldson, P.T., and Lohse, A.W. (2002). Antibodies to soluble liver antigen/liver pancreas and HLA risk factors in type 1 autoimmune hepatitis. *Am. J. Gastroenterol. 97,* 413–419.

Czaja, A.J., Shums, Z., Binder, W.L., Lewis, S.J., Nelson, V.J., and Norman, G.L. (2003). Frequency and significance of antibodies to chromatin in autoimmune hepatitis. *Dig. Dis. Sci. 48,* 1658–1664.

Dalekos, G.N., Wedemeyer, H., Obermayer-Straub, P., Kayser, A., Barut, A., Frank, H., and Manns, M.P. (1999). Epitope mapping of cytochrome P4502D6 autoantigen in patients with chronic hepatitis C under alpha-interferon treatment. *J. Hepatol. 30,* 366–75.

Dalekos, G.N., Obermayer-Straub, P., Bartels, M., Maeda, T., Kayser, A., Braun, S., Loges, S., Schmidt, E., Gershwin, M.E., and Manns, M.P. (2003). Cytochrome P450 2A6: a new hepatic autoantigen in patients with chronic hepatitis C infection. J. Hepatol. 39, 800–806.

Desmet, V.J., Gerber, M., Hoofnagle, J.H., Manns, M.P., and Scheuer, P.J. (1994). Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology 19,* 1513–1520.

Devaney K, Goodman, Z.D., and Ishak, K.G. (1992). Postinfantile giant-cell transformation in hepatitis. *Hepatology 16,* 327–333.

Dienes, H. P. (1989). *Viral and Autoimmune Hepatitis,* (pp. 47–51). Stuttgart: Fischer Verlag.

Djilali-Sariah, I., Oeulette, P., Caillar-Zucman, S., Debray, D., Kohn, J.I., and Alvarez, F. (2001). CTLA–4/CD28 region polymorphisms in children from families with autoimmune hepatitis. *Human Immunol. 62,* 1356–1362.

Doherty, D.G., Donaldson, P.T., Underhill, J.A., Farrant, J.M., Duthie, A., Mieli-Vergani, G., McFarlane, I.G., Johnson, P.J., Eddleston, A.L.W.F., Mowat, A.P., and Williams, R. (1994). Allelic sequence variation in the HLA class II genes and proteins in patients with autoimmune hepatitis. *Hepatology 19,* 609–615.

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.

Dos Remedios, C.G., Chhabra, D., Kekic, M., Dedova, M., Tsubakihara, M., Berry, D.A., and Nosworthy, N.J. (2002). Actin binding proteins: regulation of cytoskeletal microfilaments. *Physiol. Rev. 83,* 433–472.

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.

Fainboim, L., Marcos, Y., Pando, M., Capucchio, M., Reyes, G.B., Galoppo, C., Badia, I., Remondino, G., Ciocca, M., Ramonet, M., Fainboim, H., and Satz, M.L. (1994). Chronic active autoimmune hepatitis in children. Strong association with a particular HLA DR6 (DRB1*1301) haplotype. *Human Immunol. 41,* 146–150.

Fainboim. L., Velasco, V.C.C., Marcos, C.Y., Ciocca, M., Roy, A., Theiler, G., Capucchio, M., Nuncifora, S., Sala, L., and Zelazko, M. (2001). Protracted, but not acute, hepatitis A virus infection is strongly associated with HLA-DRB1*1301, a marker for pediatric autoimmune hepatitis. *Hepatology 33,* 1512–1517.

Frazer, I.H., Mackay, I.R., Bell, J., and Becker, G. (1985). The cellular infiltrate in the liver in autoimmune chronic active hepatitis: analysis with monoclonal antibodies. *Liver 5,* 162–72.

Frazer, I.H., Jordan, T.W., Collins, E.C., Andrews, P., and Mackay, I.R. (1987). Antibody to liver membrane antigens in chronic active hepatitis. *Hepatology 7,* 4–10.

Fukuda, Y., Yotsuyanagi, H., Ooka, S., Sekine, T., Kocke, J., Koike, J., Takano, T., Suzuki, M., Itoh, F., Nishioka, K., and Kato, T. (2004). Identification of a new autoantibody in patients with chronic hepatitis. *Hum. Immunol. 65,* 1530–1538.

Galkin, V.E., Vanloock, M.S., Orlova, A., and Egelman, E.H. (2002). A new internal mode in F-actin helps explain the remarkable evolutionary conservation of actin's sequence and structure. *Current Bio. 12,* 570–575.

Galle, P.R., Hofmann, W.J., Walczak, H., Shaller, H., Otto, G., Stremmel, W., Krammer, P.H., and Runkel, L. (1995). Involvement of the CD95 (APO-1/Fas) receptor and ligand in liver damage. *J. Exp. Med. 182,* 1223–1230.

Gebre-Medhin, G., Husebye, E.S., Gustafsson, J., Winqvist, O., Goksoyr, A., Rorsman, F., and Kampe, O. (1997). Cytochrome P4501A2 and aromatic L-amino acid decarboxylase are hepatic autoantigens in autoimmune polyendocrine syndrome type 1. *FEBS Lett 412,* 439–445.

Gelpi, C., Sontheimer, E.J., Rodriguez-Sanchez, J.L. (1992). Autoantibodies against a serine tRNA-protein complex implicated in cotranslational selenocysteine insertion. *Proc. Natl. Acad. Sci. U.S.A. 89,* 9739–9743.

Goldberg, A.C., Bittencourt, P.L., Mougin, B., Cancado, E.L.R., Porta, G., Carrilho, F., and Kalil, J. (2001). Analysis of HLA haplotypes in autoimmune hepatitis type 1: identifying the major susceptibility locus. *Hum. Immunol. 62,* 165–169.

González-Koch, A., Czaja, A.J., Carpenter, H.A., Roberts, S.K., Charlton, M.R., Porayko, M.K., Rosen, C.B., and Wiesner, RH. (2001). Recurrent autoimmune hepatitis after orthotopic liver transplantation. *Liver Transplant. 4,* 302–310.

Gueguen, M., Meunier-Rotival, M., Bernard, O., and Alvarez, F. (1988) Anti-liver kidney microsome antibody recognizes a cytochrome P450 from the IID subfamily. *J. Exp. Med. 168,* 801–806.

Gueguen, M., Yamamotoh, A.M., Bernard, O., and Alvarez, F. (1989). Anti-liver kidney microsome antibody type 1 recognizes human cytochrome P450 dbl. *Biochem. Biophys Res Comm 159,* 542–547.

Hardy, C.L., Bhathal, P.S., Snibson, K.J., and Adams, T.E.L. (1997). Comparison of intrahepatic lymphocytes from normal and growth hormone transgenic mice with chronic hepatitis and liver cancer. *Immunology 90,* 412–420.

Hay, J.E., Czaja, A.J., Rakela, J., and Ludwig, J. (1989). The nature of unexplained chronic aminotransferase elevations of a mild to moderate degree in asymptomatic patients. *Hepatology 9,* 193–197.

Heneghan, M.A. and McFarlane, I.G. (2002). Current and novel immunosuppressive therapy for autoimmune hepatitis. *Hepatology 35,* 7–13.

Holbrow, E.J., Asherson, G.L., Johnson, G.D., Barnes, R.D.S., and Carmichael, P.S. (1963). Antinuclear factor and other antibodies in blood and liver diseases. *Br. Med. J. 1,* 656–658.

Homberg, J-C., Abuaf, N., Bernard, O., Alvarez, F., Khalil, S.H., Poupon, R., Darnis, F., Levy, V.-G., Grippon, P., Opolon, P., Bernuau, J., Benhamou, J-P., and Alagille, D. (1987). Chronic active hepatitis with antiliver/kidney microsome antibody Type 1: A second type of autoimmune hepatitis. *Hepatology 7,* 1333–1339.

Howell, C.D. (2002) Animal models of autoimmunity. *Clin. Liver. Dis. 6,* 775–783.

Hurlburt, K.J., McMahon, B.J., Deubner, H., Hsu-Trawinski, B., Williams, J.L., and Kowdley, K.V. (2002). Prevalence of autoimmune hepatitis in Alaska natives. *Am. J. Gastroenterol. 97,* 2402–2407.

International Group of Pathologists (1977). Acute and chronic hepatitis revisited. *Lancet 310,* 914–919.

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. Immunopath. 84,* 223–243.

Johnson, P.J. and McFarlane, I.G. (1993). Meeting report: International Autoimmune Hepatitis Group. *Hepatology 18,* 998–1005.

Johnson, G.D., Holborow, E.J., and Glynn, L.E. (1965). Antibody to smooth muscle in patients with liver disease. *Lancet 2,* 878–879.

Johnson, P.J., McFarlane, I.G., and Williams, R. (1995). Azathioprine for long term maintenance of remission in autoimmune hepatitis. *N. Engl. J. Med. 333,* 958–963.

Joske, R.A. and King, W.E. (1955). The "L.E. Cell" phenomenon in active chronic viral hepatitis. *Lancet ii,* 477–480.

Kammer, A.R., van der Burg, S.H., Grabscheid, B., Hunziker, I.P., Kwappenberg, K.M.C., Reichen, J., Melief, C.J.M., and Cerny, A.

(1999). Molecular mimicry of human cytochrome P450 by hepatitis C virus at the level of cytoxic T cell recognition. *J. Exp. Med. 1990,* 169–176.

Kanzler, S., Weidemann, C., Gerken, G., Lohr, H.F., Galle, P.R., Meyer zum Büschenfelde, K.H., and Lohse, A.W. (1999). Clinical significance of autoantibodies to soluble liver antigen in autoimmune hepatitis. *J. Hepatol. 31,* 635–640.

Kanzler, S., Gerken, G., Löhr, H., Galle, P.R., Meyer zum Büschenfelde, K.-H., and Lohse, AW. (2001). Duration of immunosuppressive therapy in autoimmune hepatitis. *J. Hepatol. 34,* 354–355.

Kerkar, N., Choudhuri, K., Ma, Y., Mahmoud, A., Bogdanos, D.P., Muratori, L., Bianchi, F., Williams, R., Mieli-Vergani, G., and Vergani, D. (2003). Cytochrome P4502D6(193–212): A new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type 1-positive liver disease. *J. Immunol. 170,* 1481–1489.

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.

Kinoshita, H., Omagari, K., Whittingham, S., Kato, Y., Ishibashi, H., Sugi, K., Yano, M., Kohno, S., Nakanuma, Y., Penner, E., Wesierska-Gadek, J., Reynoso-Paz, S., Gershwin, M.E., Anderson, J., Jois, J.A., and Mackay, I.R. (1999). Autoimmune cholangitis and primary biliary cirrhosis—an autoimmune enigma. *Liver 19,* 122–128.

Kirk, A.P., Jain, S., Pocock, S., Thomas, H.C., and Sherlock, S. (1980). Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. *Gut 21,* 78–93.

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,* 1485–1501.

Kitazawa, E., Igarashi, T., Kawaguchi, N., Matsuhima, H., Kawashima, Y., Hankins, R.W., and Miyakawa, H. (2001). Differences in anti-LKM-1 autoantibody immunoreactivity to CYP2D6 antigenic sites between hepatitis C virus-negative and -positive patients. *J. Autoimmunity 17,* 243–249.

Kogan, J., Safadi, R., Ashur, Y., Shouval, D., and Ilan, Y. (2002) Prognosis of symptomatic versus asymptomatic autoimmune hepatitis. A study of 68 patients. *J. Clin. Gastroenterol. 35,* 75–81.

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.

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.

Kurki, P. and Virtanen, C. (1984). The detection of human antibodies against cytoskeletal components. *J. Immunol. Methods 67,* 209–223.

Lapierre, P., Hajoui, O., Homberg, J.-C., and Alvarez, F. (1999). Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognised by sera of patients with autoimmune hepatitis. *Gastroenterology 116,* 643–649.

Lapierre, P., Djilali-saiah, I., Vitozzi, S., and Alvarez, F. (2004). A murine model of type 2 autoimmune hepatitis: Xenoimmunization with human antigens. *Hepatology 39,* 1066–1074.

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.

Lenzi, M., Manotti, P., Muratori, L., Cataleta, M., Ballardini, G, Cassani, F., and Bianchi, F.B. (1995). Liver cytosolic 1 antigen-antibody system in type 2 autoimmune hepatitis and hepatitis C virus infection. *Gut 36,* 749–754.

Liu, Z-X. and Kaplowitz, N. (2002). Immune-mediated drug-induced liver disease. *Clin. Liver Dis. 6,* 755–774.

Lohse, A.W., Meyer zum Büschenfelde, K.-H., Franz, B., Kanzler, S., Gerken, G., and Dienes H.P. (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.

Ludwig, J., Czaja, A.J., Dickson, E.R., LaRusso, N.F., and Wiesner, R.H. (1984). Manifestations of nonsuppurative cholangitis in chronic hepatobiliary disease: morphologic spectrum, clinical correlations and terminology. *Liver 4,* 105–116.

Lunel, F., Abuaf, N., Frangeul, L., Grippon, P., Perrin, M., Le Coz, Y., Valla, D., Borotto, E., Yamamoto, A.-M., Huraux, J.-M., Opolon, P., and Homberg, J-C. (1992). Liver/kidney microsome antibody type 1 and hepatitis C virus infection. *Hepatology 16,* 630–636.

Ma, Y., Peakman, M., Lobo-Yeo, A., Wen, L., Lenzi, M., Gaken, J., Farzaneh, F., Mieli-Vergani, G., Bianchi, F.B., and Vergani, D. (1994). Differences in immune recognition of cytochrome P4502D6 by liver kidney microsomal (LKM) antibody in autoimmune hepatitis and chronic hepatitis C virus infection. *Clin. Exp. Immunol. 97,* 94–99.

Ma, Y., Okamoto, M., Thomas, M.G., Bogdanos, D.P., Lopes, A.R., Portmann, B., Underhill, J., Durr, R., Mieli-Vergani, G., and Vergani, D. (2002) Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. *Hepatology 35,* 658–664.

Mackay, I.R. (1968) Chronic hepatitis: effect of prolonged suppressive treatment and comparison of azathioprine with prednisolone. *Quart. J. Med. 37,* 379–392.

Mackay, I.R. (1972). The prognoses of chronic hepatitis. *Ann. Intern. Med. 77,* 649–651.

Mackay, I.R. (1975). Chronic active hepatitis. In: L. van der Reis (ed.), *Frontiers of Gastrointestinal Research*, vol 1, (pp. 142–187). Basel: Karger.

Mackay, I.R. (1985) Autoimmune diseases of the liver: chronic active hepatitis and primary biliary cirrhosis. In N.R. Rose and I.R. Mackay (ed.), *The Autoimmune Diseases,* (pp. 291–337) San Diego: Academic Press.

Mackay, I.R. (2000). Autoimmune disease overlaps and the liver: Two for the price of one. *J. Gastroenterol. Hepatol. 15,* 3–8.

Mackay, I.R. (2001). Antinuclear (chromatin) autoantibodies in autoimmune hepatitis. *J. Gastroenterol. Hepatol. 16,* 245–247.

Mackay, I.R. (2004). Hepatic disease and systemic lupus erythematosus: coincidence or convergence. In R.G. Lahita (ed.), *Systemic Lupus Erythematosus, 4th ed.* (pp. 993–1017). San Diego: Elsevier.

Mackay, I.R. and Gajdusek, D.C., (1958). An autoimmune reaction against human tissue antigens in certain acute and chronic diseases: II. Clinical correlation. *Arch. Intern. Med. 101,* 30–46.

Mackay, I.R. and Morris, P.J. (1972). Association of autoimmune chronic hepatitis with HLA1,8. *Lancet ii,* 793–795.

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.

Mackay, I.R. and Tait, B.D. (1994). The history of autoimmune hepatitis. In M. Nishioka, G. Toda, M. Zeniya (eds.), *Autoimmune Hepatitis* (pp. 3–23). Amsterdam: Elsevier.

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.

Mackay, I.R., Taft, L.I., and Cowling, D.C. (1956). Lupoid hepatitis. *Lancet 268,* 1323–1326.

Mackay, I.R., Weiden, S., and Hasker, J. (1965). Autoimmune hepatitis. *Ann. N.Y. Acad. Sci. 24,* 767–780.

Manns, M.P. and Bahr, M.J. (2000). Recurrent autoimmune hepatitis after liver transplantation—when non-self becomes self. *Hepatology 32,* 868–870 (Editorial).

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.

Manns, M., Gerken, G., Kyriatsoulis, A., Staritz, M., and Meyer zum Buschenfelde, K.H. (1987). Characterization of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. *Lancet, i,* 292–294.

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 is cytochrome P450db. *J. Clin. Invest. 83,* 1066–1072.

Manns, M.P., Zanger, U., Gerken, G., Sullivan, K.F., Meyer zum Büschenfelde, K.H., Meyer, U.A., and Eichelbaum, M (1990). Patients with autoimmune hepatitis type II express functionally intact cytochrome P450 DB1 which is inhibited by LKM-1 autoantibodies in vitro but not in vivo. *Hepatology 12,* 127–132.

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 P-450 monooxygenase. *J. Clin. Invest. 88,* 1370–1378.

Martini, 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.

Matsuo, I., Ikuno, N., Omagari, K., Kinoshita, H., Oka, M., Yamaguchi, H., Khono, S., and Mackay, I.R. (2000). Autoimmune reactivity of sera to hepatocyte plasma membrane in type 1 autoimmune hepatitis. *J. Gastroenterol. 35,* 226–234.

McFarlane, B. (1996). Hepatocellular membrane antigens. In I.G. McFarlane and R. Williams (eds.), *Molecular Basis of Autoimmune Hepatitis* (pp. 75–104). Austin: Landes.

McFarlane, I.G., McFarlane, B., Major, G.N., Tolley, P., and Williams, R. (1984a). Identification of the hepatic-receptor (hepatic lectin) as a component of liver specific membrane lipoprotein (LSP). *Clin. Exp. Immunol. 55,* 347–354.

McFarlane, I.G., Hegarty, J.E., McSorley, C.G., McFarlane, B.M., and Williams, R. (1984b) Antibodies to liver-specific protein predict outcome of treatment withdrawal in autoimune chronic active hepatitis. *Lancet 2,* 954–956.

McKiernan, S., Norris, S., Vaughan, R., Czaja, A.J., and Donaldson, P.T. (2002). MICA polymorphisms and susceptibility to autoimmune hepatitis: further evidence that susceptibility maps to the HLA class II region on chromosome 6p21.3 [abstract]. *Hepatology 36,* 503A.

Meyer zum Büschenfelde, K.H., Hütteroth, T.H., Manns, M., and Möller, B. (1980). The role of liver membrane antigens as targets in autoimmune type liver disease. *Springer Semin. Immunopathol. 3,* 297–315.

Michel, G., Ritter, A., Gerken, G., Meyer zum Buschenfelde, K.H., Decker, R., and Manns, M.P. (1992). Anti-GOR and hepatitis C virus in autoimmune liver diseases. *Lancet 339,* 267–269.

Mielei-Vergani G. and Vergani D. (2004). De novo autoimmune hepatitis after liver transplantation. *J. Hepatol. 40,* 3–7.

Misdraji, J., Thiim, M., and Graeme-Cook, F.M. (2004). Autoimmune hepatitis with centrilobular necrosis. *Am. J. Surg. Path. 28,* 471–478.

Miyagawa-Hayashino, A., Haga, H., Egawa, H., Hayashino, Y., Sakurai, T., Minamiguchi, S., Tanaka, K., and Manabe, T. (2004). Outcome and risk factors of de novo autoimmune hepatitis in living donor liver transplantation. *Transplantation 78,* 128–135.

Molinero, L.L., Fuertes, M.B., Rabinovich, G.A., Fainboim, L., and Zwirner, N.W. (2002). Activation-induced expression of MICA on T lymphocytes involves engagement of CD3 and CD28. *J. Leukoc. Biol. 71,* 791–797.

Muratori, L., Cataleta, M., Muratori, P., Lenzi, M., Cassani, F., and Bianchi, F.B. (1995). Detection of anti-liver cytosol antibody type 1 (anti-LC1) by immunodiffusion, counter immunoelectrophoresis and immunoblotting: comparison of different techniques. *J. Immunol. Methods 187,* 259–264.

Muratori, L., Sztul, E., Muratori, P., Gao, Y., Ripalti, A., Ponti, C., Lenzi, M., Landini, M.P., and Bianchi F.B. (2001). Distinct epitopes on formiminotransferase cyclodeaminase induce autoimmune liver cytosol antibody type 1. *Hepatology 34,* 494–501.

Muratori, P., Muratori, M., Agostinelli, D., Pappas, G., Veronesi, L., Granito, A., Cassani, F., Terlizzi, P., Lenzi, M., and Bianchi, FB. (2002). Smooth muscle antibodies and type 1 hepatitis. *Autoimmunity 35,* 497–500.

Murray-Lyon, I.M., Stern, R.B., and Williams, R. (1973). Controlled trial of prednisolone and azathioprine in active chronic hepatitis. *Lancet i,* 735–737.

Nikias, G.A., Batts, K.P., and Czaja, A.J. (1994). The nature and prognostic implications of autoimmune hepatitis with an acute presentation. *J. Hepatol. 21,* 866–871.

Obermayer-Straub, P., Strassburg, C.P., and Manns, M.P. (2000). Autoimmune hepatitis. *J. Hepatol. 32,* 181–197.

Obermayer-Straub, P., Perheentupa, J., Braun, S., Kayer, A., Barut, A., Loges, S., Harms, A., Dalekos, G., Strassburg, C.P., and Manns, M.P. (2001). Hepatic autoantigens in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Gastroenterology 121,* 668–677.

Orian, J.M., Tamakoshi, K., Mackay, I.R., and Brandon, M.R. (1990). A new murine model for hepatocellular carcinoma: metallothionine-growth hormone transgenic mice. *J. Natl. Cancer Inst. 82,* 393–398.

Page, A.R. and Good, R.A. (1960). Plasma cell hepatitis with special attention to steroid therapy. *J. Dis. Child. 99,* 288–314.

Pando, M., Larriba, J., Fernandez, G.C., Fainboim, H., Ciocca, M., Ramonet, M., Badia, I., Daruich, J., Findor, J., Tanno, H., Canero-Velasco, C., and Fainboim, L. (1999). Pediatric and adult forms of type 1 autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. *Hepatology 30,* 1374–1380.

Parker, D.R. and Kingham, J.G.C. (1997). Type 1 autoimmune hepatitis is primarily a disease of later life. *Quart. J. Med. 90,* 289–296.

Phillips, M.J., Blendis, L.M., Poucell, S., Offterson, J., Petric, M., Roberts, E., Levy, G.A., Superina, R.A., Greig, P.D., and Cameron, R. (1991). Syncytial giant-cell hepatitis. Sporadic hepatitis with distinctive pathological features, a severe clinical course, and paramyxoviral features. *N. Engl. J. Med. 324,* 455–460.

Philipp, T., Durazzo, M., Trautwein, C., Alex, B., Straub, P., Lamb, G., Johnson, E.F., Tukey, R.H., and Manns, M.P. (1994). Recognition of uridine diphosphate glucoronosyl transferases by LKM-3 antibodies in chronic hepatitis D. *Lancet 344,* 576–581.

Popper, H., Paronetto, F., and Schaffner, F. (1965). Immune processes in the pathogenesis of liver disease. *Ann. N.Y. Acad. Sci. 124,* 781–799.

Porta, G., Gayotto, L.C., and Alvarez, F. (1990). Anti-liver-kidney microsome antibody-positive autoimmune hepatitis presenting as fulminant liver failure. *J. Ped. Gastroenterol. Nutrition 11,* 138–140.

Pratt, D.S. and Kaplan, M. (2000). Evaluation of abnormal liver enzyme results in asymptomatic patients. *N. Engl. J. Med. 342,* 1266–1271.

Pratt, D.S., Fawaz, K.A., Rabson, A., Dellelis, R., and Kaplan, M.M. (1997). A novel histological lesion in glucocorticoid-responsive chronic hepatitis. *Gastroenterology 113,* 664–668.

Reddy, R.K., Krawitt, E.L., Homberg, J.C., Jeffers, L.J., deMedina, M., Chastenay, B., Poupon, R., Opolon, P., Beaugrand, M., Abuaf, N., Radick, J., Parker, T., Johanet, C., Gergeois, J., and Schiff, E.R. (1995) Absence of anti-LKM1 in hepatitis C viral infection in the United States. *J. Viral Hepat. 2,* 175–179.

Rizzetto, M., Swana, G., and Doniach, D. (1973). Microsomal antibodies in active chronic hepatitis and other disorders. *Clin. Exp. Immunol. 15,* 331–344.

Roberts, S.K., Therneau, T., and Czaja, A.J. (1996). Prognosis of histological cirrhosis in type 1 autoimmune hepatitis. *Gastroenterology 110,* 848–857.

Sacher, M., Blümel, P., Thaler, H., and Manns, M.P. (1990). Chronic active hepatitis associated with vitiligo, nail dystrophy, alopecia, and a new variant of LKM antibodies. *J. Hepatol. 10,* 364–369.

Schalm, S.W., Korman, M.G., Summerskill, W.H.J., Czaja, A.J., and Baggenstoss, A.H. (1977). Severe chronic active liver disease. Prognostic significance of initial morphologic patterns. *Am. J. Dig. Dis. 22,* 973–980.

Schramm, C., Kanzler, S., Meyer zum Buschenfelde, K-H., Galle, P.R., and Lohse, A.W. (2001). Autoimmune hepatitis in the elderly. *Am. J. Gastroenterol. 96,* 1587–1591.

Searle, J., Harmon, B.V., Bishop, C.J., and Kerr, J.F.R. (1987). Significance of single cell death by apoptosis in hepatobiliary disease. *J. Gastroenterol. Hepatol. 2,* 77–96.

Seki, T., Ota, M., Furuta, S., Fukushima, H., Kondo, T., Hino, K., Mizuki, N., Ando, A., Tsuji, K., Inoko, H., and Kiyosawa, K. (1992). HLA class II molecules and autoimmune hepatitis susceptibility in Japanese patients. *Gastroenterology 103,* 1041–1047.

Singh, R., Nair, S., Farr, G., Mason, A., and Perrillo, R. (2002) Acute autoimmune hepatitis presenting with centrizonal liver disease: case report and review of the literature. *Am. J. Gastroenterol. 97,* 2670–2673.

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.

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.

Soloway, R.D., Summerskill, W.H.J., Baggenstoss, A.H., Geall, M.G., Gitnick, G.L., Elveback, L.R., and Schoenfield, L.J. (1972). Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology 63,* 820–833.

Stechemesser, E., Klein, R., and Berg, P.A. (1993). Characterization and clinical relevance of liver-pancreas autoantibodies in autoimmune hepatitis. *Hepatology. 18,* 1–9.

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.

Strettell, M.D.J, Donaldson, P.T., Thomson, L.J., Santrach, P.J., Moore, S.B., Czaja, A.J., and Williams, R. (1997). Allelic basis for HLA-encoded susceptibility to type 1 autoimmune hepatitis. *Gastroenterology 112,* 2028–2035.

Sugimura, T., Obermayer-Straub, P., Kayser, A., Braun, S., Loges, S., Alex, B., Luttig B., Johnson, E.F., Manns, M.P., and Strassburg, C.P. (2002). A major CYP2D6 autoepitope in autoimmune hepatitis type 2 and chronic hepatitis C is a three-dimensional structure homologeous to other cytochrome P450 autoantigens. *Autoimmunity 35,* 501–513.

Swanson, N.R., Reed, W.D., Yarred, L.J., Shikin, K.B., and Joske, R.A. (1990). Autoantibodies to isolated plasma membranes in chronic active hepatitis. II. Specificity of antibodies. *Hepatology 11,* 613–621.

Tait, B., Mackay, I.R., Board, P., Coggan, M., Emery, P., and Eckardt, G. (1989). HLA A1, B8, DR3 extended haplotype in autoimmune chronic hepatitis. *Gastroenterology 97,* 479–481.

Targan, S.R., Landers, C., Vidrich, A., and Czaja, A.J. (1995). High-titer antineutrophil cytoplasmic antibodies in type 1 autoimmune hepatitis. *Gastroenterology 108,* 1159–1166.

Te, H.S., Konkoulis, G., and Ganger, D.R. (1997). Autoimmune hepatitis: a histological variant associated with nominent centrilobular necrosis. *Gut 41,* 269–271.

Terjung, B., Worman, H.J., Herzog, V., Suerbruch, T., and Spengler, U. (2001). Differentiation of antineutrophil nuclear antibodies in inflammatory bowel and autoimmune liver diseases from antineutrophil cytoplasmic antibodies (p-ANCA) using immunofluorescence microscopy. *Clin. Exp. Immunol. 126,* 37–46.

Tiegs, G., Hentschel, J., and Wendel, A. (1992). A T cell-dependent experimental liver injury in mice inducible by concanavalin *Am. J. Clin. Invest. 90,* 196–203.

Toda, G., Zeniya, M., Watanabe, F., Imawari, M., Kiyosawa, K., Nishioka, M., Tsuji, T., Omata, M., and the Japanese National Study Group of Autoimmune Hepatitis (1997). Present status of autoimmune hepatitis in Japan—correlating the characteristics with international criteria in an area with a high rate of HCV infection. *J. Hepatol. 26,* 1207–1212.

Toh, B.H. (1979). Smooth muscle autoantibodies and autoantigens. *Clin. Exp. Immunol. 38,* 621–628.

Torres-Collado, A.X., Czaja, A.J., and Gelpi, C. Anti-tRNP(Ser)Sec/SLA/LP autoantibodies. Comparative study using in-house ELISA with recombinant 48.8 kDa protein, immunoblot and analysis of immunoprecipitated RNAs. *Liver Intl. 25,* 410–419.

Treichel, U. and Meyer zum Büschenfelde, K.H. (1998). The asialoglycoprotein receptor in autoimmune liver disease. In E.L. Krawit, R.H. Wiesner and M. Nishioka (eds), *Autoimmune Liver Diseases, 2nd ed.* (pp. 273–286). Amsterdam: Elsevier.

Treichel, U., McFarlane, B., Seki, T., Krawitt E.L., Alessi N., Stickel F., McFarlane I.G., Kiyosawa K., Furuta S., Freni M.A., Gerken G., and Meyer zum Büschenfelde KH. (1994) Demographics of anti-asialoglycoprotein receptor autoantibodies in autoimmune hepatitis. *Gastroenterology 107,* 799–804.

Vazquez-Garcia, M.N., Alaez, C., Olivo, A., Debaz, H., Perez-Luque, E., Burguete, A., Cano, S., de la Rosa, G., Bautista, N., Hernandez, A,, Bandera. J., Torres, L.F., Kersenobich, D., Alvarez, F., and Gorodezky, C. (1998). MHC class II sequences of susceptibility and protection in Mexicans with autoimmune hepatitis. *J. Hepatol. 28,* 985–990.

Vergani, D., Alvarez, F., Bianchi, F.B., Cancado, E.L.R., Mackay, I.R., Manns, M.P., Nishioka, M., and Penner, E. (2004). Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis group. *Hepatology 41,* 1–7.

Vierling, J.M. (2003). Animal models of autoimmune diseases of the liver. In M.E. Gershwin, J. Vierling and Manns (eds), *Liver Immunology* (pp. 263–290). Philadelphia: Harley & Belfus.

Vivier, E., Tomasello, E., and Paul, P. (2002). Lymphocyte activation via NKG2D: towards a new paradigm in immune recognition. *Curr. Opin. Immunol. 14,* 306–311.

Vogel, A., Strassburg, C.P., and Manns, M.P. (2002). Genetic association of vitamin D receptors polymorphisms with primary biliary cirrhosis and autoimmune hepatitis. *Hepatology 35,* 126–131.

Volkmann, M., Martin, L., Baurle, A., Heid, H., Strassburg, C.P., Trautwein, C., Fiehn, W., and Manns, M.P. (2001). Soluble liver antigen: isolation of a 35-kd recombinant protein (SLA-p35) specifically recognizing sera from patients with autoimmune hepatitis. *Hepatology 33,* 591–596.

Vong, S. and Bell, B.P. (2004) Chronic disease mortality in the United States, 1990–1998. *Hepatology 39,* 476–483.

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.

Waxman, D.J., Lapenson, D.P., Krishman, 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.

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.

Whittingham, S., Mackay, I.R., and Irwin, J. (1966). Autoimmune hepatitis. Immunofluorescence reactions with cytoplasm of smooth muscle and renal glomerular cells. *Lancet 1,* 1333–1335.

Wies, I., Brunner, S., Henninger, J., Herkel, J., Kanzler, S., Meyer zum Buschenfelde, K.-H., and Lohse, A.W. (2000). Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. *Lancet 355,* 1510–1515.

Wilkinson, S.P., Portmann, B., Cochrane, A.M.G., and Williams, R. (1978). Clinical course of chronic lobular hepatitis. *Quart. J. Med. 47,* 421–429.

Woodward, J. and Neuberger J. (2001). Autoimmune overlap syndromes. *Hepatology 33,* 994–1002.

Yamamoto, A.M., Cresteil, D., Boniface, O., Clerc, F.F., and Alvarez, F. (1993a). Identification and analysis of cytochrome P4502D6 antigenic sites recognized by anti-liver-kidney microsome type–1 antibodies (LKM1). *Eur. J. Immunol. 23,* 1105–1111.

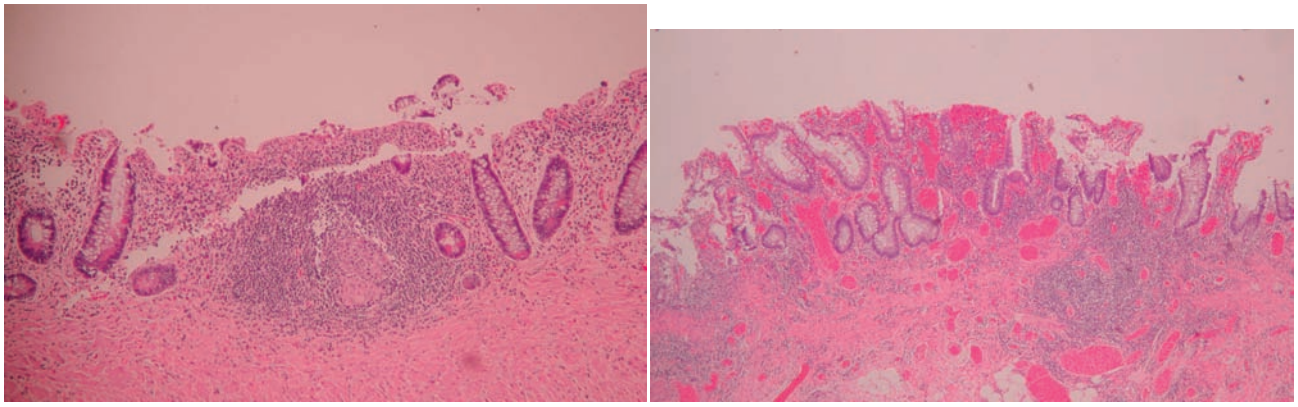
Yamamoto, A.M., Cresteil, D., Homberg, J.C., and Alvarez, F. (1993b). Characterization of the anti-liver-kidney microsome antibody (anti-LKM1) from hepatitis C virus-positive and -negative sera. *Gastroenterology 104,* 1762–1767.

Yamauchi, K., Yamaguchi, N., Furukawa, T., Takatsu, K., Nakanashi, T., Sasaki, M., Isono, E., Tokushige, K., Komatsu, T., and Shiratori, K. (2004). A novel 1gM class autoantibody to a hepatocytes-related 190 kDa molecule in patients with type 1 autoimmune hepatitis. *Hepatology 40*, 687–692.

Zamanou, A., Samiotaki, M., Panayotou, G., Margaritis, L., and Lymberi, P. (2003). Fine specificity and subclasses of IgG anti-actin autoantibodies differ in health and disease. *J. Autoimmunity 20,* 333–344.

Zampieri, S., Mahler, M., Blüthner, M., Qiu, Z., Malmegrin, K., Ghirardello, A., Doria, A., Van Venrooij, W.J., and Raats, J.M.H.L. (2003). Recombinant anti-P protein autoantibodies isolated from a human autoimmune library: reactivity, specificity and epitope recognition. *Cell Molec. Life Sci. 66,* 588–598.

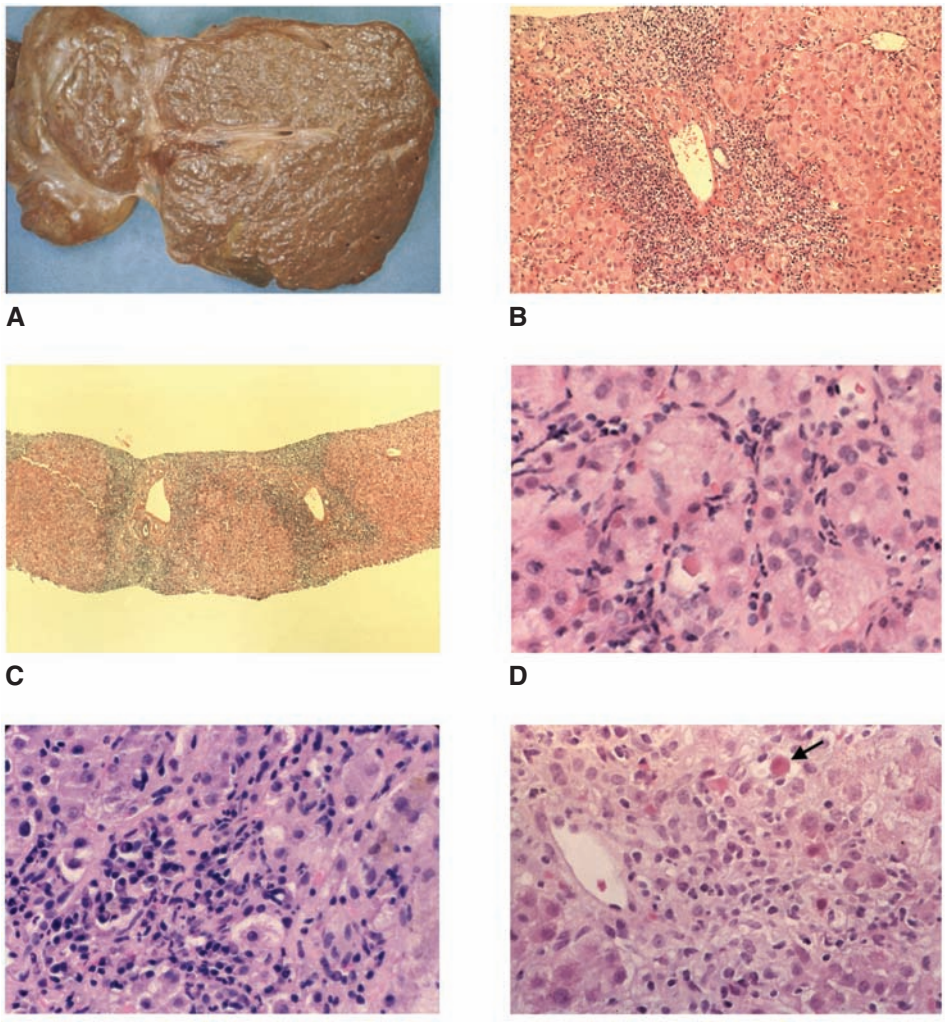
Zanger, U.M., Hauri, H.-P., Loeper, J., Homberg, J.-C., and Meyer, U.A. (1988). Antibodies against human cytochrome P–450db1 in autoimmune hepatitis type II. *Proc. Natl. Acad. Sci. U.S.A. 85,* 8256–8260.



A B

FIGURE 52.3 Histology of irritable bowel disease. *A,* Crohn disease in the left colon showing a non-necrotizing granuloma, inflammatory cell infiltrate, and Paneth cell metaplasia. *B,* Ulcerative colitis showing crypt architecture distortion, hemorrhage, ulceration, and inflammatory cell infiltrate.

Courtesy of Dr Mikhail Tismenetsky.



A B C D E F

FIGURE 53.1 Pathological features of autoimmune hepatitis (AIH) *A,* An autopsy specimen of a liver in advanced AIH illustrating coarse nodular cirrhosis and shrunken left lobe. *B–F,* photomicrographs from cases of AIH type 2. *B, Interface hepatitis* illustrating lymphocytic infiltrate in portal trail of liver abutting an adjacent liver lobules. *C, Bridging necrosis* illustrating necroinflammatory infiltrates connecting portal tracts and a likely forerunner to fibrosis and cirrhosis. *D, Lobular hepatitis* illustrating extra lobular lymphocytic infiltrates, damaged hepatocytes and prominence of (spindle-shaped) Kupffer cells. *E, Lobular hepatitis* illustrating severely degraded liver cells surrounded by lymphocytes, and prominence plasma cells. *F. Periportal hepatitis* illustrating *(arrow)* a shrunken eosinophilic remnant of an hepatocytic that has undergone apoptosis (Councilman body).

A, from Joske and King, (1955).

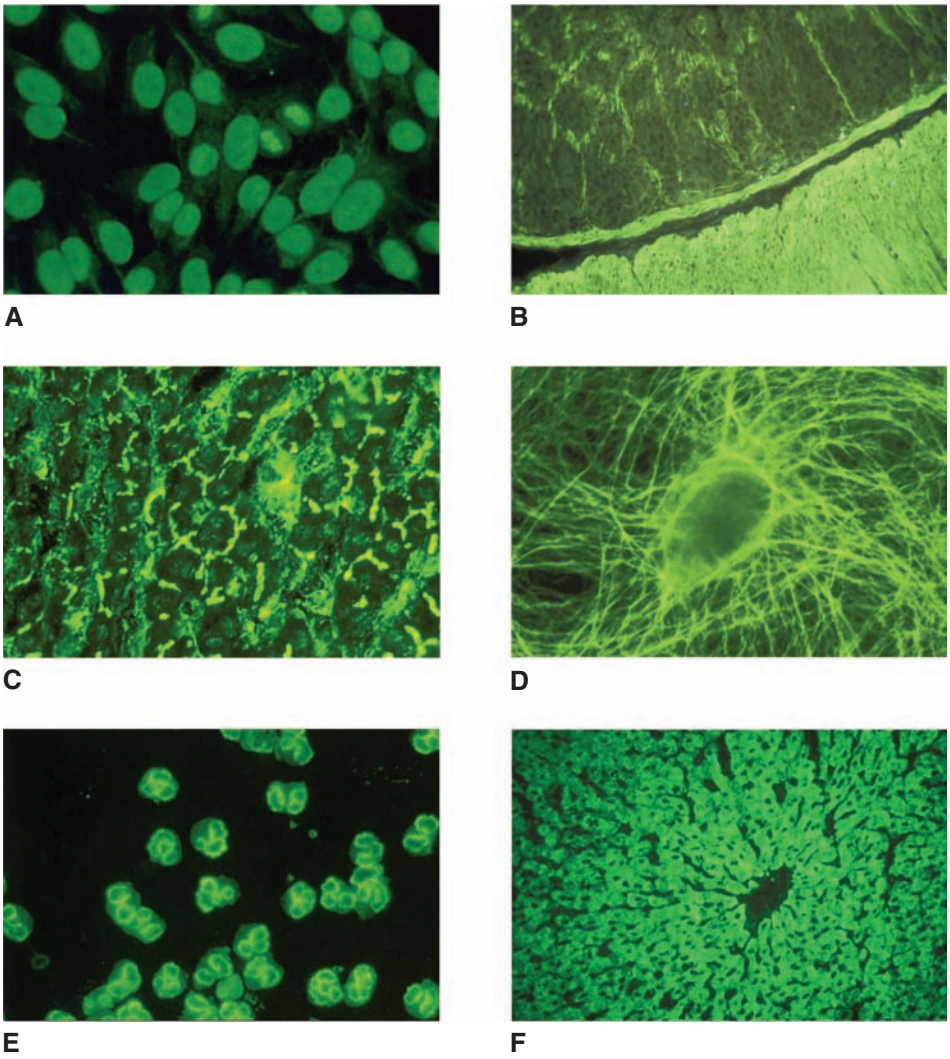


FIGURE 53.2 Serologic expressions of autoimmune hepatitis (AIH) by indirect immunofluorescence. *A,* Antinuclear antibody (ANA) from an AIH type 1 serum on cells of HEp2 cell line illustrating typical homogeneous pattern of labeling. *B,* Smooth muscle antibody (SMA) on mouse from an AIH type 1 serum illustrating labeling of submucosal smooth muscle layer musculares mucosal (thin band) and then verticle strands in mucosa suggestive of antiactin reactivity. *C,* Antiactin reactivity on mouse liver by an AIH type 1 serum illustrating polygonal pattern due to labeling of submembranous actin of hepatocytes. *D,* Anti-intermediate filament reactivity on cultured fibroblast of an SMA positive but antiactin negative serum; this reactivity is not specific for AIH. *E,* Antineutrophil cytoplasmic antibodies (ANCA) reactivity on granutocytes exposed to an ANCA-positive serum, as seen in vasculitic and other diseases (see Chapter ••); note that the perinuclear ANCA seen in AIH may be a true antineutrophil nuclear autoantibody (see text). *F,* Anti-LKM1 reactivity on mouse liver by an AIH type 2 serum illustrating evenly granular labeling of cytoplasm of hepatocytes throughout the lobule.

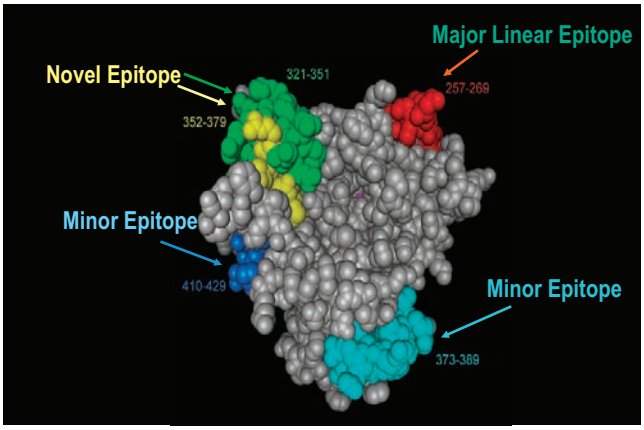


FIGURE 53.3 A three dimensional representation of cytochrome P450 2D6 showing various epitope regions. A major epitope region *(red)* at amino acids 257–269 is a reactant for autoantibodies in autoimmune hepatitis (AIH) type 2, and it has sequence homology with the intermediate-early protein IE 175 of herpes simplex virus type 1 (Manns et al., 1991). An epitope region *(green/yellow)* is a reactant for autoantibodies in AIH type 2 and chronic hepatitis C. Minor epitopes at amino acids 373–389 and amino acids 410–429 are shown in light blue and dark blue, respectively.

From Sigimura et al. (2002), and reproduced with permission from *Autoimmunity*.

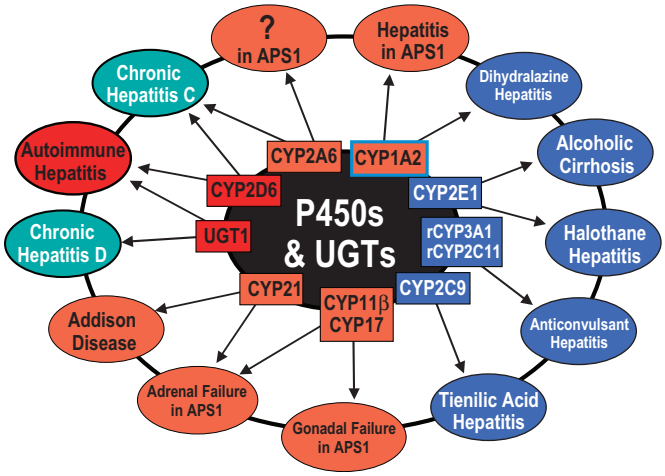


FIGURE 53.4 Cytochrome P450 isoforms (CYPs) and uridine-5′-diphosphate glucuronosyl transferase 1 (UGT1) as autoantigenic targets in various types of hepatitis occurring in spontaneous autoimmune hepatitis (AIH) type 2 *(red)*, or associated with drug reactions *(blue)* or hepatitis virus infection *(green)*, or other spontaneous autoimmune conditions *(orange)*. (APS1, autoimmune polyendocrine syndrome type 1.)

From Manns and Obermeyer-Straub (1997) and reproduced with permisstion from *Hepatology*.

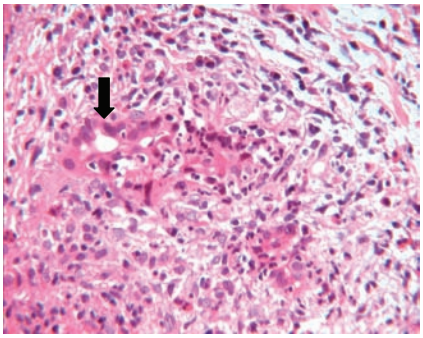


FIGURE 55.2 Portal tract inflammatory cell infiltrate in a case of juvenile sclerosing cholangitis positive for anti-nuclear and anti-smooth muscle antibodies. Plasma cells and lymphocytes cross the limiting plate invading the parenchyma (interface hepatitis). The arrow points to a damaged bile duct.

CHAPTER

54

Primary Biliary Cirrhosis

CARLO SELMI,*† ROSS L. COPPEL,‡ AND M. ERIC GERSHWIN*

**Division of Rheumatology, Allergy, and Clinical Immunology, University of California at Davis, Davis, CA. †Division of Internal Medicine, San Paolo School of Medicine, University of Milan, Italy. ‡Department of Microbiology, Monash University, Clayton, Victoria, Australia*

The first description of biliary cirrhosis, albeit possibly secondary, can be traced back to the work of the Italian pathologist Giovanni Battista Morgagni in 1761, while the first report of non-obstructive biliary cirrhosis was by Addison and Gull in 1851. For this reason, the entity was then known as the Addison–Gull syndrome (Reuben, 2003) until the term primary biliary cirrhosis (PBC) was finally accepted in medical literature (Ahrens et al., 1950). In 1959, Sherlock presented the first series of patients affected by PBC who had been followed over the previous decade and noted that patients presented with pruritus as well as with signs and symptoms of endstage liver disease including jaundice (Sherlock, 1959). In most cases, the diagnosis was made after laparotomy, once the physical examination or the referred symptoms had suggested the presence of cholestasis. The association between serum antimitochondrial antibodies (AMA) and PBC was first recognized in 1965 by Walker and colleagues who described the specific fluorescence pattern due to reactivity with antigens present in cytoplasmic organelles; such reactivity was found almost exclusively in sera from affected individuals (Walker et al., 1965). In 1987, the AMA antigens were cloned and identified as subunits of the pyruvate dehydrogenase complex (PDC) located on the inner mitochondrial membrane (Gershwin et al., 1987). This discovery led to the development of more sensitive assays for the determination of AMA, although indirect immunofluorescence remains the method of routine testing in most clinical centers.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Diagnosis

The diagnosis of PBC, although in some cases suggested by symptoms or clinical signs (see below), is based on three objective criteria. This triad consists of detectable serum AMA (titer >1:40), increased enzymes indicating cholestasis (i.e. alkaline phosphatase) for longer than 6 months, and a compatible or diagnostic liver histology (Heathcote, 2000). A classification proposed by a British group (Metcalf et al., 1997) indicates a "probable" diagnosis when two out of the three criteria (most often AMA-positivity and compatible liver histology, but normal liver enzymes) are present. Accordingly, a "definite" diagnosis can be made in the presence of all three states. This classification may be overly strict as the vast majority of asymptomatic AMA-positive individuals will eventually develop a classical picture of PBC during follow-up. Moreover, patients lacking detectable AMA (especially when indirect immunofluorescence is used) but otherwise presenting signs of PBC should be regarded as affected by "AMA-negative PBC" (or "autoimmune cholangitis"), as they appear to follow a similar natural history when compared to their AMA-positive counterparts (Invernizzi et al., 1997). Although it remains critical for the assessment of the histologic stage, the issue of whether a liver biopsy is still needed for the diagnosis of PBC is still highly debated. Currently, performing a liver biopsy seems not to be indicated when the other two diagnostic criteria are met. However, histology should be pursued to establish the diagnosis of PBC in the presence of serum AMA and serum alkaline phosphatase less than 1.5 times the upper normal level (Zein et al., 2003) or in the absence of detectable AMA.

Asymptomatic/Symptomatic Primary Biliary Cirrhosis

In a large number of cases (20–60%) the diagnosis of PBC is established in the absence of symptoms indicating a liver condition or cholestasis (Inoue et al., 1995). The number of asymptomatic patients at the time of diagnosis has been increasing with time since the earlier series descriptions when the majority of patients were diagnosed when jaundice was already present (Ahrens et al., 1950). The increasing number of symptomless patients most likely also represents the growing awareness of the syndrome as well as, perhaps more importantly, the availability of more sensitive noninvasive tests. Interestingly, however, symptomless patients are commonly older than symptomatic ones, thus possibly implying differences in the progression of PBC in these two groups (Howel et al., 2000). We note, however, that during extended clinical follow-up most AMA-positive patients will eventually develop PBC-associated symptoms, such as fatigue, pruritus, or right upper abdominal pain (Metcalf et al., 1997). In the later stages of the disease, when liver cirrhosis appears, symptoms of advanced liver disease, such as complications of portal hypertension or jaundice, may also develop. The most common symptoms accompanying PBC are fatigue and pruritus. Physical findings may include skin hyperpigmentation, hepatosplenomegaly, and xanthelasmas (caused by deposition of cholesterol). Jaundice may appear in advanced stages and is now less common than described in early reports (Sherlock and Scheuer, 1973), where patients were diagnosed when severe liver impairment had already developed. Endstage symptoms are those of all liver cirrhosis and include ascites, encephalopathy, and upper digestive bleeding. In some cases, however, endoscopic signs of portal hypertension, such as varices or gastropathy, can be encountered during what is apparently early stages PBC—i.e., without histologic evidence of liver cirrhosis—and appears to be secondary to presinusoidal fibrosis and inflammation induced by granulomas (Navasa et al., 1987).

Clinical features

Fatigue

Fatigue, roughly defined as reported severe distress and disability, may affect up to 70% of patients with PBC. The symptom may be overlooked by patients, particularly middle-aged women, who might believe it a consequence of aging. Similar prevalence rates can be observed in other autoimmune conditions including systemic lupus erythematosus in which, however, fatigue often correlates with depression rather than with immunologic markers or inflammation. In PBC, the severity of fatigue is also independent of the severity of liver disease or its other symptoms (pruritus or severe cholestasis) and independent of psychiatric factors. Impairments in central neurotransmission (Jones and Yurdaydin, 1997) or corticotropin-releasing hormone response (Swain and Maric, 1995), and, more recently, morphologic abnormalities of the central nervous system due to accumulation of manganese have been postulated as putative causes of fatigue in PBC (Forton et al., 2004). No medical treatment has been shown effective in alleviating this symptom, although fatigue has never been included as an endpoint in any of the large controlled clinical trials.

Pruritus

Pruritus is considered the second most common symptom of PBC, affecting 20–70% of patients with PBC and jaundice. Longitudinal data show that the vast majority of

patients will experience this symptom during progression of the disease, and its appearance most commonly precedes jaundice by months or years. Pruritus can be localized or diffuse, but at the time of onset it is more frequently localized to specific areas. Typically, the symptom worsens at night, following contact with certain fabrics (wool), or in warm climates. However, the pruritus of cholestasis is not mediated in the skin. The two main hypotheses to explain the pathogenesis of pruritus implicate either serum bile-acid retention secondary to chronic cholestasis, or alternatively but not exclusively, an amplified release of endogenous opioids (Bergasa et al., 2000).

Treatment of pruritus in PBC is often challenging for the clinician. Trials of antihistamines or phenobarbital for the treatment of the symptom have proven these medications to be ineffective, whereas the use of cholestyramine (4 g before and after the first meal) ameliorates pruritus. In selected cases poorly responsive to resins, rifampicin has been used to achieve rapid symptom relief; its prolonged use, however, is not recommended. Since experimental evidence indicates that the opioid neurotransmitter system, rather than bile acid retention alone, might mediate pruritus in chronic cholestasis, a central mechanism has been proposed. This hypothesis is supported by data (Jones and Bergasa, 1999) demonstrating that opioid receptor ligands with agonist properties mediate pruritus and that endogenous opioid-mediated neuromodulation in the central nervous system is increased in chronic cholestasis. For these reasons, treatment with opiate antagonists such as naltrexone 50 mg/day is currently being used for pruritus in PBC. The efficacy of naltrexone has been assessed in one controlled clinical study that has also indicated that side effects were temporary and usually did not require specific treatment (Terg et al., 2002). Studies of the behavioral consequence of the pruritus of cholestasis, scratching activity, allow for the design of clinical trials with objective and well-defined endpoints. The efficacy of sertraline, so far evaluated only in one clinical study (Browning et al., 2003), is promising but warrants further evaluation. In patients with intractable pruritus, liver transplantation is the ultimate therapeutic option.

Portal Hypertension

As mentioned above, portal hypertension is a common finding in patients with PBC, but significantly fewer patients now present with acute digestive bleeding or other signs of portal hypertension compared with the first reported series of affected individuals. Interestingly, portal hypertension in PBC does not imply the presence of liver cirrhosis. Longitudinal studies indicate that approximately 58% of untreated patients will eventually develop endoscopic signs of portal hypertension over a 4-year follow-up (Lindor et al., 1997). Signs of portal hypertensions should be monitored in individuals affected by PBC but the phenomenon should be regarded as less life-threatening than if it occurred in the context of other chronic liver conditions.

Reduction in Bone Density

A metabolic bone disease is found in PBC with accelerated bone loss due to reduced bone deposition being noted in patients compared with sex- and age-matched healthy individuals. These findings are still somewhat contentious and conflicting data have been reported. A mild reduction of bone density (osteopenia) is present in about 30% of patients while frank osteoporosis is diagnosed in 10% of patients. The bone loss can, moreover, worsen after liver transplantation, possibly due to the administration of specific immunosuppressive drugs. The mechanisms leading to metabolic bone alterations are not completely understood as no significant changes in the metabolism of calcium and vitamin D can be found in patients with PBC. Current treatment of bone loss includes oral calcium supplementation, weight-bearing activity, and oral vitamin D replacement (if a deficiency is present). Post-menopausal hormone replacement therapy should be considered as effective and as prone to cause side effects in women with PBC as in the general population. However, as estrogens have been associated with worsening of the cholestatic pattern, jaundice and signs of liver failure should be monitored closely particularly during the first months of treatment. Efficacy and safety of treatment with calcitonin, sodium fluoride, alendronate, and etidronate are under evaluation or have provided limited efficacy.

Hyperlipidemia

Alterations in the blood lipid profile are a common finding in PBC (up to 85% of patients present with hyperlipidemia) and often precede the diagnosis. Both serum cholesterol and serum triglyceride levels can be raised. Interestingly, however, such alterations do not correlate with increased incidence of cardiovascular events or atherosclerosis (Longo et al., 2002). Of note, the degree of hyperlipidemia does not correlate with the presence of cutaneous xanthelasmas. Treatment with ursodeoxycholic acid (UDCA) may reduce blood lipid levels via unknown mechanisms.

Steatorrhea and Malabsorption

Long-standing cholestasis leads to steatorrhea by inducing bacterial overgrowth syndrome in the gut. The mechanism is mediated by the impaired flow of bile acids to the small intestine and is commonly found in advanced stages of PBC (Lanspa et al., 1985). Oral replacement of medium-chain triglycerides for long-chain compounds, along with an overall reduction of fat in the diet can be offered as the treatment for symptoms. Pancreatic enzyme replacement

medications can also improve the symptoms when pancreatic insufficiency is suspected. Empirical antibiotic regimens can treat the bacterial overgrowth but their use, particularly when prolonged, should be carefully evaluated.

Malabsorption of fat-soluble vitamins is commonly found in advanced stages of PBC (Phillips et al., 2001). The most common deficiency, involving vitamin A, although almost always symptomless, is present in 20% of cases. Oral replacement therapy can overcome impaired absorption, and monitoring of serum concentrations is recommended after 6–12 months to avoid potential hepatotoxicity or overcorrection. In less common deficiencies such as vitamin E (potentially leading to ataxia), vitamin K (influencing coagulation), and vitamin D (see metabolic bone loss), oral or parenteral supplementations are safe and effective.

Associated Conditions

Various disorders, particularly other autoimmune syndromes, have been reported as associated with PBC. As many as 70% of patients with PBC will present another autoimmune disease (Talwalkar and Lindor, 2003). Table 54.1 lists the most commonly associated diseases and conditions and their prevalence in PBC. Some degree of impaired alveolar function and renal tubular acidosis, in both cases rarely burdened by symptoms or clinical significance, were found in 39% and 50% of PBC cases, respectively (Costa et al., 1995; Pares et al., 1981). Among autoimmune conditions, keratoconjunctivitis sicca and Raynaud phenomenon are most commonly associated with PBC but also systemic sclerosis is not uncommon. Among autoimmune diseases rarely associated with PBC, cases have been reported for systemic lupus erythematosus and multiple sclerosis, while the prevalence among PBC cases of inflammatory bowel diseases and pulmonary fibrosis is in both cases below 5%.

Malignancies

Similarly to other chronic liver conditions that lead to cirrhosis, PBC at the stage of cirrhosis can be complicated by the occurrence of hepatocellular carcinoma (HCC) and patients should be periodically monitored (Findor et al., 2002). From the clinical perspective, therefore, this implies that in PBC patients with cirrhosis, the screening for HCC should be performed using ultrasonography (and computed tomography in selected cases) twice a year to estimate the prognosis and to choose among therapeutic alternatives, particularly when orthotopic liver transplantation (OLT) is being evaluated. Apart from liver cirrhosis, there seem not to be any PBC-specific risk factors for the development of HCC (Findor et al., 2002). The treatment of HCC in PBC should follow the same guidelines as in other chronic liver diseases. No association with cholangiocellular carcinoma or other malignancies has been identified.

Natural History

The clinical course of PBC presents a very wide range of progression rates, with some patients remaining stable and asymptomatic for decades and others rapidly advancing towards endstage liver disease in just a few years. For descriptive purposes, however, the natural history of the disease can be divided into three time periods. First, patients experience an asymptomatic stage, lasting up to 20 years, in which the diagnosis is usually made based on occasional tests or referral for investigation of non-specific symptoms. Later on, a symptomatic phase, lasting up to 5 years, can be observed in which mild jaundice may also appear. Ultimately, patients show a rapid pre-terminal stage characterized by severe jaundice and complications of liver cirrhosis. Although the diagnosis is now made more frequently within the first stage, it is clear that the high variability of presentation time-points militates against the development of

TABLE 54.1 Prevalence of disorders associated with primary biliary cirrhosis (PBC) derived from clinical studies. Other associations suggested by limited case reports are not indicated herein

Disorder	Prevalence in PBC (%)	Reference
Keratoconjunctivitis sicca	75	Tsianos et al., 1990
Renal tubular acidosis*	50	Pares et al., 1981
Reduced alveolar diffusion capacity*	39	Costa et al., 1995
Raynaud phenomenon	32	Marasini et al., 2001
Hashimoto's disease	11	Elta et al., 1983
Celiac disease	6	Kingham and Parker, 1998
Systemic sclerosis	12	Marasini et al., 2001

*Usually of limited or no clinical significance.

prognostic models or the reaching of definitive conclusions about the natural history of the disease. Studies on patients followed over a long period of time, however, have provided indications on the progression of PBC.

Asymptomatic/Symptomatic Primary Biliary Cirrhosis

The presence of symptoms at the time of presentation, as indicated above, should be regarded as a major factor determining survival rates of patients with PBC.

Initial studies indicated that symptomless PBC is accompanied by survival rates over 10 years similar to the sex- and age-matched general population. However, longitudinal studies indicate that 67% of precirrhotic patients will develop liver cirrhosis over a 7-year period (Roll et al., 1983), while up to 70% of asymptomatic patients will develop symptoms over a longer follow-up (Mitchison et al., 1986). Further, regression models indicate that asymptomatic patients with PBC present significantly lower survival rates during long-term observation when compared with the general population. At the present state of knowledge, it appears reasonable to conclude that, among asymptomatic patients, survival should be considered shorter than the general population once symptoms develop during follow-up, while patients with PBC who remain symptomless over a 5-year follow-up have a survival rate equal to same-sex unaffected individuals of similar age (Springer et al., 1999). We also note that a recent report has suggested that the shorter survival of asymptomatic patients over time might be due to a higher prevalence of nonhepatic causes of death in such groups, compared with deaths related to PBC (31% vs. 57%, respectively) (Prince et al., 2004). These observations, albeit interesting and possibly compatible with data on comorbidity in PBC, present potential implications in the clinical management that need further study.

Patients with symptomatic PBC show a more rapid progression to late-stage disease and a worse prognosis than their asymptomatic counterparts. It should be noted, however, that such progression is to be considered slow as the symptomatic phase may last for several years. The mean survival time among these patients is estimated in the 6–10 year range (Pares and Rodes, 2003). Multivariate analysis among symptomatic patients with PBC has indicated several factors that can negatively influence the prognosis including clinical features (older age, presence of ascites, edema, or hepatic encephalopathy), analytical features (hyperbilirubinemia, hypoalbuminemia), and histologic features (presence of fibrosis or cirrhosis) (Pares and Rodes, 2003).

Mathematical Prognostic Models

Several prognostic models have been proposed for PBC over the past decades, but convincing validation has been obtained for only a few. Currently, the model based on the Mayo score is the most widely accepted and utilized for estimating expected survival (Grambsch et al., 1989). The formula takes into account clinical (age, presence of ascites), as well as biochemical variables, based on cholestasis (bilirubin levels) and liver function (prothrombin time, albumin). It should be noted that the major limitation to wide clinical application of this as well as other models is the fact that they rely on single-point measures and variables, thus providing a static representation of a dynamic entity. Attempts have been made to remove this limitation by deriving a numerical factor for the yearly increase in a risk score for PBC, but this often fails to account for the highly variable course of the disease. It is also debatable whether pharmacologic therapies, such as UDCA, actually influence the natural history of the disease over long periods (see below).

HISTOLOGY

The utility of histologic assessment in PBC has been widely debated. We believe that obtaining a liver biopsy specimen provides important data to determine the stage of the disease, both at presentation and during the follow-up (Heathcote, 2000). As a compatible histology is one of the three internationally accepted diagnostic criteria, it should be pursued in those in whom the diagnosis is suspected but AMAs are negative and alkaline phosphatase levels normal. The possibility of a sampling error should be considered and, in case of variable staging within a single biopsy, the highest stage should be accepted (Ludwig, 2000).

According to Ludwig's classification (Ludwig et al., 1978), histology identifies four stages of PBC. Morphologically, stage I shows portal-tract inflammation with predominantly lymphoplasmacytic infiltrates, resulting in vanishing septal and interlobular bile ducts (diameter $<100\,\mu m$). At this stage, the diagnosis of PBC should be regarded as highly probable if duct obliteration and granulomas are observed, in the absence of sarcoidosis or tuberculosis. In stage II, a periportal inflammatory infiltrate is observed. Signs of cholangitis, granulomas, and florid proliferation of ductules are typical in this stage. Stage III is characterized by the appearance of septal or bridging fibrosis, with ductopenia (over half of the visible interlobular bile ducts having vanished). As in other cholestatic conditions, copper deposition in periportal and paraseptal hepatocytes can be present at this stage. Stage IV corresponds to frank cirrhosis. Peculiar characteristics of PBC that can be found at any histologic stage include epithelioid granulomas with no signs of caseous necrosis such as in tuberculosis. A large retrospective study has demonstrated that 23.8% cases of granulomas encountered in unselected liver biopsies were to be attributed to PBC. The mechanisms leading to granuloma

formation are still largely unknown, although experimental findings suggest that gram-positive bacteria through lipoteichoic acid might initiate the process (Tsuneyama et al., 2001) while osteopontin might also mediate the recruitment of mononuclear cells (Harada et al., 2003). The observation of eosinophils in the portal tract is a specific finding in PBC histology (Goldstein et al., 2001), although its significance, alongside a peripheral hypereosinophilia, is currently poorly understood (Neuberger, 1999).

AUTOIMMUNE FEATURES

Several clinical and experimental findings strongly imply an autoimmune pathogenesis for PBC (Selmi et al., 2004). Common features of other autoimmune conditions seem to apply only in part to PBC. First, autoantibodies should be present in patients with the disease. Primary biliary cirrhosis is characterized by the presence of detectable AMA in approximately 90% of affected individuals (Mackay et al., 2000), although we note that patients lacking AMA can present with a similar disease picture and progression as found in AMA-positive subjects (Invernizzi et al., 1997) seemingly arguing against a pathogenic role for these autoantibodies. Autoreactive T cells, both $CD4^+$ and $CD8^+$ have been identified in AMA-negative PBC and such lymphocytes and AMA recognize overlapping epitopes within the mitochondrial antigens (Ishibashi et al., 2003). Second, autoantibodies should interact with the target antigen, the passive transfer of autoantibodies should reproduce the clinical features, and experimental immunization with the antigen should produce a model disease. As mentioned above, no direct proof has yet been provided for a direct pathogenic role of AMA in the bile duct injury observed in PBC. Similarly, no convincing animal model has been described (see below), although AMA can be generated in experimental animals following immunization. Thirdly, in autoimmune diseases the reduction of autoantibody levels should ameliorate the disease; this criterion is poorly fulfilled in PBC, where there is no correlation between the pattern or titer of AMA and progression of the disease (Bogdanos et al., 2003). Finally, it is well-established that most autoimmune diseases are responsive to immunosuppressive therapy. In PBC, all immunosuppressive agents have proven relatively ineffective (see below).

Antimitochondrial Antibodies

Antimitochondrial antibodies are directed against components of the 2-oxoacid dehydrogenase (2-OADC) family of enzymes within the mitochondrial respiratory chain (Gershwin et al., 2000). Specifically, the main recognized antigens are the E2 and E3 binding protein (E3BP) components of the pyruvate dehydrogenase complex PDC (frequently presenting cross-reactivity) and the E2 components of the 2-oxoglutarate dehydrogenase and branched-chain 2-oxoacid dehydrogenase complexes (OGDC and BCOADC). The mapping of AMA epitopes within such molecules has been achieved using partial constructs and combined peptides. Data indicated that AMA recognize closely-related conformational epitopes including the inner lipoylated domain of PDC-E2, BCOADC-E2, and OGDC-E2 (Gershwin et al., 2000). In all three molecules, in fact, contact sites contain the three amino acid motif DKA, with lipoic acid covalently bound to the lysine (K) residue. It is not clear whether lipoic acid participates in the epitope recognition by AMA; apparently conflicting results indicated that AMA bind to both the lipoylated and unlipoylated forms of PDC-E2 (Quinn et al., 1993), while they can also bind to lipoic acid attached to a non-2-OADC peptide (Bruggraber et al., 2003).

Antinuclear Antibodies

As many as 50% of patients with PBC present serum antinuclear antibodies (ANA). The pathogenic role of ANA in PBC is still poorly understood, or perhaps it could be said that their pathogenic role is no better understood than that of AMA. The two immunofluorescence patterns most often encountered in PBC are "nuclear rim" and "multiple nuclear dots," based on the recognition by the autoantibodies of gp210 and nucleoporin 62 (within the nuclear pore complex) and of Sp100 and promyelocytic leukemia protein (PML), respectively. Interestingly, the presence of serum reactivity against proteins from the nuclear pore complex (gp210, p62) in PBC was found associated with a more severe disease in a cross-sectional study (Invernizzi et al., 2001). On the other hand, the nuclear body proteins Sp100 and PML have been shown to be complexed with small ubiquitin-like molecules (SUMO) for cell transport regulation, and SUMO are in turn independent antigens specific for ANA-positive PBC (Janka et al., 2005).

Autoreactive T cells

A number of mononuclear cells can be found in the area surrounding damaged bile ducts in PBC. T-helper ($CD4^+$), $TCR\alpha\beta^+$, and $CD8^+$ T cells are most commonly seen among such cells, perhaps secondary to high levels of IFN-γ acting as a chemotaxic stimulus. Autoreactive T cells have been well characterized in PBC from both the liver and peripheral blood of affected patients. PDC-E2-specific autoreactive $CD4^+$ T-cell (T-helper) clones were isolated by *in vitro* stimulation of intrahepatic or peripheral lymphocytes to PDC-E2 (Van de Water et al., 1991; 1995). The autoepitope for T cells overlaps with the B-cell (AMA) epitope and includes the lipoyl-lysine residue located at amino acid residue 174 of the inner lipoylated domain of the protein (Shimoda et al., 1995). Interestingly, a specific increase of between 100- and 150-fold in the frequency of PDC-

$E2_{163–176}$-specific $CD4^+$ T-cells in the hilar lymph nodes and liver when compared with that in periphery is observed in PBC (Shimoda et al., 1998). Autoreactive cytotoxic T lymphocytes (CTLs) are also well characterized in PBC and currently considered major effectors in the tissue injury encountered in PBC. The MHC class I restricted epitope for CTLs, namely amino acids 159–167, also maps in close vicinity to the epitopes recognized by $CD4^+$ cells and by AMA (Kita et al., 2002b) . Similarly to $CD4^+$ cells, moreover, the recent use of tetramer technology has shown a 10-fold higher prevalence of PDC-$E2_{159–167}$-specific CTLs in the liver compared with peripheral blood of patients with PBC (Kita et al., 2002c). Figure 54.1 illustrates the structure of tetramers used for MHC-peptide coupling in cell-immunity studies.

EPIDEMIOLOGY

Epidemiology and Geoepidemiology

Most of the epidemiologic data used to determine the incidence and prevalence rates of PBC are descriptive, with some studies containing methodological flaws such as non-uniform case definition. The performance of a population-based study to ascertain the prevalence of PBC is challenging, as a noninvasive highly sensitive marker is not currently available (the most specific candidate, AMA, being negative in 5–10% of patients) and the absence of a register of patients does not allow the recognition of all cases. Primary biliary cirrhosis is considered to be most prevalent in England, Scandinavia, and specific areas of the United States, although a factitious prevalence due to more exhaustive epidemiologic studies from these countries compared with other areas cannot be excluded. Table 54.2 indicates a synopsis of the epidemiologic data available (Selmi et al., 2004). Prevalence rates for PBC vary widely in different geographic areas and have been reported to be as high as 402 in 1 million.

Sex Differences and Gravidity

Similarly to other autoimmune diseases, women, primarily middle-aged, outnumber men by as much as 10 : 1 among patients with PBC (see Table 54.2). The prevailing expla-

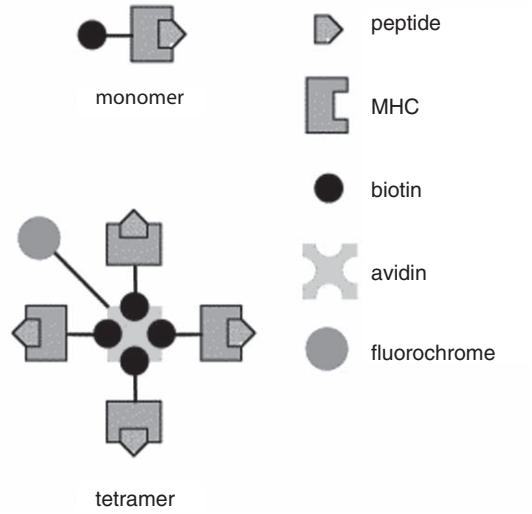


Figure 54.1 Structure of peptide-MHC tetramers used for the isolation of specific autoreactive T cells. The tetramer based on streptavidin-biotin is the most widely used and is represented. Recombinant MHC molecules (HLA class I, II, or non-typical II can be used), are incubated with peptide to form peptide-MHC complex, which is biotinylated. Only autoreactive T cells specifically reacting with the presented peptide recognize the tetramer, and the presence of a fluorochrome allows detection of such cells by flow cytometry.

TABLE 54.2 Synopsis of population-based epidemiologic studies of primary biliary cirrhosis

Year	Location	No of cases	Annual incidence (per million)	Prevalence (per million)	Gender ratio (M/F)
1980	Sheffield, UK	34	5.8	54	1 : 16
1980	Dundee, UK	21	10.6	40.2	1 : 9.5
1983	Newcastle, UK	117	10	37–144	1 : 14
1984	Malmoe, Sweden	33	4–24	28–92	1 : 3
1984	Western Europe	569	4	23 (5–75)	1 : 10
1985	Orebro, Sweden	18	14	128	1 : 3.5
1987	Glasgow, UK	373	11–15	70–93	–
1990	Umea, Sweden	111	13.3	151	1 : 6
1990	Ontario, Canada	225	3.26	22.4	1 : 13
1990	Northern England	347	19	129–154	1 : 9
1995	Victoria, Australia	84	–	19.1	1 : 11
1995	Estonia	69	2.27	26.9	1 : 22
1997	Newcastle, UK	160	14–32	240	1 : 10
2000	Olmsted County, MN, USA	46	27	402	1 : 8

Modified from Selmi et al. (2004b).

nation for the striking predominance of disease in females is the effects of sex hormones on the immune system, although conclusive data are not available. It has also been reported that female PBC cases have significantly more pregnancies than controls, although no explanation is available for this observation (Parikh-Patel et al., 2002).

GENETIC FEATURES

Familial Primary Biliary Cirrhosis

Primary biliary cirrhosis is more frequent in relatives of affected individuals and the term "familial PBC" has been coined to indicate families that have more than one case. Variable rates of familial PBC are seen in different geographic areas, possibly due once again to different methods of case definition. In general, data indicate that 1–6% of PBC cases have at least one family member presenting the disease (Selmi et al., 2004). Such familial prevalence rates are significantly higher than general population prevalence estimates (see Table 54.2), thus indicating a genetic predisposition to the disease. However, the difficulty in evaluating these data is that prevalence rates in the general population are still uncertain, and control groups are not always included in the family studies.

Twin Studies

The concordance rate observed among monozygotic twins for PBC is 63%, among the highest reported in autoimmunity, reinforcing the idea of an important role of genetics in disease susceptibility (Selmi et al., 2004).

Association Studies

Several studies have attempted to identify genes associated with PBC. No family study of genetic linkage has been performed, possibly because PBC is a relatively rare disease and it is, therefore, difficult to obtain DNA samples from a large number of representative families. All available studies were designed in a controlled, cross-sectional fashion but were prone to multiple sampling errors and biases of incorrect estimations. A multi-hit genetic model seems to apply to PBC, with different genetic variants conferring susceptibility (first hit) and others influencing disease progression (second hit). For this reason, most authors investigating genetic factors in PBC have studied the role of such factors in susceptibility to the disease (comparing allele and genotype frequencies in patients and controls), as well as in its severity (through the analysis of clinical characteristics of patients carrying different genotypes or alleles). No definitive association of PBC susceptibility or progression could be identified in these studies (Jones and Donaldson, 2003). When an association has been found, it has proven to be weak or limited to specific geographic areas. We note, moreover, that this also applies to the study of the variants of MHC (including type I, II, and III loci) in which, different from most autoimmune diseases, reported associations were often weak or limited to specific geographic areas (Invernizzi et al., 2005). Similar findings were also reported from the study of the genetic variants of immunomodulatory molecules, enzymes producing vasoactive compounds, bile-acid transporters. Among immunomodulatory molecules, we note that weak associations have been reported with polymorphisms of genes coding for chemokines IP-10 and MIG and chemokine receptors CXCR5 and CXCR3, toll-like receptor 9, and cytotoxic T lymphocyte associated antigen-4 (CTLA-4). In the future, definitive indications on the genetics of PBC may be obtained by methods based on inheritance by descent techniques on large series of affected and unaffected family members and should be encouraged. Such an approach will lead to more reliable findings compared with the use of cross-sectional association studies based on the comparison of allelic frequencies in cases and controls.

Sex Chromosomes

Similar to other autoimmune diseases more commonly diagnosed in women following reproductive years (Lambert and Nelson, 2003), fetal microchimerism has been suggested in PBC, with the hypothesis of a higher prevalence of small amounts of fetal (paternal) DNA found in mothers with PBC. First, it was suggested that the presence of fetal DNA in the liver of affected women years after pregnancy might predispose to PBC (Fanning et al., 2000); independent findings, however, have not confirmed such hypothesis (Invernizzi et al., 2000; Tanaka et al., 1999a).

Genes mapping on the X chromosome are critical to the maintenance of physiological sex hormone levels and, more importantly, of immune responsiveness. Invernizzi and colleagues reported an age-dependent enhanced monosomy X in the peripheral white blood cells of women with PBC (Invernizzi et al., 2004b). This observation seems to indicate a polygenic model for PBC with an X-linked major locus of susceptibility in which genes escaping inactivation are the major candidates. On the other hand, it can also be hypothesized that susceptibility to PBC is the result of a multigenic complex inheritance model where Y-linked genes might exert a protective role.

ENVIRONMENTAL INFLUENCES

Risk Factors

Although genetics should be regarded as the major determinant in susceptibility to PBC, several other factors have

Box 54.1
Proposed risk factors and their association with primary biliary cirrhosis (PBC)

Significant associations

- Female gender
- Non-PBC autoimmune disease (particularly autoimmune thyroiditis)
- Vaginal or urinary tract infection
- Previous tonsillectomy
- Chronic bronchitis

Doubtful associations

- History of shingles
- Previous hepatitis A infection
- Previous surgery (particularly appendectomy, uterine surgery)
- Former smoking habit

No association

- Dietary fat intake
- Childhood diseases

Modified from Parikh-Patel et al. (2001).

Box 54.2
Environmental agents proposed to be important in primary biliary cirrhosis onset

Infections
Chemicals and xenobiotics
Medicinal drugs
Contraceptive pills
Foods
Vaccines
Deficient sunlight/vitamin D
Pregnancies/microchimerism

been proposed, including a history of previous infections, comorbidity with other autoimmune diseases, and lifestyle factors, such as smoking and a high-fat diet. Most evidence was obtained from large questionnaire-based studies, which showed associations of PBC with past smoking habit, the presence of other autoimmune diseases, previous tonsillectomy and a history of vaginal or urinary tract infection in females only (Parikh-Patel et al., 2001). Box 54.1 lists the proposed risk factors for the development of PBC.

Proposed Environmental Factors

The lack of strong genetic associations for PBC has meant that environmental factors have received attention as possible triggers of autoimmunity in PBC. A list of proposed factors is given in Box 54.2. Attention has focused on two main agents, infectious (bacteria and viruses) and chemical (xenobiotics).

The ability of infectious agents, particularly bacteria, to induce autoimmune responses in experimental settings has been documented, and molecular mimicry is the most widely studied mechanism explaining these observations (Van de Water et al., 2001). Briefly, this paradigm suggests that microbes present peptides sharing different degrees of homology with self-proteins, thus leading to a promiscuous antibody and cell-mediated immune response capable of reacting with both microbial and self-epitopes. T-cell activation produces cross-reacting T cells leading to self-tissue destruction, thus perpetuating the autoimmune response, possibly through the degeneracy of the T-cell receptor and cross-priming. Of the bacterial strains suggested to lead to PBC through molecular mimicry (Selmi and Gershwin, 2004), most evidence has been reported for *Escherichia coli*, mostly based on the reports of an increased prevalence of urinary tract infections in patients with PBC (Parikh-Patel et al., 2001). We also note that, based on serum cross-reactivity, several infectious agents have been proposed for the initiation of PBC, including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella minnesota*, *Mycobacterium gordonae*, *Neisseria meningitidis*, and *Trypanosoma brucei* (Van de Water et al., 2001). More recently, the common commensal yeast *Saccharomyces cerevisiae* has also been investigated in PBC, based on the expression of AMA antigens in extra-mitochondrial sites (Ghadiminejad et al., 1988) but serologic studies indicated that the reactivity against the yeast (anti-*Saccharomyces cerevisiae* antibodies; ASCA) was not specific for the disease (Muratori et al., 2003). Interestingly, contrasting evidence has been collected on the role of *Chlamydia pneumoniae* in the pathogenesis of PBC. Abdulkarim and colleagues detected the bacterial antigen and RNA in 100% of PBC explanted liver sections compared with 8.5% of controls (Abdulkarim et al., 2004) while a different immunologic and molecular approach could not confirm this hypothesis (Leung et al., 2003a). Finally, our group has recently provided serologic data suggesting that a ubiquitous xenobiotic-metabolizing gram-negative bacterium, *Novosphingobium aromaticivorans*, is the best candidate yet for the induction of PBC, as it elicits a specific antibody reaction (estimated to be 100- to 1000-fold higher than against *E. coli*) and its 16S rRNA-specific sequences were detected in human fecal samples (Selmi et al., 2003). For completeness, we also note that a novel human beta-retrovirus has been identified in lymph nodes and other samples from patients with PBC (Xu et al., 2003), thus suggesting that this mouse mammary tumor (MMTV)-like virus might play a role in the patho-

genesis of PBC. However, our laboratory failed to confirm this hypothesis using a different molecular and immunologic approach on a large series of patients and controls (Selmi and Gershwin, 2004), therefore discouraging the use of any anti-retroviral therapy in PBC (Gershwin and Selmi, 2004).

Xenobiotics are foreign compounds that may either alter or complex to defined self or non-self proteins, inducing a change in the molecular structure of the native protein sufficient to induce an immune response. Such immune responses may then result in the cross-recognition of the self form, which could in turn perpetuate the immune response, thus leading to chronic autoimmunity. A role for specific compounds has been proposed in a number of autoimmune conditions (Table 54.3). Figure 54.2 depicts the proposed pathogenic pathway by which xenobiotics may induce PBC in genetically susceptible individuals. Interestingly, most xenobiotics are metabolized in the liver, thereby increasing the potential for liver-specific alteration of proteins. Experiments showed that specific organic structures attached to the mitochondrial antigens were recognized by sera from PBC patients with a higher affinity than native forms of such antigens (Long et al., 2001). Such findings indicated for the first time that an organic compound may serve as a mimotope for an autoantigen, thus further providing evidence for a potential mechanism by which environmental organic compounds may cause PBC. One halogenated compound was capable of inducing AMA production in animal models (Leung et al., 2003b). This model did not lead to production of liver lesions; however, it appeared to simulate the stage of the disease in humans where AMA is present prior to the appearance of liver damage. The AMA-positivity, moreover, was reversible after the immunization ceased (Amano et al., 2004).

It is of note that the vast majority of data on molecular mimicry in PBC were obtained from the study of humoral immunity (i.e., AMA), either in patient sera or animal models, while the study of cellular autoimmunity is limited. An extensive study of autoreactive $CD4^+$ T-cell clones by Shimoda et al. (2003) has demonstrated that molecular mimicry takes place between T-cell epitopes of PDC-E2 and gp210 (an ANA antigen in PBC), thus suggesting that immunospreading may occur from mitochondrial proteins to nuclear proteins, similar to that hypothesized for bacterial antigens. Supporting evidence was obtained by our group also for autoreactive $CD8^+$ T cells. Results indicated that a peptide derived from the gram-negative *Pseudomonas aeruginosa* (belonging to the same family as *N. aromaticivorans*) showed a higher binding affinity to the T-cell HLA than PDC-E2 (Kita et al., 2002a).

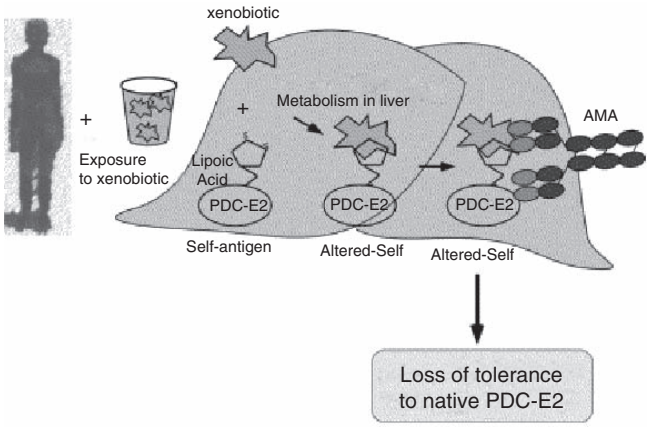


Figure 54.2 Proposed pathway by which xenobiotics might break tolerance and give rise to primary biliary cirrhosis (PBC). According to such theory, xenobiotics are transported to the liver through the portal circulation. In the liver, xenobiotics are then metabolized (possibly through the cytochrome P450 pathways) and their metabolites form adducts with the lipoylated domains of the mitochondrial antigens (PDC-E2). Such adducts in turn induce changes in the structure of the native proteins, which are now recognized as non-self, thus raising a humoral response (AMA) against both the modified and the unmodified forms and perpetuating the autoimmune response.

TABLE 54.3 The role of xenobiotics in autoimmune disease

Compound	Associated autoimmune condition
Mercury (Bagenstose et al., 1999; Yoshida and Gershwin, 1993)	Immune complex formation Glomerulonephritis
Iodine (Rose and Burek, 2000)	Autoimmune thyroiditis
Vinyl chloride (D'Cruz, 2000)	Scleroderma-like disease
Contaminated L-tryptophan (Hess, 1999; Simat et al., 1999)	Eosinophilia myalgia syndrome
Toxic oil (Rao and Richardson, 1999; Yoshida et al., 1993)	Scleroderma-like disease
Silica (Steenland and Goldsmith, 1995)	Rheumatoid arthritis Systemic lupus erythematosus (SLE) Scleroderma
Halothane (Obermayer-Straub et al., 2000; Pumford et al., 1997)	Autoimmune hepatitis
Canavanine (Babu et al., 1999)	SLE-like syndromes
6-bromohexanoate (Selmi et al., 2003)	Primary biliary cirrhosis

ANIMAL MODELS

The development of an animal model would be extremely helpful in elucidating the undoubtedly multifactorial causation and progression of PBC. Several models, mostly murine, have been proposed. The first reported animal model for PBC was observed in an inbred rabbit strain with spontaneous nonsuppurative cholangitis. However, the AMA response was weak (Tison et al., 1982). In later attempts, PBC epithelial cells and purified PDC-E2 with lipopolysaccharide (LPS) or recombinant polypeptides of PDC-E2 were injected intravenously into neonatally thymectomized A/J mice (Kobashi et al., 1994) and C57BL/6 mice (Hayashi et al., 1989), respectively. Antimitochondrial antibodies were induced in both these models, but no PBC-like pathologic changes in the liver were observed. A graft-versus-host disease (GvHD) model has also been developed based on the observation that the histology of PBC shared some characteristics with human GvHD, but no specific serologic response was generated against mitochondrial antigens (Vierling et al., 1989). The injection of PBC cell repertoire into human severe combined immunodeficient (SCID) as well as knockout murine models, has also been pursued, with conflicting and non-definitive results (Krams et al., 1989). In 2000, a British group reported that the immunization of SJL/J mice with PDC-E2 led to autoimmune cholangitis associated with T cells that display a mixed cytokine profile similar to that observed in PBC. Such findings were later proven to be nonspecific, as bile duct inflammation was found also after immunization with control peptides under the same conditions (Sasaki et al., 2002). More recently, our group has reported that immunization of rabbits with a specific halogenated compound coupled with albumin could elicit AMA development (Selmi et al., 2003), but not liver lesions.

A number of obstacles still militate against the development of a consistent and reliable animal model for PBC. An important one is likely to be the long latent period between AMA and liver pathology appearance (as observed in humans), which is at odds with the short life expectancy of the animals used (particularly mice). Moreover, and possibly more importantly, we need to take into account that an enormous "constellation" of genetic conditions must be met in order to produce a specific phenotype, as indicated by the growing experience with the non-obese diabetic (NOD) mouse model for type 1 diabetes (Johansson et al., 2003).

PATHOGENIC MECHANISMS

Several theories have been proposed for the pathogenesis of the immune-mediated tissue injury observed in PBC. In all cases, such theories should not be regarded as independent from other etiologic factors leading to PBC susceptibility (i.e., genetic background, environmental triggers), but rather as effector mechanisms leading to the clinical manifestations.

Expression of PDC-Like Antigens on Cell Membranes

The highly selective destruction of biliary epithelial cells (BEC) is one of the unique features of PBC. As all nucleated cells in the body contain at least some mitochondria, the target antigens are continually being presented in association with MHC class I in all tissues. Why then are BECs singled out for destruction? An explanation for this comes from the observation that certain anti-PDC-E2 antisera react not only with PDC-E2 in mitochondria but also additional material located at the apical end of the BEC in close apposition to the surface (Van de Water et al., 1993). The degree of staining is quite intense, suggesting large amounts of this apically located material. Such a staining pattern is not seen in any other cells of the body, other than salivary duct epithelial cells, which are frequently a target of immune attack in PBC. Thus, there appears to be aberrant expression of a molecule that shares epitopes with PDC-E2 exclusively in the cells targeted by the immune system (Joplin et al., 1997). Further, this apical staining occurs very early in the natural history of PBC, even before the appearance of MHC class II on BECs (Tsuneyama et al., 1995a), and it is not detected in other liver diseases such as autoimmune hepatitis or primary sclerosing cholangitis. Finally, this aberrant apical staining is detected in liver samples from patients with AMA-negative PBC (Tsuneyama et al., 1995b). What is the identity of this apically and surface-located material in the BECs of PBC patients? This is still mysterious, and the difficulty of obtaining liver samples has significantly slowed progress. Livers from patients obtained at the time of transplantation have very few intact intrahepatic bile ducts so that laser dissection of BECs yields very few cells. Studies that have been performed have shown that the material is not immunologically identical to PDC-E2 as only some murine anti-human PDC-E2 monoclonal antibodies react with it. However, it may be a truncated product or it may be PDC-E2 that is complexed with other molecules (perhaps xenobiotics) in a way that masks epitopes. There is no significant difference in the amount of PDC-E2 transcript present in PBC liver compared with normal liver, suggesting that the observation may not be secondary to overexpression of PDC-E2. However, there is seldom a good correlation in eukaryotic cells between mRNA transcript levels and abundance of protein in the cell. Mass spectrometric studies of BECs from PBC suggested that the material had a different molecular mass to PDC-E2, making it either a breakdown product or a molecular mimic (Yip et al., 1996). The matter

rests there at present but the increasing sensitivity of proteomics methods may eventually provide a means to sequence or otherwise fingerprint the material. In the interim it is only possible to speculate. If the material is derived from PDC-E2, perhaps there are sequence variants common in PBC patients that lead to an altered turnover of the molecule, thus leading to the accumulation of PDC-E2 only in specific BECs. In this scenario, moreover, chemicals (xenobiotics) metabolized by the liver could significantly modify PDC-E2, possibly leading to the production of such variants (Long et al., 2002). Perhaps abnormal splicing during synthesis of PDC-E2 mRNA could result in an endoplasmic reticulum targeting signal instead of a mitochondrial targeting signal at the N-terminus of PDC-E2. This mechanism would be responsible for the delivery of PDC-E2 into the endoplasmic reticulum and Golgi apparatus via the secretory route. Although fascinating, this theory is currently supported only by observations using transmission electron microscopy (Jorge et al., 2002). The other alternative is that it is a molecular mimic perhaps associated with microbial infection or left over from prior infection. Whatever its identity, it is noteworthy that the material is found in association with IgA and the polymeric Ig receptor (Fukushima et al., 2002; Migliaccio et al., 1998) on the cell membranes of BECs.

The Role of Immunoglobulin A

The IgA isotype of immunoglobulin is the most common form found on all epithelial surfaces, including the biliary epithelium. It is, therefore, possible that the selective injury in PBC is mediated by AMA-IgA binding to the mitochondrial antigen, thus inducing cell dysfunction and death. Our group has demonstrated that IgA from PBC patients co-localizes with the PDC-E2 (or cross-reactive material) both in the cell cytoplasm and on the apical membrane of BECs, whereas no co-localization could be observed with antibodies from healthy subjects (Fukushima et al., 2002). The IgA that is found on the mucosal surface arrives there by uptake and trafficking across the BEC in association with the polymeric Ig receptor. It is possible that during this transport process, IgA with antimitochondrial specificity may contact its cognate antigen and that the complex may lead to deleterious effects. Evidence for this is provided by a study in which IgA was purified from PBC patient sera and incubated with canine kidney cells transfected with the human polymeric Ig receptor. Uptake of IgA into the cells led to a significant activation of caspases suggesting that apoptosis was being induced. Incubation with control IgA did not lead to caspase stimulation, providing evidence for a direct pathogenic role of anti-PDC-E2 IgA. IgA titers were directly correlated with the degree of caspase activation. (Matsumura et al., 2004). Presumably, in PBC patients, further cellular damage arises with time as AMA IgA transcytoses the BEC.

Molecular Mimicry by Infectious Agents

The cross-reactivity of AMA with prokaryotic antigens (particularly with the microbial respiratory-chain enzymes) has been reported for a number of microbes, including *E. coli*. This cross-reactivity is not particularly surprising given the conserved sequence of PDC-E2 across all species, from eubacteria to mammals (Van de Water et al., 2001). Indeed it is proposed that mitochondria originated following uptake of bacteria into the precursors of eukaryotic cells and maintenance as intracellular symbionts. Thus, it becomes difficult to tease out a causal role for microbial proteins in pathogenesis given their phylogenetic relationship to the human autoantigen. One line of argument that we have taken is that the breaking of tolerance and induction of autoimmunity would be more likely to occur when the microbial protein is extremely similar in sequence. We have recently suggested that a gram-negative ubiquitous bacterium, *N. aromaticivorans*, sharing the highest homology with human PDC-E2 yet described and capable of metabolizing xenobiotics, is the best candidate so far identified for the induction of PBC via molecular mimicry (Selmi et al., 2003). A necessary requirement of such a scenario would be the exposure of the patient to the candidate bacteria, either accompanied by overt signs of infection or not. A number of studies have tried to search for bacterial species within the liver and biliary tract of patients with PBC, but data have so far failed to define bacteria specific only to PBC liver (Tanaka et al., 1999b). *A priori* however, it is not clear that the bacteria would necessarily need to be present in these tissues as infection, and tolerance breakdown may occur anywhere including the urinary tract. Further the bacteria may well have disappeared by the time the patient presents with PBC, further complicating the search.

Researchers from one group have reported the presence of a beta-retrovirus in the liver and the lymph nodes of some patients with PBC, and that the culture of normal BECs in the presence of an homogenate of such PBC lymph nodes induced the expression of a PDC-E2-like antigen on cell membrane (Xu et al., 2003). Although intriguing, this latter observation has not been confirmed (Selmi et al., 2004).

Briefly, we can summarize our theory on molecular mimicry in PBC as follows. The microorganism (possibly the ubiquitous *N. aromaticivorans*) enters the human system through the digestive mucosa. Bacterial mimics containing lipoic acid residues at this point might be modified by xenobiotics to form immunoreactive adducts. This modification would be then sufficient to trigger the innate immune system to initiate a cascade of local inflammatory events, via Toll-like receptors, for example, thus resulting in local dendritic cell activation and antigen processing. Mucosal antigen-presenting cells (APC) in turn activate autoreactive T and B cells that are directed to the liver through the portal system.

T cells participate directly in the autoimmune injury and/or further recruit autoreactive lymphocytes. B cells, on the other hand, secrete AMA, particularly of the IgA type. These antimitochondrial IgA are then transported to the vascular side of the bile duct cell, where they react with the PDC-E2-like molecules located on the luminal surface cell membrane. This binding then initiates the apoptotic signaling cascade. Ultimately, the immune complexes of post-apoptotic PDC-E2 and IgG-AMA and the direct cytopathic effects of autoreactive T cells (and possibly AMA) contribute to the tissue injury observed in PBC.

IMMUNOLOGIC MARKERS FOR DIAGNOSIS

Antimitochondrial Antibodies

Antimitochondrial antibodies are highly specific for PBC and can be detected in up to 95% of patients, depending on the diagnostic methodology used. In most cases, indirect immunofluorescence techniques are used for initial screening of cases. It should be noted, however, that such methods have a limited sensitivity and, particularly at lower dilutions of sera, low specificity for PBC, with the latter probably responsible for the encountered cases of non-PBC AMA positivity. Commercially available enzyme-linked immunosorbent assays (ELISAs) can also be used to detect AMA, although such assays are less sensitive than immunofluorescence. When AMA are tested with more recently developed techniques, based on the use of recombinant mitochondrial antigens (with immunoblotting), the sensitivity and specificity of the test are significantly higher, approaching 100% (Miyakawa et al., 2001). However, these methods are currently only performed at selected centers and are not readily available to the physician for diagnostic purposes. Accordingly, immunofluorescence continues to be available as the main diagnostic test for use in commercial laboratories.

Antinuclear Antibodies

In a subset of patients with PBC approaching in some cases 50%, autoantibodies are directed at nuclear antigens. Patients who are ANA-positive are more frequently AMA-negative, possibly because of the lack of a masking effect of these latter antibodies in such sera. Antinuclear antibodies are currently tested by immunofluorescence, although commercial ELISA kits for individual antigens are becoming available. Cumulatively, such "nuclear rim" and "nuclear dots" patterns should be considered as highly specific for PBC (Worman and Courvalin, 2003), thus being an important diagnostic tool particularly in AMA-negative patients. Finally, we note that patients with PBC and CREST syndrome (but not overt systemic sclerosis) present anticentromere antibodies (ACA) in 10–15% of cases (Bernstein et al., 1982).

TREATMENT AND OUTCOME

Several medical treatments have been investigated in patients with PBC. Currently, ursodeoxycholic acid (UDCA) is the only accepted therapy and has received US Food and Drug Administration approval.

Ursodeoxycholic Acid

Ursodeoxycholic acid accounts for 4% of the bile acid pool in human bile. Compared with other bile acids, such as chenodeoxycholic and deoxycholic acids, UDCA is more hydrophilic. Its absorption (3–60% following oral dose) occurs mainly in the small intestine, and its presence decreases cholesterol secretion into bile, possibly lowering its conversion to bile acids. Cumulatively, the proposed mechanism of action of UDCA in PBC is based on modification of the bile-acid pool, reduction in pro-inflammatory cytokines, effects on apoptosis and on vasoactive mediators (Lazaridis et al., 2001). However, since UDCA anti-inflammatory effects are found only in bile ducts, it has been assumed that its effect is mediated by the modification of the bile acid pool. Doses of UDCA ranging from 13–20 mg/kg lead to optimum bile enrichment. Long-term survival with UDCA therapy has been noted to be higher than predictions based on mathematical models for PBC and an extensive meta-analysis of published trials indicated that such increased survival is obtained only when an adequate dose (>13 mg/kg) is used (Gluud and Christensen, 2002). A complete biochemical response to UDCA (normalization of serum liver tests in absence of cirrhosis) is achieved in approximately 40% of treated patients (Leuschner et al., 2000).

Other Drugs

Based on success rates observed in other autoimmune diseases, the use of immunosuppressive drugs was attempted in PBC; however, efficacy was poor (Oo and Neuberger, 2004). Corticosteroids, azathioprine, cyclosporine, methotrexate, penicillamine, and colchicine have not been shown to influence the severity or progression of the disease or are associated with serious side effects. Their use should be considered only in combination with UDCA in selected cases (i.e., when features of autoimmune hepatitis overlap with PBC). In the event of an unsatisfactory response to UDCA alone, these drugs are still considered, but the lack of efficacy and the risk of serious side effects makes their use highly debatable. Inconclusive

results from pilot studies or case reports of single or UDCA-combinations of bezafibrate (Itakura et al., 2004), mycophenolate mofetil (Talwalkar et al., 2005), and tamoxifen (Invernizzi et al., 2004a; Reddy et al., 2004) require future long-term studies, possibly not limited to patients unresponsive to UDCA treatment.

Orthotopic Liver Transplantation

Orthotopic liver transplantation is considered the ultimate treatment for end-stage PBC, and PBC is among the most common indications for transplantation worldwide (MacQuillan and Neuberger, 2003). Post-OLT survival rates are 92% and 85% at 1 and 5 years, respectively. A retransplantation is needed in fewer than 10% of patients. Orthotopic liver transplantation appears to be beneficial also with regard to PBC symptoms of fatigue and pruritus, as well as bone loss. The AMA status does not change after OLT, and recurrence of PBC following OLT is found in 25% of patients after 5 years (Liermann Garcia et al., 2001). Interestingly, however, this seems to reproduce a new start of the disease with a long latency between AMA appearance and clinical manifestation of the disease (Balan et al., 1993). Recurrence rates could be influenced by the use of specific immunosuppressive post-OLT regimens. Interestingly, the frequency of OLT for PBC in a large series from the United Kingdom was reported as decreasing over the past decades, alongside an increased age at the time of transplantation (Liermann Garcia et al., 2001). Cumulatively, such data could once again indicate that the natural history of PBC might be influenced by earlier diagnosis or medical treatment. The use of UDCA in transplanted patients is currently considered safe, and no contraindications have been identified so far.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Primary biliary cirrhosis should be considered a unique disease within the range of autoimmunity. Future efforts should be dedicated to overcoming some of these conceptual and logistic difficulties. First, only study of a very large number of representative families will unravel the genetic basis of PBC. Given the relatively rare prevalence of the disease, only a worldwide effort will allow the collection of a population of families large enough (and with two or three generations available) to guarantee enough statistical power for a linkage analysis. Second, the role of xenobiotics and infectious agents in the onset of PBC should be further probed, particularly with respect to the efforts for the development of an animal model and using detailed epidemiologic studies to ascertain the exposure to specific environmental factors. Third, it is crucial to determine the pathogenic role of AMA in the bile duct damage of PBC. Once again, the development of an animal model appears to be the only way to provide a clear demonstration of such pathogenic mechanism. Finally, from a clinical standpoint, new clinical trials are needed to identify novel therapies in the long-term treatment of PBC. Together with the already present trend towards an earlier diagnosis of the disease, a better efficacy of medical treatment, possibly using specific monoclonal antibodies or hematopoietic stem cell transplant, will be the cornerstone to reducing the need for OLT in patients affected by PBC.

References

Abdulkarim, A.S., Petrovic, L.M., Kim, W.R., Angulo, P., Lloyd, R.V., and Lindor, K.D. (2004). Primary biliary cirrhosis: an infectious disease caused by Chlamydia pneumoniae? *J. Hepatol. 40,* 380–384.

Ahrens, E.H., Jr., Payne, M.A., Kunkel, H.G., Eisenmenger, W.J., and Blondheim, S.H. (1950). Primary biliary cirrhosis. *Medicine (Baltimore) 29,* 299–364.

Amano, K., Leung, P.S., Xu, Q., Marik, J., Quan, C., Kurth, M.J., Nantz, M.H., Ansari, A.A., Lam, K.S., Zeniya, M., Coppel, R.L., and Gershwin, M.E. (2004). Xenobiotic-induced loss of tolerance in rabbits to the mitochondrial autoantigen of primary biliary cirrhosis is reversible. *J. Immunol. 172,* 6444–6452.

Babu, B.R., Frey, C., and Griffith, O.W. (1999). L-arginine binding to nitric-oxide synthase. The role of H-bonds to the nonreactive guanidinium nitrogens. *J. Biol. Chem. 274,* 25218–25226.

Bagenstose, L.M., Salgame, P., and Monestier, M. (1999). Murine mercury-induced autoimmunity: a model of chemically related autoimmunity in humans. *Immunol. Res. 20,* 67–78.

Balan, V., Batts, K.P., Porayko, M.K., Krom, R.A., Ludwig, J., and Wiesner, R.H. (1993). Histological evidence for recurrence of primary biliary cirrhosis after liver transplantation. *Hepatology 18,* 1392–1398.

Bergasa, N.V., Mehlman, J.K., and Jones, E.A. (2000). Pruritus and fatigue in primary biliary cirrhosis. *Baillières Best Pract. Res. Clin. Gastroenterol.* 14, 643–655.

Bernstein, R.M., Callender, M.E., Neuberger, J.M., Hughes, G.R., and Williams, R. (1982). Anticentromere antibody in primary biliary cirrhosis. *Ann. Rheum. Dis. 41,* 612–614.

Bogdanos, D.P., Baum, H., and Vergani, D. (2003). Antimitochondrial and other autoantibodies. *Clin. Liver Dis. 7,* 759–777.

Browning, J., Combes, B., and Mayo, M.J. (2003). Long-term efficacy of sertraline as a treatment for cholestatic pruritus in patients with primary biliary cirrhosis. *Am. J. Gastroenterol. 98,* 2736–2741.

Bruggraber, S.F., Leung, P.S., Amano, K., Quan, C., Kurth, M.J., Nantz, M.H., Benson, G.D., Van de Water, J., Luketic, V., Roche, T.E., Ansari, A.A., Coppel, R.L., and Gershwin, M.E. (2003). Autoreactivity to lipoate and a conjugated form of lipoate in primary biliary cirrhosis. *Gastroenterology 125,* 1705–1713.

Costa, C., Sambataro, A., Baldi, S., Modena, V., Todros, L., Libertucci, D., Coni, F., Fusaro, E., Revello, F., Murgia, A., et al. (1995). Primary biliary cirrhosis: lung involvement. *Liver 15,* 196–201.

D'Cruz, D. (2000). Autoimmune diseases associated with drugs, chemicals and environmental factors. *Toxicol. Lett. 112–113,* 421–432.

Elta, G.H., Sepersky, R.A., Goldberg, M.J., Connors, C.M., Miller, K.B., and Kaplan, M.M. (1983). Increased incidence of hypothyroidism in primary biliary cirrhosis. *Dig. Dis. Sci. 28,* 971–975.

Fanning, P.A., Jonsson, J.R., Clouston, A.D., Edwards-Smith, C., Balderson, G.A., Macdonald, G.A., Crawford, D.H., Kerlin, P., Powell, L.W., and Powell, E.E. (2000). Detection of male DNA in the liver of female patients with primary biliary cirrhosis. *J. Hepatol. 33,* 690–695.

Findor, J., He, X.S., Sord, J., Terg, R., and Gershwin, M.E. (2002). Primary biliary cirrhosis and hepatocellular carcinoma. *Autoimmun. Rev. 1,* 220–225.

Forton, D.M., Patel, N., Prince, M., Oatridge, A., Hamilton, G., Goldblatt, J., Allsop, J.M., Hajnal, J.V., Thomas, H.C., Bassendine, M., Jones, D.E., and Taylor-Robinson, S.D. (2004). Fatigue and primary biliary cirrhosis: association of globus pallidus magnetisation transfer ratio measurements with fatigue severity and blood manganese levels. *Gut 53,* 587–592.

Fukushima, N., Nalbandian, G., Van De Water, J., White, K., Ansari, A.A., Leung, P., Kenny, T., Kamita, S.G., Hammock, B.D., Coppel, R.L., Stevenson, F., Ishibashi, H., and Gershwin, M.E. (2002). Characterization of recombinant monoclonal IgA anti-PDC-E2 autoantibodies derived from patients with PBC. *Hepatology 36,* 1383–1392.

Gershwin, M.E. and Selmi, C. (2004). Apocalypsal versus apocryphal: the role of retroviruses in primary biliary cirrhosis. *Am. J. Gastroenterol. 99,* 2356–2358.

Gershwin, M.E., Mackay, I.R., Sturgess, A., and Coppel, R.L. (1987). Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. *J. Immunol. 138,* 3525–3531.

Gershwin, M.E., Ansari, A.A., Mackay, I.R., Nakanuma, Y., Nishio, A., Rowley, M.J., and Coppel, R.L. (2000). Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. *Immunol. Rev. 174,* 210–225.

Ghadiminejad, I., Wilkie, D., and Baum, H. (1988). Expression of the primary biliary cirrhosis antigens in yeast: aspects of mitochondrial control. *J. Bioenerg. Biomembr. 20,* 243–259.

Gluud, C. and Christensen, E. (2002). Ursodeoxycholic acid for primary biliary cirrhosis. *Cochrane Database Syst. Rev*.: CD000551.

Goldstein, N.S., Soman, A., and Gordon, S.C. (2001). Portal tract eosinophils and hepatocyte cytokeratin 7 immunoreactivity helps distinguish early-stage, mildly active primary biliary cirrhosis and autoimmune hepatitis. *Am. J. Clin. Pathol. 116,* 846–853.

Grambsch, P.M., Dickson, E.R., Kaplan, M., LeSage, G., Fleming, T.R., and Langworthy, A.L. (1989). Extramural cross-validation of the Mayo primary biliary cirrhosis survival model establishes its generalizability. *Hepatology 10,* 846–850.

Harada, K., Ozaki, S., Sudo, Y., Tsuneyama, K., Ohta, H., and Nakanuma, Y. (2003). Osteopontin is involved in the formation of epithelioid granuloma and bile duct injury in primary biliary cirrhosis. *Pathol. Int. 53,* 8–17.

Hayashi, Y., Utsuyama, M., Kurashima, C., and Hirokawa, K. (1989). Spontaneous development of organ-specific autoimmune lesions in aged C57BL/6 mice. *Clin. Exp. Immunol. 78,* 120–126.

Heathcote, E.J. (2000). Management of primary biliary cirrhosis. The American Association for the Study of Liver Diseases practice guidelines. *Hepatology 31,* 1005–1013.

Hess, E.V. (1999). Are there environmental forms of systemic autoimmune diseases? *Environ. Health Perspect. 107*(Suppl 5), 709–711.

Howel, D., Fischbacher, C.M., Bhopal, R.S., Gray, J., Metcalf, J.V., and James, O.F. (2000). An exploratory population-based case-control study of primary biliary cirrhosis. *Hepatology 31,* 1055–1060.

Inoue, K., Hirohara, J., Nakano, T., Seki, T., Sasaki, H., Higuchi, K., Ohta, Y., Onji, M., Muto, Y., and Moriwaki, H. (1995). Prediction of prognosis of primary biliary cirrhosis in Japan. *Liver 15,* 70–77.

Invernizzi, P., Crosignani, A., Battezzati, P.M., Covini, G., De Valle, G., Larghi, A., Zuin, M., and Podda, M. (1997). Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. *Hepatology 25,* 1090–1095.

Invernizzi, P., De Andreis, C., Sirchia, S.M., Battezzati, P.M., Zuin, M., Rossella, F., Perego, F., Bignotto, M., Simoni, G., and Podda, M. (2000). Blood fetal microchimerism in primary biliary cirrhosis. *Clin. Exp. Immunol. 122,* 418–422.

Invernizzi, P., Podda, M., Battezzati, P.M., Crosignani, A., Zuin, M., Hitchman, E., Maggioni, M., Meroni, P.L., Penner, E., and Wesierska-Gadek, J. (2001). Autoantibodies against nuclear pore complexes are associated with more active and severe liver disease in primary biliary cirrhosis. *J. Hepatol. 34,* 366–372.

Invernizzi, P., Alvaro, D., Crosignani, A., Gaudio, E., and Podda, M. (2004a). Tamoxifen in treatment of primary biliary cirrhosis. *Hepatology 39,* 1175–1176.

Invernizzi, P., Miozzo, M., Battezzati, P.M., Bianchi, I., Grati, F.R., Simoni, G., Selmi, C., Watnik, M., Gershwin, M.E., and Podda, M. (2004b). Frequency of monosomy X in women with primary biliary cirrhosis. *Lancet 363,* 533–535.

Invernizzi, P., Selmi, C., Mackay, I.R., Podda, M., and Gershwin, M.E. (2005). From bases to basis: linking genetics to causation in primary biliary cirrhosis. Clin. Gastroenterol. Hepatol. 3, 401–410.

Ishibashi, H., Nakamura, M., Shimoda, S., and Gershwin, M.E. (2003). T cell immunity and primary biliary cirrhosis. Autoimmun. Rev. 2, 19–24.

Itakura, J., Izumi, N., Nishimura, Y., Inoue, K., Ueda, K., Nakanishi, H., Tsuchiya, K., Hamano, K., Asahina, Y., Kurosaki, M., Uchihara, M., and Miyake, S. (2004). Prospective randomized crossover trial of combination therapy with bezafibrate and UDCA for primary biliary cirrhosis. *Hepatol. Res. 29,* 216–222.

Janka, C., Selmi, C., Gershwin, M.E., Will, H., and Sternsdorf, T. (2005). Small ubiquitin-related modifiers: A novel and independent class of autoantigens in primary biliary cirrhosis. *Hepatology 41,* 609–616.

Johansson, A.C., Lindqvist, A.K., Johannesson, M., and Holmdahl, R. (2003). Genetic heterogeneity of autoimmune disorders in the nonobese diabetic mouse. *Scand. J. Immunol. 57,* 203–213.

Jones, D.E. and Donaldson, P.T. (2003). Genetic factors in the pathogenesis of primary biliary cirrhosis. *Clin. Liver Dis. 7,* 841–864.

Jones, E.A. and Bergasa, N.V. (1999). The pathogenesis and treatment of pruritus and fatigue in patients with PBC. *Eur. J. Gastroenterol. Hepatol. 11,* 623–631.

Jones, E.A. and Yurdaydin, C. (1997). Is fatigue associated with cholestasis mediated by altered central neurotransmission? *Hepatology 25,* 492–494.

Joplin, R.E., Wallace, L.L., Lindsay, J.G., Palmer, J.M., Yeaman, S.J., and Neuberger, J.M. (1997). The human biliary epithelial cell plasma membrane antigen in primary biliary cirrhosis: pyruvate dehydrogenase X? *Gastroenterology 113,* 1727–1733.

Jorge, A.D., Gutierrez, L.S., Jorge, O., and Burgos, M.H. (2002). Ultrastructural and cytochemical changes in the liver of primary biliary cirrhosis patients. *Biocell 26,* 253–262.

Kingham, J.G. and Parker, D.R. (1998). The association between primary biliary cirrhosis and coeliac disease: a study of relative prevalences. *Gut 42,* 120–122.

Kita, H., Matsumura, S., He, X.S., Ansari, A.A., Lian, Z.X., Van de Water, J., Coppel, R.L., Kaplan, M.M., and Gershwin, M.E. (2002a). Analysis of TCR antagonism and molecular mimicry of an HLA-A0201-restricted CTL epitope in primary biliary cirrhosis. *Hepatology 36,* 918–926.

Kita, H., Matsumura, S., He, X.S., Ansari, A.A., Lian, Z X., Van de Water, J., Coppel, R.L., Kaplan, M.M., and Gershwin, M.E. (2002b). Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. *J. Clin. Invest. 109,* 1231–1240.

Kita, H., Naidenko, O.V., Kronenberg, M., Ansari, A.A., Rogers, P., He, X.S., Koning, F., Mikayama, T., Van De Water, J., Coppel, R.L., Kaplan, M., and Gershwin, M.E. (2002c). Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. *Gastroenterology 123,* 1031–1043.

Kobashi, H., Yamamoto, K., Yoshioka, T., Tomita, M., and Tsuji, T. (1994). Nonsuppurative cholangitis is induced in neonatally thymectomized

mice: a possible animal model for primary biliary cirrhosis. *Hepatology 19,* 1424–1430.

Krams, S.M., Dorshkind, K., and Gershwin, M.E. (1989). Generation of biliary lesions after transfer of human lymphocytes into severe combined immunodeficient (SCID) mice. *J. Exp. Med. 170,* 1919–1930.

Lambert, N. and Nelson, J.L. (2003). Microchimerism in autoimmune disease: more questions than answers? *Autoimmun. Rev. 2,* 133–139.

Lanspa, S.J., Chan, A.T., Bell, J.S., 3rd, Go, V.L., Dickson, E.R., and DiMagno, E.P. (1985). Pathogenesis of steatorrhea in primary biliary cirrhosis. *Hepatology 5,* 837–842.

Lazaridis, K.N., Gores, G.J., and Lindor, K.D. (2001). Ursodeoxycholic acid 'mechanisms of action and clinical use in hepatobiliary disorders'. *J. Hepatol. 35,* 134–146.

Leung, P.S., Park, O., Matsumura, S., Ansari, A.A., Coppel, R.L., and Gershwin, M.E. (2003a). Is there a relation between Chlamydia infection and primary biliary cirrhosis? *Clin. Dev. Immunol. 10,* 227–233.

Leung, P.S., Quan, C., Park, O., Van de Water, J., Kurth, M.J., Nantz, M.H., Ansari, A.A., Coppel, R.L., Lam, K.S., and Gershwin, M.E. (2003b). Immunization with a xenobiotic 6-bromoriexanoate bovine serum albumen conjugate induces antimitochondrial antibodies. *J. Immunol. 170*, 5326–5332.

Leuschner, M., Dietrich, C.F., You, T., Seidl, C., Raedle, J., Herrmann, G., Ackermann, H., and Leuschner, U. (2000). Characterisation of patients with primary biliary cirrhosis responding to long term ursodeoxycholic acid treatment. *Gut 46,* 121–126.

Liermann Garcia, R.F., Evangelista Garcia, C., McMaster, P., and Neuberger, J. (2001). Transplantation for primary biliary cirrhosis: retrospective analysis of 400 patients in a single center. *Hepatology 33,* 22–27.

Lindor, K.D., Jorgensen, R.A., Therneau, T.M., Malinchoc, M., and Dickson, E.R. (1997). Ursodeoxycholic acid delays the onset of esophageal varices in primary biliary cirrhosis. *Mayo Clin. Proc. 72,* 1137–1140.

Long, S.A., Quan, C., Van de Water, J., Nantz, M.H., Kurth, M.J., Barsky, D., Colvin, M.E., Lam, K.S., Coppel, R.L., Ansari, A., and Gershwin, M.E. (2001). Immunoreactivity of organic mimeotopes of the E2 component of pyruvate dehydrogenase: connecting xenobiotics with primary biliary cirrhosis. *J. Immunol. 167,* 2956–2963.

Long, S.A., Van de Water, J., and Gershwin, M.E. (2002). Antimitochondrial antibodies in primary biliary cirrhosis: the role of xenobiotics. *Autoimmun. Rev. 1,* 37–42.

Longo, M., Crosignani, A., Battezzati, P.M., Squarcia Giussani, C., Invernizzi, P., Zuin, M., and Podda, M. (2002). Hyperlipidaemic state and cardiovascular risk in primary biliary cirrhosis. *Gut 51,* 265–269.

Ludwig, J. (2000). The pathology of primary biliary cirrhosis and autoimmune cholangitis. *Baillières Best Pract. Res. Clin. Gastroenterol. 14,* 601–613.

Ludwig, J., Dickson, E.R., and McDonald, G.S. (1978). Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch. Pathol. Anat. Histol. 379,* 103–112.

Mackay, I.R., Whittingham, S., Fida, S., Myers, M., Ikuno, N., Gershwin, M.E., and Rowley, M.J. (2000). The peculiar autoimmunity of primary biliary cirrhosis. *Immunol. Rev. 174,* 226–237.

MacQuillan, G.C. and Neuberger, J. (2003). Liver transplantation for primary biliary cirrhosis. *Clin. Liver Dis. 7,* 941–956, ix.

Marasini, B., Gagetta, M., Rossi, V., and Ferrari, P. (2001). Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. *Ann. Rheum. Dis. 60*, 1046–1049.

Matsumura, S., Van De Water, J., Leung, P., Odin, J.A., Yamamoto, K., Gores, G.J., Mostov, K., Ansari, A.A., Coppel, R.L., Shiratori, Y., and Gershwin, M.E. (2004). Caspase induction by IgA antimitochondrial antibody: IgA-mediated biliary injury in primary biliary cirrhosis. *Hepatology 39,* 1415–1422.

Metcalf, J.V., Bhopal, R.S., Gray, J., Howel, D., and James, O.F. (1997). Incidence and prevalence of primary biliary cirrhosis in the city of Newcastle upon Tyne, England. *Int. J. Epidemiol. 26,* 830–836.

Migliaccio, C., Nishio, A., Van de Water, J., Ansari, A.A., Leung, P.S., Nakanuma, Y., Coppel, R.L., and Gershwin, M.E. (1998). Monoclonal antibodies to mitochondrial E2 components define autoepitopes in primary biliary cirrhosis. *J. Immunol. 161,* 5157–5163.

Mitchison, H.C., Bassendine, M.F., Hendrick, A., Bennett, M.K., Bird, G., Watson, A.J., and James, O.F. (1986). Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis? *Hepatology 6,* 1279–1284.

Miyakawa, H., Tanaka, A., Kikuchi, K., Matsushita, M., Kitazawa, E., Kawaguchi, N., Fujikawa, H., and Gershwin, M.E. (2001). Detection of antimitochondrial autoantibodies in immunofluorescent AMA-negative patients with primary biliary cirrhosis using recombinant autoantigens. *Hepatology 34,* 243–248.

Muratori, P., Muratori, L., Guidi, M., Maccariello, S., Pappas, G., Ferrari, R., Gionchetti, P., Campieri, M., and Bianchi, F.B. (2003). Anti-*Saccharomyces cerevisiae* antibodies (ASCA) and autoimmune liver diseases. *Clin. Exp. Immunol. 132,* 473–476.

Navasa, M., Pares, A., Bruguera, M., Caballeria, J., Bosch, J., and Rodes, J. (1987). Portal hypertension in primary biliary cirrhosis. Relationship with histological features. *J. Hepatol. 5,* 292–298.

Neuberger, J. (1999). Eosinophils and primary biliary cirrhosis–stoking the fire? *Hepatology 30,* 335–337.

Obermayer-Straub, P., Strassburg, C.P., and Manns, M.P. (2000). Autoimmune hepatitis. *J. Hepatol. 32,* 181–197.

Oo, Y.H. and Neuberger, J. (2004). Options for treatment of primary biliary cirrhosis. *Drugs 64,* 2261–2271.

Pares, A., Rimola, A., Bruguera, M., Mas, E., and Rodes, J. (1981). Renal tubular acidosis in primary biliary cirrhosis. *Gastroenterology 80,* 681–686.

Pares, A. and Rodes, J. (2003). Natural history of primary biliary cirrhosis. *Clin. Liver Dis. 7,* 779–794.

Parikh-Patel, A., Gold, E.B., Worman, H., Krivy, K E., and Gershwin, M.E. (2001). Risk factors for primary biliary cirrhosis in a cohort of patients from the united states. *Hepatology 33,* 16–21.

Parikh-Patel, A., Gold, E., Utts, J., and Gershwin, M.E. (2002). The association between gravidity and primary biliary cirrhosis. *Ann. Epidemiol. 12,* 264–272.

Phillips, J.R., Angulo, P., Petterson, T., and Lindor, K.D. (2001). Fat-soluble vitamin levels in patients with primary biliary cirrhosis. *Am. J. Gastroenterol. 96,* 2745–2750.

Prince, M.I., Chetwynd, A., Craig, W.L., Metcalf, J.V., and James, O.F. (2004). Asymptomatic primary biliary cirrhosis: clinical features, prognosis, and symptom progression in a large population based cohort. *Gut 53,* 865–870.

Pumford, N.R., Halmes, N.C., and Hinson, J.A. (1997). Covalent binding of xenobiotics to specific proteins in the liver. *Drug Metab. Rev. 29,* 39–57.

Quinn, J., Diamond, A.G., Palmer, J.M., Bassendine, M.F., James, O.F., and Yeaman, S.J. (1993). Lipoylated and unlipoylated domains of human PDC-E2 as autoantigens in primary biliary cirrhosis: significance of lipoate attachment. *Hepatology 18,* 1384–1391.

Rao, T. and Richardson, B. (1999). Environmentally induced autoimmune diseases: potential mechanisms. *Environ. Health Perspect. 107*(Suppl 5), 737–742.

Reddy, A., Prince, M., James, O.F., Jain, S., and Bassendine, M.F. (2004). Tamoxifen: a novel treatment for primary biliary cirrhosis? *Liver Int. 24,* 194–197.

Reuben, A. (2003). The serology of the Addison-Gull syndrome. *Hepatology 37,* 225–228.

Roll, J., Boyer, J.L., Barry, D., and Klatskin, G. (1983). The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. *N. Engl. J. Med. 308,* 1–7.

Rose, N.R. and Burek, C.L. (2000). Autoantibodies to thyroglobulin in health and disease. *Appl. Biochem. Biotechnol. 83,* 245–251; discussion 251–244, 297–313.

Sasaki, M., Allina, J., Odin, J.A., Thung, S.N., Coppel, R., Nakanuma, Y., and Gershwin, M.E. (2002). Autoimmune cholangitis in the SJL/J mouse is antigen non-specific. *Dev. Immunol. 9,* 103–111.

Selmi, C., Ross, S.R., Ansari, A.A., Invernizzi, P., Podda, M., Coppel, R.L., and Gershwin, M.E. (2004). Lack of immunological or molecular evidence for a role of mouse mammary tumor retrovirus in primary biliary cirrhosis. *Gastroenterology. 7*, 493–501.

Selmi, C., Balkwill, D.L., Invernizzi, P., Ansari, A.A., Coppel, R.L., Podda, M., Leung, P.S., Kenny, T.P., Van De Water, J., Nantz, M.H., Kurth, M.J., and Gershwin, M.E. (2003). Patients with primary biliary cirrhosis react against a ubiquitous xenobiotic-metabolizing bacterium. *Hepatology 38,* 1250–1257.

Selmi, C., Invernizzi, P., Keefe, E.B., Coppel, R.L., Podda, M., Rossaro, L., Ansari, A.A., and Gershwin, M.E. (2004). Epidemiology and pathogenesis of primary biliary cirrhosis. *J. Clin. Gastroenterol. 38,* 264–271.

Sherlock, S. (1959). Primary billiary cirrhosis (chronic intrahepatic obstructive jaundice). *Gastroenterology 37,* 574–586.

Sherlock, S. and Scheuer, P.J. (1973). The presentation and diagnosis of 100 patients with primary biliary cirrhosis. *N. Engl. J. Med. 289,* 674–678.

Shimoda, S., Nakamura, M., Ishibashi, H., Hayashida, K., and Niho, Y. (1995). HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. *J. Exp. Med. 181,* 1835–1845.

Shimoda, S., Van de Water, J., Ansari, A., Nakamura, M., Ishibashi, H., Coppel, R.L., Lake, J., Keeffe, E.B., Roche, T.E., and Gershwin, M.E. (1998). Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. *J. Clin. Invest. 102,* 1831–1840.

Shimoda, S., Nakamura, M., Ishibashi, H., Kawano, A., Kamihira, T., Sakamoto, N., Matsushita, S., Tanaka, A., Worman, H.J., Gershwin, M.E., and Harada, M. (2003). Molecular mimicry of mitochondrial and nuclear autoantigens in primary biliary cirrhosis. *Gastroenterology 124,* 1915–1925.

Simat, T.J., Kleeberg, K.K., Muller, B., and Sierts, A. (1999). Synthesis, formation, and occurrence of contaminants in biotechnologically manufactured L-tryptophan. *Adv. Exp. Med. Biol. 467,* 469–480.

Springer, J., Cauch-Dudek, K., O'Rourke, K., Wanless, I.R., and Heathcote, E.J. (1999). Asymptomatic primary biliary cirrhosis: a study of its natural history and prognosis. *Am. J. Gastroenterol. 94,* 47–53.

Steenland, K. and Goldsmith, D.F. (1995). Silica exposure and autoimmune diseases. *Am. J. Ind. Med. 28,* 603–608.

Swain, M.G. and Maric, M. (1995). Defective corticotropin-releasing hormone mediated neuroendocrine and behavioral responses in cholestatic rats: implications for cholestatic liver disease-related sickness behaviors. *Hepatology 22,* 1560–1564.

Talwalkar, J.A. and Lindor, K.D. (2003). Primary biliary cirrhosis. *Lancet 362,* 53–61.

Talwalkar, J.A., Angulo, P., Keach, J.C., Petz, J.L., Jorgensen, R.A., and Lindor, K.D. (2005). Mycophenolate mofetil for the treatment of primary biliary cirrhosis in patients with an incomplete response to ursodeoxycholic acid. *J. Clin. Gastroenterol. 39,* 168–171.

Tanaka, A., Lindor, K., Gish, R., Batts, K., Shiratori, Y., Omata, M., Nelson, J.L., Ansari, A., Coppel, R., Newsome, M., and Gershwin, M.E. (1999a). Fetal microchimerism alone does not contribute to the induction of primary biliary cirrhosis. *Hepatology 30,* 833–838.

Tanaka, A., Prindiville, T.P., Gish, R., Solnick, J.V., Coppel, R.L., Keeffe, E.B., Ansari, A., and Gershwin, M.E. (1999b). Are infectious agents involved in primary biliary cirrhosis? A PCR approach. *J. Hepatol. 31,* 664–671.

Terg, R., Coronel, E., Sorda, J., Munoz, A.E., and Findor, J. (2002). Efficacy and safety of oral naltrexone treatment for pruritus of cholestasis, a crossover, double blind, placebo-controlled study. *J. Hepatol. 37,* 717–722.

Tison, V., Callea, F., Morisi, C., Mancini, A.M., and Desmet, V.J. (1982). Spontaneous "primary biliary cirrhosis" in rabbits. *Liver 2,* 152–161.

Tsianos, E.V., Hoofnagle, J.H., Fox, P.C., Alspaugh, M., Jones, E.A., Schafer, D.F., and Moutsopoulos, H.M. (1990). Sjogren's syndrome in patients with primary biliary cirrhosis. *Hepatology 11,* 730–734.

Tsuneyama, K., Van de Water, J., Leung, P.S., Cha, S., Nakanuma, Y., Kaplan, M., De Lellis, R., Coppel, R., Ansari, A., and Gershwin, M.E. (1995a). Abnormal expression of the E2 component of the pyruvate dehydrogenase complex on the luminal surface of biliary epithelium occurs before major histocompatibility complex class II and BB1/B7 expression. *Hepatology 21,* 1031–1037.

Tsuneyama, K., Van De Water, J., Van Thiel, D., Coppel, R., Ruebner, B., Nakanuma, Y., Dickson, E.R., and Gershwin, M.E. (1995b). Abnormal expression of PDC-E2 on the apical surface of biliary epithelial cells in patients with antimitochondrial antibody-negative primary biliary cirrhosis. *Hepatology 22,* 1440–1446.

Tsuneyama, K., Harada, K., Kono, N., Hiramatsu, K., Zen, Y., Sudo, Y., Gershwin, M.E., Ikemoto, M., Arai, H., and Nakanuma, Y. (2001). Scavenger cells with gram-positive bacterial lipoteichoic acid infiltrate around the damaged interlobular bile ducts of primary biliary cirrhosis. *J. Hepatol. 35,* 156–163.

Van de Water, J., Ansari, A.A., Surh, C.D., Coppel, R., Roche, T., Bonkovsky, H., Kaplan, M., and Gershwin, M.E. (1991). Evidence for the targeting by 2-oxo-dehydrogenase enzymes in the T cell response of primary biliary cirrhosis. *J. Immunol. 146,* 89–94.

Van de Water, J., Turchany, J., Leung, P.S., Lake, J., Munoz, S., Surh, C.D., Coppel, R., Ansari, A., Nakanuma, Y., and Gershwin, M.E. (1993). Molecular mimicry in primary biliary cirrhosis. Evidence for biliary epithelial expression of a molecule cross-reactive with pyruvate dehydrogenase complex-E2. *J. Clin. Invest. 91,* 2653–2664.

Van de Water, J., Ansari, A., Prindiville, T., Coppel, R., Ricalton, N., Kotzin, B.L., Liu, S., Roche, T.E., Krams, S.M., Munoz, S., and Gershwin, M.E. (1995). Heterogeneity of autoreactive T cell clones specific for the E2 component of the pyruvate dehydrogenase complex in primary biliary cirrhosis. *J. Exp. Med. 181,* 723–733.

Van de Water, J., Ishibashi, H., Coppel, R.L., and Gershwin, M.E. (2001). Molecular mimicry and primary biliary cirrhosis: premises not promises. *Hepatology 33,* 771–775.

Vierling, J.M., Ruderman, W.B., Jaffee, B.D., Fennell, R.H., Jr., and Claman, H.N. (1989). Hepatic lesions in murine chronic graft-versus-host disease to minor histocompatibility antigens. A reproducible model of nonsuppurative destructive cholangitis. *Transplantation 48,* 717–718.

Walker, J.G., Doniach, D., Roitt, I.M., and Sherlock, S. (1965). Serological tests in diagnosis of primary biliary cirrhosis. *Lancet 39,* 827–831.

Worman, H.J. and Courvalin, J.C. (2003). Antinuclear antibodies specific for primary biliary cirrhosis. *Autoimmun. Rev. 2,* 211–217.

Xu, L., Shen, Z., Guo, L., Fodera, B., Keogh, A., Joplin, R., O'Donnell, B., Aitken, J., Carman, W., Neuberger, J., and Mason, A. (2003). Does a betaretrovirus infection trigger primary biliary cirrhosis? *Proc. Natl. Acad. Sci. U.S.A. 100,* 8454–8459.

Yip, T.T., Van de Water, J., Gershwin, M.E., Coppel, R.L., and Hutchens, T.W. (1996). Cryptic antigenic determinants on the extracellular pyruvate dehydrogenase complex/mimeotope found in primary biliary cirrhosis. A probe by affinity mass spectrometry. *J. Biol. Chem. 271,* 32825–32833.

Yoshida, S. and Gershwin, M.E. (1993). Autoimmunity and selected environmental factors of disease induction. *Semin. Arthritis Rheum. 22,* 399–419.

Zein, C.O., Angulo, P., and Lindor, K.D. (2003). When is liver biopsy needed in the diagnosis of primary biliary cirrhosis? *Clin. Gastroenterol. Hepatol. 1,* 89–95.

CHAPTER

55

Primary Sclerosing Cholangitis

DIEGO VERGANI AND GIORGINA MIELI-VERGANI
Institute of Liver Studies, King's College Hospital, London, UK

Sclerosing cholangitis (SC) is a chronic cholestatic disorder of unknown etiology, characterized by inflammation and progressive obliterative fibrosis of the intrahepatic and/or extrahepatic bile ducts. It progresses slowly and often asymptomatically to biliary cirrhosis, liver failure, and/or cholangiocarcinoma. The latter can occur any time during the course of the disease.

HISTORIC BACKGROUND

Sclerosing cholangitis was first described in 1924 by a Parisian surgeon, P.L.E. Delbet (Delbet, 1924), who reported a patient with "irregular fibrosis and stenosis of the biliary tree." In 1927, R.T.J. Miller referred to a similar condition as a "benign stricture of common bile duct" (Miller, 1927). Until the 1960s, SC remained largely undiagnosed, with only few cases reported in the literature (Delbet, 1924; Lafourcade, 1925; Judd, 1926). In 1964 (Holubitsky and McKenzie, 1964), the condition was called for the first time "primary sclerosing cholangitis" to distinguish it from SC secondary to lesions of the bile ducts or systemic disease like primary or secondary immunodeficiencies. Until the advent of endoscopic retrograde cholangiopancreatography and percutaneous transhepatic cholangiography, recognition was rare, being made at laparotomy in patients with persistent jaundice. With the availability of cholangiography the disease is now recognized in anicteric patients, and its reported prevalence in both adults and children is increasing. The association of SC with ulcerative colitis (UC) was first clearly established in 1966 (Warren et al., 1966), though inflammatory involvement of the liver parenchyma in inflammatory bowel disease (IBD) had been described as early as 1899 by Lister (Lister, 1899).

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Clinical Features

Primary SC has long been considered a disease confined to adulthood, but it is now clear that it occurs in all age groups, with some features being unique to children. In adult primary SC, the majority of patients are male. They are often initially asymptomatic, and identified on the basis of abnormal liver function tests, in particular elevated levels of alkaline phosphatase (AP) and/or γ-glutamyltransferase (GGT). These patients may also have increased levels of immunoglobulins G and M (IgG, IgM) and positive auto-

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

antibodies. Some patients may present with entirely normal AP (Balasubramaniam, et al., 1988). Further investigations are usually prompted by the persistence of laboratory abnormalities. Cholangiography shows the characteristic changes of SC, i.e., multifocal strictures and dilatations, usually involving both the intrahepatic and extrahepatic bile ducts (Figure 55.1). In early stages, ulcerations of the common bile duct similar to those seen in the colon in early ulcerative colitis may be seen. Some patients, usually with associated IBD, have chronic cholestasis and hepatic histology compatible with primary SC but normal findings on cholangiography. This condition, called "small duct SC" (Wee and Ludwig, 1985), may represent an early stage of classic primary SC and is associated with a significantly better long-term prognosis. Patients with primary SC may have advanced radiologic and histologic disease without overt symptoms. Increase in serum bilirubin with a decrease in serum albumin heralds impending liver failure. The most common symptoms of decompensation are jaundice, fatigue, itching, and weight loss. Fever and acute cholangitis are uncommon, being present in 10–15% of the cases (Kaplan, 1991). Primary SC is a risk factor for the development of colorectal adenocarcinoma that is independent with or without the presence of IBD.

In childhood, idiopathic SC is characterized by florid autoimmune features, including elevated titers of autoantibodies, in particular antinuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA), elevated IgG, and interface hepatitis (Figure 55.2) (Gregorio et al., 2001). Since these features are shared in common with autoimmune hepatitis (AIH), in the absence of cholangiographic studies, many of these children are diagnosed with and treated for AIH, though the diagnosis of SC may become apparent during follow-up. To ascertain the relative prevalence of SC and AIH in childhood, a prospective study was conducted over a period of 16 years, in which all children presenting with liver disease and elevated levels of aspartate aminotransferase (AST) and IgG and positive autoantibodies underwent cholangiography and liver biopsy both at presentation and follow-up (Gregorio et al., 2001). Approximately 50% of the patients had alterations of the bile ducts characteristic of SC at presentation, though less advanced than in adult SC (Figure 55.3), often in the absence of histologic features suggesting bile duct involvement. Fifty-five percent were female. Presentation ranged from symptoms indistinguishable from those of acute hepatitis to a prolonged history of liver dysfunction or the incidental finding of abnormal liver function tests. Inflammatory bowel disease was diagnosed in 44%, but also in 18% of the children with AIH. Cholestatic biochemistry was rare, with most children having normal GGT and AP. A laboratory index significantly different between SC and AIH was an increased AP/AST ratio in SC. Ninety seven percent of the children with SC had ANA and SMA. One child with normal cholangiography at presentation progressed to severe SC 8 years later.

Overlap between primary SC and AIH has been described also in adult patients (Rabinovitz et al., 1992; Lawrence, 1994; Schrumpf and Boberg, 2001), who similarly to children have elevated levels of circulating immunoglobulins and non-organ-specific autoantibodies and interface hepatitis on liver biopsy. In these patients, treatment with corticosteroids may, at least temporarily, induce biochemical and clinical improvement in selected cases. (Boberg et al., 1996).

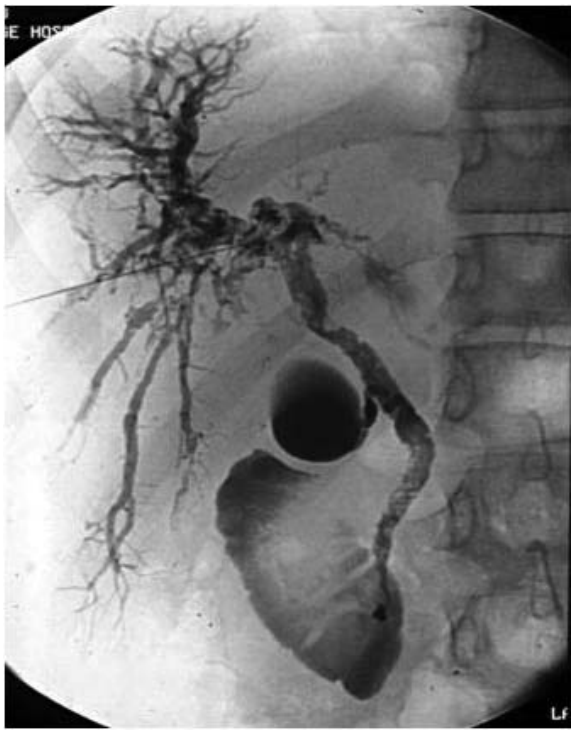


Figure 55.1 Cholangiogram of an adult patient with primary sclerosing cholangitis showing severe widespread strictures and dilatations of the intra- and extrahepatic bile ducts.

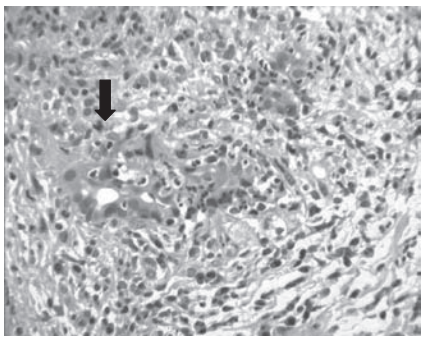


Figure 55.2 Portal tract inflammatory cell infiltrate in a case of juvenile sclerosing cholangitis positive for anti-nuclear and anti-smooth muscle antibodies. Plasma cells and lymphocytes cross the limiting plate invading the parenchyma (interface hepatitis). The arrow points to a damaged bile duct.

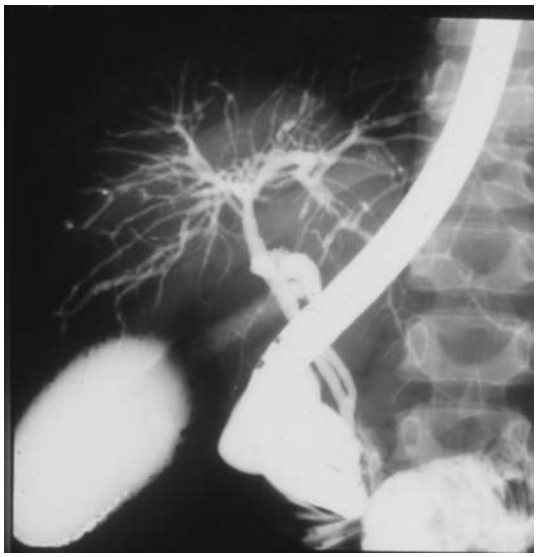


Figure 55.3 Cholangiogram of a child with sclerosing cholangitis showing moderate strictures and dilatations of the intrahepatic bile ducts.

Figure 55.4 'Onionskin' fibrosis around a damaged bile duct. This is the characteristic lesion of sclerosing cholangitis, but is rarely seen in percutaneous liver biopsies.

Histological Features

Histologically, primary SC has been divided into four stages, though this classification is used more as a research tool than for clinical management (Ludwig et al., 1986). In Stage 1, there is degeneration of the bile duct epithelial cells and infiltration of the bile ducts by mononuclear cells, with occasional neutrophils. The portal tracts may be expanded because of fibrosis and edema, and may show proliferation of bile ducts, vacuolization of ductular epithelial cells, and concentric layers of connective tissue surrounding individual bile ducts (onionskin fibrosis). Stage 2 is characterized by more widespread fibrosis and inflammation, which infiltrates the periportal parenchyma with damage of periportal hepatocytes (interface hepatitis). A prominent feature is bile ductopenia. This becomes more severe in Stage 3, where bile ducts may be absent and porto-portal bridging fibrosis prominent, with cholestasis in periportal and paraseptal hepatocytes. Stage 4 is characterized by frank biliary cirrhosis.

The typical onionskin fibrosis lesion (Figure 55.4) is rarely seen on a percutaneous liver biopsy, where paucity of normal bile ducts with nonspecific portal tract fibrosis and inflammation are the most common findings. In view of the variable and largely nonspecific histologic lesions, the diagnosis of SC rests on cholangiographic imaging.

Epidemiologic Features

Adult primary SC affects mainly patients in their third to fourth decade of life with a male-to-female ratio of 2:1 (Lee and Kaplan, 1995). There is a strong association between primary SC and IBD, particularly ulcerative colitis (Fausa et al., 1991; Olsson, 1991). Patients with primary SC and without IBD are more likely to be female (Rabinovitz, 1990) ranging from 21% in Japan to 82% in Northern Europe (Schrumpf and Boberg, 2001). Of the primary SC patients with IBD, some 85% have UC, and the remainder have Crohn's colitis. Conversely, approximately 4% of patients with IBD will either have or develop primary SC.

An early Norwegian population study showed an incidence of primary SC of 1.3 and a prevalence of 8.5 for 100,000 inhabitants (Boberg et al., 1998). These values were considered higher than for the rest of Europe and the United States. However, a recent report from Minnesota, USA (Bambha et al., 2003), shows an incidence of primary SC in men of 1.25 and in women of 0.54 per 100,000 person-years. The prevalence of primary SC in 2000 was 20.9 per 100,000 men and only 6.3 per 100,000 women.

Little is known of the epidemiology of childhood SC, though genders appear to be equally represented (Gregorio et al., 2001).

AUTOIMMUNE FEATURES

A high proportion of patients with primary SC have circulating autoantibodies, including ANA, SMA, and antineutrophil cytoplasmic antibodies (ANCA) (Cullen and Chapman, 2003). Possibly of pathogenic relevance is the observation that sera from two-thirds of patients with primary SC have circulating autoantibodies against amino acid sequences shared in common by colon and biliary epithelial cells (Mandal et al., 1994). Other autoantibodies described in primary SC include anti-endothelial cell

antibody (35%; Gur et al., 1995), anticardiolipin antibody (4–66%; Angulo et al., 2000), antithyroperoxidase (7–16%; Angulo et al., 2000), antithyroglobulin (4%; Gur et al., 1995), and rheumatoid factor (15%; Angulo et al., 2000). The presence of these autoantibodies in association to abnormalities of the complement system (Senaldi et al., 1989) and elevated circulating immune complexes (Bodenheimer et al., 1983; Minuk et al., 1985) suggest a possible immune pathogenesis in primary SC. This is supported by the early histologic findings of a mononuclear inflammatory infiltrate in the portal tracts—comprising lymphocytes, macrophages, and plasma cells—with interface hepatitis. This histologic picture, particularly evident in the juvenile form of SC, is undistinguishable from that of AIH (Gregorio et al., 2001).

GENETIC FEATURES

The occurrence of primary SC in members of the same family suggests a genetic predisposition (Quigley et al., 1983). Immunogenetic studies have contributed towards the understanding of the influence of the immune system on the development and progression of primary SC. Small, early studies identified significant associations with both HLA B8 and DR3 (Schrumpf et al., 1982; Chapman et al., 1983; Shepherd et al., 1983). Subsequent investigations (Donaldson et al., 1991; Farrant et al., 1992; Mehal et al., 1994; Chapman, 1995; Olerup et al., 1995; Spurkland et al., 1995; Moloney et al., 1998; Bernal et al., 1999; Mitchell et al., 2001b; Norris et al., 2001; Wiencke et al., 2001; Donaldson and Norris, 2002) in different European populations uncovered three potential susceptibility haplotypes: the first being *A1-Cw*0701-B8-MICA*008-TNFA*2-DRB3*0101-DRB1*0301-DQA1*0501-DQB1*0201*, the second *DRB3*0101-DRB1*1301-DQA1*0103-DQB1*0603*, and the third *MICA*008-DRB5*0101-DRB1*1501-DQA1*0102-DQB1*0602*, and three different protective haplotypes, the first encoding *DRB4*0103-DRB1*0401-DQA1*03-DQB1*0302*, the second *DRB4-DRB1*0701-DQA1*0201-DQB1*0303*, and the third the *MICA*002* allele. A key role in conferring susceptibility and resistance appears to be played by specific amino acids at position DQβ-87 and DQβ-55, respectively (Donaldson and Norris, 2002).

A range of non-major histocompatibility complex (MHC) immunoregulatory genes have been studied in relation to primary SC, including cytotoxic lymphocyte antigen-4 (Saarinen et al., 1998; Agarwal et al., 2000), tumor necrosis factor-α (Bernal et al., 1999; Mitchell et al., 2001b), and interleukins 1 and 10 (IL-1 and IL-10) (Donaldson et al., 2000), with controversial results. Since the end result of inflammation in primary SC is periductal fibrosis, the reported association between a polymorphism of *MMP*-3 (stromelysin) (Satsangi et al., 2001), a gene involved in the regulation of the production and destruction of extracellular matrix, and susceptibility to primary SC and its progression is of interest. Susceptibility and resistance to primary SC are likely not to be linked to individual loci, but to the contribution of different alleles according to the model of a "complex genetic disease" (Nepom, 1990).

ENVIRONMENTAL INFLUENCES

A number of environmental factors have been proposed in the pathogenesis of the bile duct injury typical of SC, including chronic portal bacteremia (Brooke et al., 1961), toxic bile acid metabolites produced by the enteric flora (Carey, 1964), toxins produced directly by the enteric bacteria (Palmer, 1972), chronic infections (Finegold and Carpenter, 1982; Fox et al., 1998; Ponsioen et al., 2002), and ischemic vascular damage (Ludwig et al., 1989). The close association between primary SC and UC has led to the hypothesis that the initiating step in primary SC is the access of intestinal bacteria, through an inflamed and leaky bowel wall, to the portal circulation (Boden et al., 1959; Warren et al, 1966). Though one study did demonstrate portal bacteremia in patients with UC after colonic surgery (Brooke et al., 1961), this observation has not been confirmed. The role of toxic bile acid metabolites generated by the gut flora is also uncertain, since no major abnormalities in the composition and concentration of bile acids have been found in the bile and portal blood of patients with either primary SC or chronic IBD (Siegel et al., 1977).

ANIMAL MODELS

Several animal models of SC have been developed, none, however, faithfully mimicking the human condition. They include models involving: 1) bacterial cell-wall components or colitis; 2) biliary epithelial and endothelial cell injury; and 3) toxic intraluminal injury of the biliary tract.

Bacterial Cell Wall Components or Colitis

Bile duct lesions similar to SC have been reproduced in rats with small bowel bacterial overgrowth (Lichtman and Sartor, 1990; 1991; Lichtman et al., 1991a; 1991b; 1991c; Sartor et al., 1996) The disease develops only in susceptible strains of rat, suggesting a crucial influence of the genetic make up. The innate immune response appears to play a major role, with production of IL-12, interferon-γ, macrophage and natural killer cell activation, and secretion of proinflammatory cytokines.

Focal strictures of intrahepatic bile ducts follow intraperitoneal injection of peptidoglycan-polysaccharide prepared

from the cell wall of group A streptococcus in rats (Cromartie et al., 1977).

Pericholangitis and periductular fibrosis can be induced in rabbits by injecting muramyl dipeptide (MDP), a bacterial cell-wall fragment, into the submucosa of the rectum and colon (Kuroe et al., 1996a; 1996b). If MDP is injected with complete Freund's adjuvant the rabbit develops granulomatous colitis (Kuroe et al., 1996a), while if it is injected with long-chain fatty acid the animal develops colitis without granulomas (Kuroe et al., 1996b).

Cholangitis is induced by *N*-formyl L-methionine L-leucine L-tyrosine (fMLT) in rats with colitis. In this model, colitis is induced in rats by intrarectal administration of 15% acetic acid, followed by daily intrarectal infusion of fMLT (Yamada et al., 1994), a pro-inflammatory peptide secreted by *Escherichia coli*, which undergoes enterohepatic circulation and is a strong chemo-attractant for neutrophils and macrophages. The fMLT appears in bile within 3 hours. One day after injection, the portal tracts are infiltrated by macrophages and neutrophils. By day 4, $CD4^+$ and $CD8^+$ T cells are present in the peribiliary infiltrates and are attached to the biliary epithelia (Yamada et al., 1999). Carageenan treatment, which reduces the number of macrophages, also reduces the cholangitic lesions, suggesting that bacterial chemotactic peptides, normally sequestered in the gut, can cause small duct cholangitis if they gain access to the portal circulation through an inflamed colonic mucosa.

Biliary Epithelial and Endothelial Cell Injury

Ueno et al. showed that immunization with highly purified cholangiocytes leads to the development of nonsuppurative cholangitis (Ueno et al., 1996). Immunohistochemical studies revealed that portal tract infiltrates around bile ducts consisted of $CD3^+$ T-lymphocytes, some expressing MHC class II antigen, a sign of activation.

Induction of experimental graft-versus-host disease across minor histocompatibility antigens in the mouse results in a severe involvement of both intrahepatic and extrahepatic bile ducts, with features of nonsuppurative cholangitis (Ueno et al., 1998; Vierling et al., 1989) 2–3 months post-transplantation, which persists long term.

Intra-arterial infusion of floxuridine in dogs or rhesus monkeys causes fibrous inflammation and diffuse focal stricturing of intra- and/or extrahepatic bile ducts (Dikengil et al., 1986). Similarly, infusion of ethanol into the hepatic arteries of rhesus monkeys induces diffuse focal strictures and dilatation of intrahepatic bile ducts, lymphocytic infiltration of the portal tracts, and portal fibrosis (Doppman and Girton, 1984), with cholangiographic features similar to those of human SC. These experiments corroborate the clinical observations in humans that direct injury of the hepatic arterial branches or capillary plexi can trigger mechanisms leading to SC, as seen in periarteritis nodosa of the hepatic artery (Le Thi Huong et al., 1989) or in the bile duct damage after infusion of floxuridine into the hepatic artery (Ludwig et al., 1989).

Hepatobiliary injury has been induced in female Lewis rats by portal vein injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS), a chemical hapten that binds to lysine residues and facilitates immune responses against haptenated proteins. The histopathology is characterized by periportal inflammation and bile ductular proliferation. In addition, ANCA with perinuclear and cytoplasmic staining is observed in 40% of the rats (Orth et al., 1999).

Toxic Intraluminal Injury of the Biliary Tract

In both male Sprague-Dawley rats (Mourelle et al., 1995) and in female Lewis rats (Orth et al., 2000) retrograde instillation of TNBS into the distal biliary tract stimulates inflammatory and immunologic events culminating in portal tract inflammation and fibrosis, cytokine production, diffuse focal stricturing of the intra- and extrahepatic bile ducts, and development of ANCA and SMA reactivities.

Histologic and cholangiographic features suggestive of SC are seen in male Sprague-Dawley rats fed with α-naphthylisothiocyanate (ANIT), a substance toxic to intrahepatic bile ducts (Lichtman et al., 1995; Tjandra et al., 2000). Portal tract inflammatory infiltrate with macrophages and lymphocytes appears by day 4, progression of portal inflammation by day 7, and extensive fibrosis by day 14.

Summary

While no animal model fulfils the attributes of an ideal primary SC model, those induced by bacterial cell-wall components and biliary infusion of the hapten TNBS exhibit several characteristics of primary SC and recapitulate the strong association between primary SC and IBD and the production of pANCA.

PATHOGENIC MECHANISMS

Primary SC is associated with a variety of immunologic changes. Early findings showed that T cells were decreased in the circulation but increased in the portal tract (Whiteside et al., 1985; Lindor et al., 1987; Snook et al., 1989) and that the $CD4^+/CD8^+$ lymphocytes ratio and the B-lymphocytes in the circulation were increased (Lindor, 1997). A sensitization to biliary antigens and enhanced expression of class II antigens on biliary epithelial cells were described (McFarlane et al., 1979; Chapman et al., 1988; Broome et al., 1990). The relevance of these mainly descriptive findings has been questioned. For example, the mere ligation of bile ducts in rats enhances the expression of class II antigens on bile-duct cells (Hu et al., 1996). The strong associ-

ation between primary SC and IBD has led to the hypothesis that the initial insult in primary SC is the reaction of a genetically susceptible host to bacterial cell-wall products entering the portal circulation through a permeable gut wall, either due to colitis or during episodes of intestinal infection (Vierling, 1998). This may lead to activation of Kupffer cells in the liver, with the consequent production of peribiliary cytokines and chemokines that attract inflammatory cells. As a result of this peribiliary inflammatory process, concentric fibrosis around the bile ducts would ensue, with consequent ischemia and atrophy of the biliary epithelial cells. Bile duct loss would then lead to progressive cholestasis, and eventually to biliary cirrhosis. Primary SC, however, exists also in the absence of IBD, may present several years before IBD or after colectomy, and colectomy does not alter the course of the disease (Cangemi et al., 1989; Fausa et al., 1991).

An interesting recent hypothesis, supported by some experimental evidence, proposes a role for an enterohepatic circulation of lymphocytes, whereby intestinal lymphocytes produced in the gut mucosa during active inflammation recirculate as memory cells through the liver (Grant et al., 2002). Activated intestinal lymphocytes express $\alpha 4\beta 7$, a gut-specific homing receptor that binds its coreceptor mucosal addressin cell adhesion molecule-1 (MAdCAM-1), which is gut specific. The liver endothelial cells in primary SC express aberrantly MAdCAM-1, providing a docking station for lymphocytes originating from the gut. Thus, it would not be the microorganism or product thereof that triggered the immune-mediated liver inflammation of primary SC, but memory T cells activated in the gut and capable of microbe/self cross-reactivity. This process would initiate in the colon, since primary SC is associated with UC and Crohn's colitis. The long life of memory T cells would explain why the disease may occur a long time after colectomy and why colectomy does not affect the course of primary SC. This hypothesis is attractive, but does not explain why primary SC can exist without IBD, though subclinical intestinal inflammation may be sufficient to trigger the process.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

Patients with primary SC often have elevated levels of IgG and IgM and circulating autoantibodies, in particular ANA, SMA, and ANCA. The relevance of these autoantibodies in the diagnosis of primary SC is a matter of debate. The presence of ANA has been described in 7–77% (Wiesner and LaRusso, 1980; Chapman 1986; Zauli et al., 1987; Klein et al., 1991; Gur et al., 1995; Angulo and Lindor, 1999), SMA in 13–20% (Wiesner and LaRusso, 1980; Chapman et al., 1986; Klein et al., 1991), and ANCA in up to 88% (Cullen and Chapman, 2003) of the patients. In the juvenile form of autoimmune SC, 97% of the patients are positive for ANA and/or SMA (Gregorio et al., 2001). The variability of the reported prevalences is almost certainly due to the lack of standardization in autoantibody detection. It would be advisable to apply to primary SC the same guidelines used in the diagnosis of AIH, where ANA and SMA are considered positive at a titer of more than 1:40 in adults (>1 : 20 in children) when tested on frozen composite sections of rodent liver, kidney, and stomach (Johnson and McFarlane, 1993; Alvarez et al., 1999).

Antineutrophil cytoplasmic antibodies are a fairly consistent feature of primary SC, but are also detected in a high proportion of patients with other autoimmune diseases, in particular UC (Frenzer et al., 1998) and type 1 AIH (Targan et al., 1995; Zauli et al., 1997). The ANCA associated with primary SC and AIH type 1 are distinct from cytoplasmic ANCA (c-ANCA) and classical perinuclear ANCA (pANCA) (Terjung and Worman, 2000), which are diagnostic seromarkers for Wegener granulomatosis and microscopic polyangitis, respectively. Primary SC, UC, and AIH are associated with "atypical pANCA," which have a distinct staining pattern on indirect immunofluorescence microscopy (Terjung et al., 2001). Recent evidence indicates that the target of "atypical pANCA" is located within the nuclear membrane, and for this reason it has been proposed to rename it perinuclear antineutrophil nuclear antibody (p-ANNA) (Terjung et al., 2000).

TREATMENT AND OUTCOME

Several choleretic, immunosuppressive, and antifibrotic agents have been used to treat adult primary SC, but no drug has been shown to alter its natural history. Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that stabilizes liver cell membranes exposed to toxic concentrations of the naturally occurring chenodeoxycholic acid. A prospective randomized double-blind placebo-controlled trial in adult patients with primary SC showed an improvement in laboratory tests in patients receiving 13–15 mg/kg/day UDCA, but this was not accompanied by long-term beneficial changes (Lindor, 1997). It has been suggested (Mitchell et al., 2001a), and some evidence provided (Harnois et al., 2001), that higher doses of UDCA (25–30 mg/kg/day) can be of benefit.

Despite anecdotal reports of improvement with glucocorticoids and azathioprine in adult patients with primary SC (Myers et al., 1970; Javett, 1971), their use is controversial. Other drugs that have not been found effective include penicillamine, methotrexate, colchicine, cyclosporine, cholestyramine, nicotine, and pentoxifylline. Tacrolimus was reported to improve liver function tests in a small pilot study, which, however, was uncontrolled, of short

duration, and lacked histologic and cholangiographic data (Van Thiel et al., 1995).

In the juvenile form of SC, which has strong autoimmune features, treatment with steroids and azathioprine akin to that used in AIH, has been reported to be beneficial in abating the parenchymal inflammatory lesion, but to be less effective in controlling the bile-duct disease (Gregorio et al., 2001). The positive response to immunosuppression observed in these young patients contrasts with the disappointing results reported in adult primary SC, possibly because the latter present with a much more advanced disease.

The median reported survival in adult patients with primary SC is 10–12 years from diagnosis (Wiesner et al., 1989; Farrant et al., 1991). The disease runs a progressive course, and most asymptomatic patients eventually develop symptoms of chronic cholestasis and biliary cirrhosis (Porayko et al., 1990). Older age, high serum bilirubin and AST, low albumin, hepatosplenomegaly, variceal bleeding, presence of IBD, and advanced histologic stage are independent predictors of a poor prognosis (Angulo and Lindor, 1999).

For patients with advanced SC, liver transplantation is the only effective therapeutic option, and, for optimal results, it should be offered when the disease is not too advanced. Current 3-year survival rates are 85–90%. Interestingly, patients transplanted for SC have a significantly higher rate of developing non-anastomotic biliary strictures after surgery (Sheng et al., 1993; Feller et al., 1996). This, together with the frequent finding of histologic features consistent with SC in post-transplant biopsies (Harrison et al., 1994; Narumi et al., 1995), has suggested that the disease can recur in the graft, though diagnosis of recurrence is made difficult by other post-transplant complications that can affect the bile ducts. A definition for recurrent SC has been proposed by the Mayo Clinic (Graziadei et al., 1999) and includes: 1) diagnosis of SC before transplant; 2) cholangiographic features of SC less than 90 days after surgery or histology showing fibrous cholangitis and/or fibro-obliterative lesions with or without ductopenia, biliary fibrosis, or biliary cirrhosis; 3) exclusion of hepatic artery thrombosis, chronic ductopenic rejection, anastomotic stricture, non-anastomotic stricture less than 90 days from surgery, donor/recipient ABO incompatibility. Using this definition, SC recurrence has been reported in 20% of transplanted patients (Graziadei et al., 1999). In general, recurrent SC does not appear to have a major negative impact on medium-term patient or graft survival. The activity of IBD after transplantation is variable, but has been reported to be more severe, especially in units where steroid immunosuppression is withdrawn early (Papatheodoridis et al., 1998; Gow and Chapman et al., 2000). Colorectal cancer affects 5–7% of patients with primary SC after liver transplantation, in particular those with a long history of ulcerative colitis and pan-colitis (Loftus et al., 1998; MacLean et al., 2003; Vera et al., 2003). To prevent this life-threatening complication, annual surveillance and colectomy in selected high-risk patients with longstanding severe colitis have been advocated (Loftus et al., 1998; Gow PJ, Chapman, 2000; Vera et al., 2003).

CONCLUDING REMARKS— FUTURE PROSPECTS

There is a large body of evidence suggesting that primary SC is an immune-mediated disease. Yet, the consensus among physicians is that adult primary SC does not respond to immunosuppressive treatment. It can be argued that the juvenile form of SC, characterized by unambiguous serologic and histologic features of autoimmunity, and responsive to immunosuppression, represents the early stage of adult primary SC. In contrast to juvenile SC, adult primary SC has an insidious onset, being often completely asymptomatic, and could represent a late "burnt out" stage of the same condition, too advanced to respond to treatment. The discrepancy in sex distribution between juvenile SC, which is slightly more common in females, and adult primary SC, which has a male preponderance, could be due to the fact that the disease is more progressive in males. A plausible alternative is that the course of the disease, more aggressive and symptomatic in young females frequently diagnosed as having AIH, is favorably modified by early immunosuppressive treatment.

Both SC and AIH could lie within the spectrum of the same disease process. Future studies should determine how frequently AIH evolves to SC and what is the ultimate outcome of juvenile SC treated with immunosuppression. The composition of the mononuclear cell infiltrate should be analyzed in both AIH and SC, and the possible intestinal origin of the liver-infiltrating lymphocytes investigated. In view of the strong association of SC with UC, a condition deemed to have a T-helper 2 profile (Bouma and Strober, 2003), the T-helper profile of adult primary SC, juvenile SC, and AIH should be determined. Moreover, regulatory T cells that are key to preventing manifestations of autoimmunity should be analyzed, also in view of an early report indicating that $CD4^+CD25^+$ regulatory T-cells are decreased in both juvenile SC and AIH (Longhi et al., 2004).

References

Agarwal, K., Hudson, M., and Harnson, P. (2000). No association between primary sclerosing cholangitis (PSC) and CTLA-4 gene polymorphism: evidence against an autoimmune aetiology? *J. Hepatol. 32,* 173A.

Alvarez, F., Berg, P.A., Bianchi, F.B., Bianchi, L., Burroughs, A.K., Cancado, E.L., Chapman, R.W., Cooksley, W.G., Czaja, A.J., Desmet,

V.J., Donaldson, P.T., Eddleston, A.L., Fainboim, L., Heathcote, J., Homberg, J.C., Hoofnagle, J.H., Kakumu, S., Krawitt, E.L., Mackay, I.R., MacSween, R.N., Maddrey, W.C., Manns, M.P., McFarlane, I.G., Meyer zum Buschenfelde, K.H., and Zeniya, M. (1999) International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol. 31*(5), 929–938.

Angulo, P. and Lindor, K.D. (1999). Primary sclerosing cholangitis. *Hepatology 30*(1), 325–332.

Angulo, P., Peter, J.B., Gershwin, M.E., DeSotel, C.K., Shoenfeld, Y., Ahmed, A.E., and Lindor, K.D. (2000). Serum autoantibodies in patients with primary sclerosing cholangitis. *J. Hepatol. 32*(2), 182–187.

Balasubramaniam, K., Wiesner, R.H., and LaRusso, N.F. (1988). Primary sclerosing cholangitis with normal serum alkaline phosphatase activity. *Gastroenterology 95*(5), 1395–1358.

Bambha, K., Kim, W.R., Talwalkar, J., Torgerson, H., Benson, J.T., Therneau, T.M., Loftus, E.V., Jr., Yawn, B.P., Dickson, E.R., and Melton, L.J., 3rd. (2003). Incidence, clinical spectrum, and outcomes of primary sclerosing cholangitis in a United States community. *Gastroenterology 125*(5), 1364–1369.

Bernal, W., Moloney, M., Underhill, J., and Donaldson, P.T. (1999). Association of tumor necrosis factor polymorphism with primary sclerosing cholangitis. *J. Hepatol. 30*(2), 237–241.

Boberg, K.M., Fausa, O., Haaland, T., Holter, E., Mellbye, O.J., Spurkland, A., and Schrumpf, E. (1996). Features of autoimmune hepatitis in primary sclerosing cholangitis: an evaluation of 114 primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. *Hepatology 23*(6), 1369–1376.

Boberg, K.M., Aadland, E., Jahnsen, J., Raknerud, N., Stiris M., and Bell, H. (1998). Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand. J. Gastroenterol. 33*(1), 99–103.

Boden, R.W., Rankin, J.G., Goulston, S.J., and Morrow, W. (1959). The liver in ulcerative colitis; the significance of raised serum-alkaline-phosphatase levels. *Lancet 2,* 245–248.

Bodenheimer, H.C., Jr., LaRusso, N.F., Thayer, W.R., Jr., Charland, C., Staples, P.J., and Ludwig, J. (1983). Elevated circulating immune complexes in primary sclerosing cholangitis. *Hepatology 3*(2), 150–154.

Bouma, G. and Strober, W. (2003). The immunological and genetic basis of inflammatory bowel disease. *Nat. Rev. Immunol. 3*(7), 521–533.

Brooke, B.N., Dykes, P.W., and Walker, F.C. (1961). A study of liver disorder in ulcerative colitis. *Postgrad. Med. J. 37,* 245–251.

Broome, U., Glaumann, H., Hultcrantz, R., and Forsum, U. (1990). Distribution of HLA-DR, HLA-DP, HLA-DQ antigens in liver tissue from patients with primary sclerosing cholangitis. *Scand. J. Gastroenterol. 25*(1), 54–58.

Cangemi, J.R., Wiesner, R.H., Beaver, S.J., Ludwig, J., MacCarty, R.L., Dozois, R.R., Zinsmeister, A.R., and LaRusso, N.F. (1989). Effect of proctocolectomy for chronic ulcerative colitis on the natural history of primary sclerosing cholangitis. *Gastroenterology 96*(3), 790–794.

Carey, J.B. (1964). Bile acids, cirrhosis, and human evolution. *Gastroenterology 46,* 490–493.

Chapman, R. (1995). Does HLA status influence prognosis in primary sclerosing cholangitis? *Gastroenterology 108*(3), 937–940.

Chapman, R.W., Varghese, Z., Gaul, R., Patel, G., Kokinon, N., and Sherlock, S. (1983). Association of primary sclerosing cholangitis with HLA-B8. *Gut 24*(1), 38–41.

Chapman, R.W., Cottone, M., Selby, W.S., Shepherd, H.A., Sherlock, S., and Jewell, D.P. (1986). Serum autoantibodies, ulcerative colitis and primary sclerosing cholangitis. *Gut 27*(1), 86–91.

Chapman, R.W., Kelly, P.M., Heryet, A., Jewell, D.P., and Fleming, K.A. (1988). Expression of HLA-DR antigens on bile duct epithelium in primary sclerosing cholangitis. *Gut 29*(4), 422–427.

Cromartie, W.J., Craddock, J.G., Schwab, J.H., Anderle, S.K., and Yang, C.H. (1977). Arthritis in rats after systemic injection of streptococcal cells or cell walls. *J. Exp. Med. 146*(6), 1585–1602.

Cullen, S. and Chapman, R. (2003). Primary sclerosing cholangitis. *Autoimmun. Rev. 2*(6), 305–312.

Delbet, P. (1924). Retrecissement du choledoque: cholecystoduodenostomie. *Bull. Mem. Soc. Nat. Chir. 50,* 1144–1146.

Dikengil, A., Siskind, B.N., Morse, S.S., Swedlund, A., Bober-Sorcinelli, K.E., and Burrell, M.I. (1986). Sclerosing cholangitis from intraarterial floxuridine. *J. Clin. Gastroenterol. 8*(6), 690–693.

Donaldson, P.T. and Norris, S. (2002). Evaluation of the role of MHC class II alleles, haplotypes and selected amino acid sequences in primary sclerosing cholangitis. *Autoimmunity 35*(8), 555–564.

Donaldson, P.T., Farrant, J.M., Wilkinson, M.L., Hayllar, K., Portmann, B.C., and Williams, R. (1991). Dual association of HLA DR2 and DR3 with primary sclerosing cholangitis. *Hepatology 13*(1), 129–133.

Donaldson, P.T., Norris, S., Constantini, P.K., Bernal, W., Harrison, P., and Williams, R. (2000). The interleukin-1 and interleukin-10 gene polymorphisms in primary sclerosing cholangitis: no associations with disease susceptibility/resistance. *J. Hepatol. 32*(6), 882–886.

Doppman, J.L. and Girton, M.E. (1984). Bile duct scarring following ethanol embolization of the hepatic artery: an experimental study in monkeys. *Radiology 152*(3), 621–626.

Farrant, J.M., Hayllar, K.M., Wilkinson, M.L., Karani, J., Portmann, B.C., Westaby, D., and Williams, R. (1991). Natural history and prognostic variables in primary sclerosing cholangitis. *Gastroenterology 100*(6), 1710–1717.

Farrant, J.M., Doherty, D.G., Donaldson, P.T., Vaughan, R.W., Hayllar, K.M., Welsh, K.I., Eddleston, A.L., and Williams, R. (1992). Amino acid substitutions at position 38 of the DR beta polypeptide confer susceptibility to and protection from primary sclerosing cholangitis. *Hepatology 16*(2), 390–395.

Fausa, O., Schrumpf, E., and Elgjo, K. (1991). Relationship of inflammatory bowel disease and primary sclerosing cholangitis. *Semin. Liver Dis. 11*(1), 31–39.

Feller, R.B., Waugh, R.C., Selby, W.S., Dolan, P.M., Sheil, A.G., McCaughan, G.W. (1996). Biliary strictures after liver transplantation: clinical picture, correlates and outcomes. *J. Gastroenterol. Hepatol. 11*(1), 21–25.

Finegold, M.J. and Carpenter, R.J. (1982). Obliterative cholangitis due to cytomegalovirus: a possible precursor of paucity of intrahepatic bile ducts. *Hum. Pathol. 13*(7), 662–665.

Fox, J.G., Dewhirst, F.E., Shen, Z., Feng, Y., Taylor, N.S., Paster, B.J., Ericson, R.L., Lau, C.N., Correa, P., Araya, J.C., and Roa, I. (1998). Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology 114*(4), 755–763.

Frenzer, A., Fierz, W., Rundler, E., Hammer, B., and Binek, J. (1998). Atypical, cytoplasmic and perinuclear anti-neutrophil cytoplasmic antibodies in patients with inflammatory bowel disease. *J. Gastroenterol. Hepatol. 13*(9), 950–954.

Gow, P.J. and Chapman, R.W. (2000). Liver transplantation for primary sclerosing cholangitis. *Liver 20*(2), 97–103.

Grant, A.J., Lalor, P.F., Salmi, M., Jalkanen, S., and Adams, D.H. (2002). Homing of mucosal lymphocytes to the liver in the pathogenesis of hepatic complications of inflammatory bowel disease. *Lancet 359*(9301), 150–157.

Graziadei, I.W., Wiesner, R.H., Marotta, P.J., Porayko, M.K., Hay, J.E., Charlton, M.R., Poterucha, J.J., Rosen, C.B., Gores, G.J., LaRusso, N.F., and Krom, R.A. (1999). Long-term results of patients undergoing liver transplantation for primary sclerosing cholangitis. *Hepatology 30*(5), 1121–1127.

Gregorio, G.V., Portmann, B., Karani, J., Harrison P., Donaldson, P.T., Vergani, D., et al. (2001). Autoimmune hepatitis/sclerosing cholangitis

overlap syndrome in childhood: a 16-year prospective study. *Hepatology 33*(3), 544–553.

Gur, H., Shen, G., Sutjita, M., Terrberry, J., Alosachie, I., Barka, N., Lin, H.C., Peter, J.B., Meroni, P.L., and Kaplan, M. (1995). Autoantibody profile of primary sclerosing cholangitis. *Pathobiology 63*(2), 76–82.

Harnois, D.M., Angulo, P., Jorgensen, R.A., Larusso, N.F., and Lindor, K.D. (2001). High-dose ursodeoxycholic acid as a therapy for patients with primary sclerosing cholangitis. *Am. J. Gastroenterol. 96*(5), 1558–1562.

Harrison, R.F., Davies, M.H., Neuberger, J.M., and Hubscher, S.G. (1994). Fibrous and obliterative cholangitis in liver allografts: evidence of recurrent primary sclerosing cholangitis? *Hepatology 20*(2), 356–361.

Holubitsky, I.B. and McKenzie, A.D. (1964). Primary sclerosing cholangitis of the extrahepatic bile ducts. *Can. J. Surg. 115,* 277–283.

Hu, W., Blazar, B.R., Manivel, J.C., Paradis, K., and Sharp, H.L. (1996). Phenotypical and functional characterization of intrahepatic bile duct cells from common duct ligated mice. *J. Lab. Clin. Med. 128*(6), 536–544.

Javett, S.L. (1971). Azathioprine in primary sclerosing cholangitis. *Lancet 1*(7703), 810.

Johnson, P.J. and McFarlane, I.G. (1993). Meeting report: International Autoimmune Hepatitis Group. *Hepatology 18*(4), 998–1005.

Judd, E. (1926). Stricture of the common bile duct. *Ann. Surg. 84,* 404.

Kaplan, M.M. (1991). Medical approaches to primary sclerosing cholangitis. *Semin. Liver Dis. 11*(1), 56–63.

Klein, R., Eisenburg, J., Weber, P., Seibold, F., and Berg, P.A. (1991). Significance and specificity of antibodies to neutrophils detected by western blotting for the serological diagnosis of primary sclerosing cholangitis. *Hepatology 14*(6), 1147–1152.

Kuroe, K., Haga, Y., Funakoshi, O., Mizuki, I., Kanazawa, K., and Yoshida, Y. (1996a). Extraintestinal manifestations of granulomatous enterocolitis induced in rabbits by long-term submucosal administration of muramyl dipeptide emulsified with Freund's incomplete adjuvant. *J. Gastroenterol. 31*(2), 199–206.

Kuroe, K., Haga, Y., Funakoshi, O., Kanazawa, K., Mizuki, I., and Yoshida, Y. (1996b). Pericholangitis in a rabbit colitis model induced by injection of muramyl dipeptide emulsified with a long-chain fatty acid. *J. Gastroenterol. 31*(3), 347–352.

Lafourcade, R. (1925). Deux observations d'obliteration cicatricielle du choledoque. Anastomose laterale entre le choledoque et le duodenum dans le premier cas. Recontruction par prothese avec tube de caoutchouc dans le second. *Bull. Mem. Soc. Nat. Chir. 51,* 828–831.

Lawrence, S.P., Sherman, K.E., Lawson, J.M., and Goodman, Z.D. (1994). A 39 year old man with chronic hepatitis. *Semin. Liver Dis. 14*(1), 97–105.

Le Thi Huong, D., de Gennes, C., Guillevin, L., Wechsler, B., Etienne, S.D., Bletry, O., and Godeau, P. (1989). [The liver and periarteritis nodosa]. *Gastroenterol. Clin. Biol. 13*(2), 141–148.

Lee, Y.M. and Kaplan, M.M. (1995). Primary sclerosing cholangitis. *N. Engl. J. Med. 332*(14), 924–933.

Lichtman, S.N. and Sartor, R.B. (1991). Hepatobiliary injury associated with experimental small-bowel bacterial overgrowth in rats. *Immunol. Res. 10*(3–4), 528–531.

Lichtman, S.N., Sartor, R.B., Keku, J., and Schwab, J.H. (1990). Hepatic inflammation in rats with experimental small intestinal bacterial overgrowth. *Gastroenterology 98*(2), 414–423.

Lichtman, S.N., Keku, J., Clark, R.L., Schwab, J.H., and Sartor, R.B. (1991a). Biliary tract disease in rats with experimental small bowel bacterial overgrowth. *Hepatology 13*(4), 766–772.

Lichtman, S.N., Keku, J., Schwab, J.H., and Sartor, R.B. (1991b). Evidence for peptidoglycan absorption in rats with experimental small bowel bacterial overgrowth. *Infect. Immun. 59*(2), 555–562.

Lichtman, S.N., Keku, J., Schwab, J.H., and Sartor, R.B. (1991c). Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. *Gastroenterology 100*(2), 513–519.

Lichtman, S.N., Okoruwa, E.E., Keku, J., Schwab, J.H., and Sartor, R.B. (1992). Degradation of endogenous bacterial cell wall polymers by the muralytic enzyme mutanolysin prevents hepatobiliary injury in genetically susceptible rats with experimental intestinal bacterial overgrowth. *J. Clin. Invest. 90*(4), 1313–1322.

Lichtman, S.N., Wang, J., and Clark, R.L. (1995). A microcholangiographic study of liver disease models in rats. *Acad. Radiol. 2*(6), 515–521.

Lindor, K.D. (1997). Ursodiol for primary sclerosing cholangitis. Mayo Primary Sclerosing Cholangitis-Ursodeoxycholic Acid Study Group. *N. Engl. J. Med. 336*(10), 691–695.

Lindor, K.D., Wiesner, R.H., Katzmann, J.A., LaRusso, N.F., and Beaver, S.J. (1987). Lymphocyte subsets in primary sclerosing cholangitis. *Dig. Dis. Sci. 32*(7), 720–725.

Lister, T. (1899). A specimen of diffuse ulcerative colitis with secondary diffuse hepatitis. *Trans. Pathol. Soc. Lon. 50,* 130–134.

Loftus, E.V., Jr., Aguilar, H.I., Sandborn, W.J., Tremaine, W.J., and Krom, R.A., Zinsmeister, A.R., Graziadei, I.W., and Wiesner, R.H. (1998). Risk of colorectal neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis following orthotopic liver transplantation. *Hepatology 27*(3), 685–690.

Longhi, M.S., Ma, Y., Bogdanos, D.P., Cheeseman, P., Mieli-Vergani, G., and Vergani, D. (2004). Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. *J. Hepatol. 41*(1), 31–37.

Ludwig, J., La Russo, N., and Wiesner, R.H. (1986). Primary sclerosing cholangitis. In R.L. Peters and J.R. Craig (eds.), *Contemporary views in surgical pathology.* New York: Churchill Livingstone.

Ludwig, J., Kim C.H., Wiesner, R.H., and Krom, R.A. (1989). Floxuridine-induced sclerosing cholangitis: an ischemic cholangiopathy? *Hepatology 9*(2), 215–218.

MacLean, A.R., Lilly, L., Cohen, Z., O'Connor, B., and McLeod, R.S. (2003). Outcome of patients undergoing liver transplantation for primary sclerosing cholangitis. *Dis. Colon Rectum 46*(8), 1124–1128.

Mandal, A., Dasgupta, A., Jeffers, L., Squillante, L., Hyder, S., Reddy, R., et al. (1994). Autoantibodies in sclerosing cholangitis against a shared peptide in biliary and colon epithelium. *Gastroenterology 106*(1), 185–192.

McFarlane, I.G., Wojcicka, B.M., Tsantoulas, D.C., Portmann, B.C., Eddleston, A.L., and Williams, R. (1979). Leukocyte migration inhibition in response to biliary antigens in primary biliary cirrhosis, sclerosing cholangitis, and other chronic liver diseases. *Gastroenterology 76*(6), 1333–1340.

Mehal, W.Z., Lo, Y.M., Wordsworth, B.P., Neuberger, J.M., Hubscher, S.C., Fleming, K.A., and Chapman, R.W. (1994). HLA DR4 is a marker for rapid disease progression in primary sclerosing cholangitis. *Gastroenterology 106*(1), 160–167.

Miller, R. (1927). Benign stricture of common bile duct. *Ann. Surg. 86,* 296–303.

Minuk, G.Y., Angus, M., Brickman, C.M., Lawley, T.J., Frank, M.M., Hoofnagle, J.H., and Jones, E.A. (1985). Abnormal clearance of immune complexes from the circulation of patients with primary sclerosing cholangitis. *Gastroenterology 88*(1 Pt 1), 166–170.

Mitchell, S.A., Bansi, D.S., Hunt, N., Von Bergmann, K., Fleming, K.A., and Chapman, R.W. (2001a). A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. *Gastroenterology 121*(4), 900–907.

Mitchell, S.A., Grove, J., Spurkland, A., Boberg, K.M., Fleming, K.A., Day, C.P., Schrumpf, E., and Chapman, R.W. (2001b). Association of the tumour necrosis factor alpha -308 but not the interleukin 10 -627 promoter polymorphism with genetic susceptibility to primary sclerosing cholangitis. *Gut 49*(2), 288–294.

Moloney, M.M., Thomson, L.J., Strettell, M.J., Williams, R., and Donaldson, P.T. (1998). Human leukocyte antigen-C genes and

susceptibility to primary sclerosing cholangitis. *Hepatology 28*(3), 660–662.

Mourelle, M., Salas A., Vilaseca, J., Guarner, F., and Malagelada, J.R. (1995). Induction of chronic cholangitis in the rat by trinitrobenzenesulfonic acid. *J. Hepatol. 22*(2), 219–225.

Myers, R.N., Cooper, J.H., and Padis, N. (1970). Primary sclerosing cholangitis. Complete gross and histologic reversal after long-term steroid therapy. *Am. J. Gastroenterol. 53*(6), 527–538.

Narumi, S., Roberts, J.P., Emond, J.C., Lake, J., and Ascher, N.L. (1995). Liver transplantation for sclerosing cholangitis. *Hepatology 22*(2), 451–457.

Nepom, G.T. (1990). A unified hypothesis for the complex genetics of HLA associations with IDDM. *Diabetes 39*(10), 1153–1157.

Norris, S., Kondeatis, E., Collins, R., Satsangi, J., Clare, M., Chapman, R., Stephens, H., Harrison, P., Vaughan, R., and Donaldson, P. (2001). Mapping MHC-encoded susceptibility and resistance in primary sclerosing cholangitis: the role of MICA polymorphism. *Gastroenterology 120*(6), 1475–1482.

Olerup, O., Olsson, R., Hultcrantz, R., and Broome, U. (1995). HLA-DR and HLA-DQ are not markers for rapid disease progression in primary sclerosing cholangitis. *Gastroenterology 108*(3), 870–878.

Olsson, R., Danielsson, A., Jarnerot, G., Lindstrom, E., Loof, L., Rolny, P., Ryden, B.O., Tysk, C., and Wallerstedt, S. (1991). Prevalence of primary sclerosing cholangitis in patients with ulcerative colitis. *Gastroenterology 100*(5 Pt 1), 1319–1323.

Orth, T., Neurath, M., Schirmacher, P., Treichel, U., Meyer zum Buschenfelde, K.H., and Mayet, W. (1999). Anti-neutrophil cytoplasmic antibodies in a rat model of trinitrobenzenesulphonic acid-induced liver injury. *Eur. J. Clin. Invest. 29*(11), 929–939.

Orth, T., Neurath, M., Schirmacher, P., Galle, P.R., and Mayet, W.J. (2000). A novel rat model of chronic fibrosing cholangitis induced by local administration of a hapten reagent into the dilated bile duct is associated with increased TNF-alpha production and autoantibodies. *J. Hepatol. 33*(6), 862–872.

Palmer, R.H. (1972). Bile acids, liver injury, and liver disease. *Arch. Intern. Med. 130*(4), 606–617.

Papatheodoridis, G.V., Hamilton, M., Mistry, P.K., Davidson, B., Rolles, K., and Burroughs, A.K. (1998). Ulcerative colitis has an aggressive course after orthotopic liver transplantation for primary sclerosing cholangitis. *Gut 43*(5), 639–644.

Ponsioen, C.Y., Defoer, J., Ten Kate, F.J., Weverling, G.J., Tytgat, G.N., Pannekoek, Y., and Wertheim-Dillen, P.M. (2002). A survey of infectious agents as risk factors for primary sclerosing cholangitis: are Chlamydia species involved? *Eur. J. Gastroenterol. Hepatol. 14*(6), 641–648.

Porayko, M.K., Wiesner, R.H., LaRusso, N.F., Ludwig, J., MacCarty, R.L., Steiner, B.L., Twomey, C.K., and Zinsmeister, A.R. (1990). Patients with asymptomatic primary sclerosing cholangitis frequently have progressive disease. *Gastroenterology 98*(6), 1594–1602.

Quigley, E.M., LaRusso, N.F., Ludwig, J., MacSween, R.N., Birnie, G.G., and Watkinson, G. (1983). Familial occurrence of primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology 85*(5), 1160–1165.

Rabinovitz, M., Gavaler, J.S., Schade, R.R., Dindzans, V.J., Chien, M.C., and Van Thiel, D.H. (1990). Does primary sclerosing cholangitis occurring in association with inflammatory bowel disease differ from that occurring in the absence of inflammatory bowel disease? A study of sixty-six subjects. *Hepatology 11*(1), 7–11.

Rabinovitz, M., Demetris, A.J., Bou-Abboud, C.F., and Van Thiel, D.H. (1992). Simultaneous occurrence of primary sclerosing cholangitis and autoimmune chronic active hepatitis in a patient with ulcerative colitis. *Dig. Dis. Sci. 37*(10), 1606–1611.

Saarinen, S., Broome, U., and Ligers, A. (1998). CTLA-4 gene polymorphism associated with primary sclerosing cholangitis (PSC). *J. Hepatol. 28,* 125A.

Sartor, R.B., Rath, H.C., Lichtman, S.N., and van Tol, E.A. (1996). Animal models of intestinal and joint inflammation. *Baillières Clin. Rheumatol. 10*(1), 55–76.

Satsangi, J., Chapman, R.W., Haldar, N., Donaldson, P., Mitchell, S., Simmons, J., Norris, S., Marshall, S.E., Bell, J.I., Jewell, D.P., and Welsh, K.I. (2001). A functional polymorphism of the stromelysin gene (MMP–3) influences susceptibility to primary sclerosing cholangitis. *Gastroenterology 121*(1), 124–130.

Schrumpf, E., Fausa, O., Forre, O., Dobloug, J.H., Ritland, S., and Thorsby, E. (1982). HLA antigens and immunoregulatory T cells in ulcerative colitis associated with hepatobiliary disease. *Scand. J. Gastroenterol. 17*(2), 187–191.

Schrumpf, E. and Boberg, K.M. (2001). Epidemiology of primary sclerosing cholangitis. *Best Pract. Res. Clin. Gastroenterol. 15*(4), 553–562.

Senaldi, G., Donaldson, P.T., Magrin, S., Farrant, J.M., Alexander, G.J., Vergani, D., and Williams, R. (1989). Activation of the complement system in primary sclerosing cholangitis. *Gastroenterology 97*(6), 1430–1434.

Shepherd, H.A., Selby, W.S., Chapman, R.W., Nolan, D., Barbatis, C., McGee, J.O., and Jewell, D.P. (1983). Ulcerative colitis and persistent liver dysfunction. *Quart. J. Med. 52*(208), 503–513.

Sheng, R., Zajko, A.B., Campbell, W.L., and Abu-Elmagd, K. (1993). Biliary strictures in hepatic transplants: prevalence and types in patients with primary sclerosing cholangitis vs those with other liver diseases. *AJR Am. J. Roentgenol. 161*(2), 297–300.

Siegel, J.H., Barnes, S., and Morris, J.S. (1977). Bile acids in liver disease associated with inflammatory bowel disease. *Digestion 15*(6), 469–481.

Snook, J.A., Chapman, R.W., Sachdev, G.K., Heryet, A., Kelly, P.M., Fleming, K.A., and Jewell, D.P. (1989). Peripheral blood and portal tract lymphocyte populations in primary sclerosing cholangitis. *J. Hepatol. 9*(1), 36–41.

Spurkland, A., Saarinen, S., Boberg, K.M., Mitchell, S., Broome, U., Caballeria, L., Ciusani, E., Chapman, R., Ercilla, G., Fausa, O., Knutsen, I., Pares, A., Rosina, F., Olerup, O., Thorsby, E., and Schrumpf, E. (1999). HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. *Tissue Antigens 53*(5), 459–469.

Targan, S.R., Landers, C., Vidrich, A., and Czaja, A.J. (1995). High-titer antineutrophil cytoplasmic antibodies in type-1 autoimmune hepatitis. *Gastroenterology 108*(4), 1159–1166.

Terjung, B. and Worman, H.J. (2000). "ANCA" in liver diseases. In M.P. Manns, P.G. and Leuschner U., (eds.), *Immunology and Liver*, (pp. 137–151). London: Kluwer.

Terjung, B., Spengler, U., Sauerbruch, T., and Worman, H.J. (2000). "Atypical p-ANCA" in IBD and hepatobiliary disorders react with a 50-kilodalton nuclear envelope protein of neutrophils and myeloid cell lines. *Gastroenterology 119*(2), 310–322.

Terjung, B., Worman, H.J., Herzog, V., Sauerbruch, T., and Spengler, U. (2001). Differentiation of antineutrophil nuclear antibodies in inflammatory bowel and autoimmune liver diseases from antineutrophil cytoplasmic antibodies (p-ANCA) using immunofluorescence microscopy. *Clin. Exp. Immunol. 126*(1), 37–46.

Tjandra, K., Sharkey, K.A., and Swain, M.G. (2000). Progressive development of a Th1-type hepatic cytokine profile in rats with experimental cholangitis. *Hepatology 31*(2), 280–290.

Ueno, Y., Phillips, J.O., Ludwig, J., Lichtman, S.N., and LaRusso, N.F. (1996). Development and characterization of a rodent model of immune-mediated cholangitis. *Proc. Natl. Acad. Sci. U.S.A. 93*(1), 216–220.

Ueno, A., Kono, N., Minato, H., and Nakanuma, Y. (1998). Diffuse biliary tract involvement mimicking primary sclerosing cholangitis in an experimental model of chronic graft-versus-host disease in mice. *Pathol. Int. 48*(6), 421–427.

Van Thiel, D.H., Carroll, P., Abu-Elmagd, K., Rodriguez-Rilo, H., Irish, W., McMichael, J., and Starzl, T.E. (1995). Tacrolimus (FK 506), a treatment for primary sclerosing cholangitis: results of an open-label preliminary trial. *Am. J. Gastroenterol. 90*(3), 455–459.

Vera, A., Gunson, B.K., Ussatoff, V., Nightingale, P., Candinas, D., Radley, S., Mayer, A.D., Buckels, J.A., McMaster, P., Neuberger, J., and Mirza, D.F. (2003). Colorectal cancer in patients with inflammatory bowel disease after liver transplantation for primary sclerosing cholangitis. *Transplantation 75*(12), 1983–1988.

Vierling, J.M. (1998). Aetiopathogenesis of primary sclerosing cholangitis. In M.P. Manns, A. Stieihl, and R.H. Wiesner (eds.), *Primary sclerosis cholangitis* (p. 9). London: Kluwer Academic Publishers.

Vierling, J.M., Ruderman, W.B., Jaffee, B.D., Fennell, R.H., Jr., and Claman, H.N. (1989). Hepatic lesions in murine chronic graft-versus-host disease to minor histocompatibility antigens. A reproducible model of nonsuppurative destructive cholangitis. *Transplantation 48*(4), 717–718.

Warren, K.W., Athanassiades, S., and Monge, J.I. (1966). Primary sclerosing cholangitis. A study of forty-two cases. *Am. J. Surg. 111*(1), 23–38.

Wee, A. and Ludwig, J. (1985). Pericholangitis in chronic ulcerative colitis: primary sclerosing cholangitis of the small bile ducts? *Ann. Intern. Med. 102*(5), 581–587.

Whiteside, T.L., Lasky, S., Si L., and Van Thiel, D.H. (1985). Immunologic analysis of mononuclear cells in liver tissues and blood of patients with primary sclerosing cholangitis. *Hepatology 5*(3), 468–474.

Wiencke, K., Spurkland, A., Schrumpf, E., and Boberg, K.M. (2001). Primary sclerosing cholangitis is associated to an extended B8-DR3 haplotype including particular MICA and MICB alleles. *Hepatology 34*(4 Pt 1), 625–630.

Wiesner, R.H. and LaRusso, N.F. (1980). Clinicopathologic features of the syndrome of primary sclerosing cholangitis. *Gastroenterology 79*(2), 200–206.

Wiesner, R.H., Grambsch, P.M., Dickson, E.R., Ludwig, J., MacCarty, R.L., Hunter, E.B., Fleming, T.R., Fisher, L.D., Beaver, S.J., and LaRusso, N.F. (1989). Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology 10*(4), 430–436.

Yamada, S., Ishii M., Liang, L.S., Yamamoto, T., and Toyota, T. (1994). Small duct cholangitis induced by N-formyl L-methionine L-leucine L-tyrosine in rats. *J. Gastroenterol. 29*(5), 631–636.

Yamada, S., Ishii, M., Kisara, N., Nagatomi, R., and Toyota, T. (1999). Macrophages are essential for lymphocyte infiltration in formyl peptide-induced cholangitis in rat liver. *Liver 19*(3), 253–258.

Zauli, D., Schrumpf, E., Crespi, C., Cassani, F., Fausa, O., and Aadland, E. (1987). An autoantibody profile in primary sclerosing cholangitis. *J. Hepatol. 5*(1), 14–18.

Zauli, D., Ghetti, S., Grassi, A., Descovich, C., Cassani, F., Ballardini, G., Muratori, L., and Bianchi, F.B. (1997). Anti-neutrophil cytoplasmic antibodies in type 1 and 2 autoimmune hepatitis. *Hepatology 25*(5), 1105–1107.

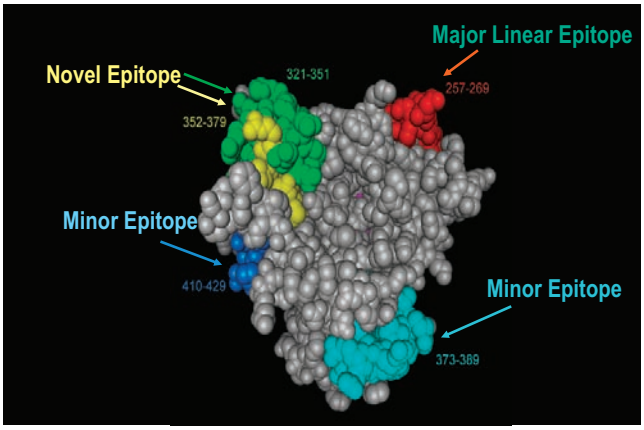


FIGURE 53.3 A three dimensional representation of cytochrome P450 2D6 showing various epitope regions. A major epitope region *(red)* at amino acids 257–269 is a reactant for autoantibodies in autoimmune hepatitis (AIH) type 2, and it has sequence homology with the intermediate-early protein IE 175 of herpes simplex virus type 1 (Manns et al., 1991). An epitope region *(green/yellow)* is a reactant for autoantibodies in AIH type 2 and chronic hepatitis C. Minor epitopes at amino acids 373–389 and amino acids 410–429 are shown in light blue and dark blue, respectively.

From Sigimura et al. (2002), and reproduced with permission from *Autoimmunity*.

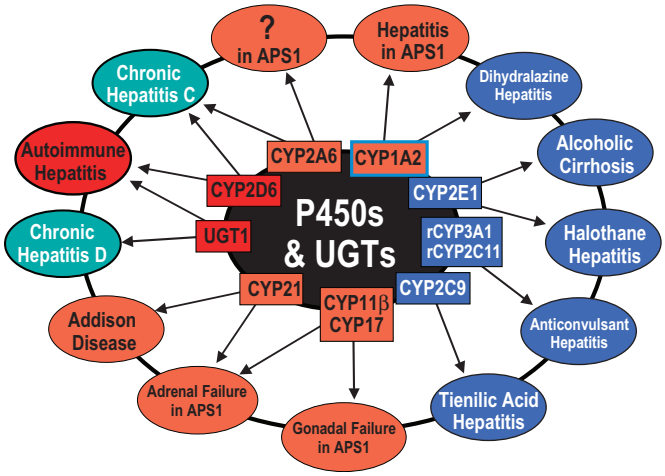


FIGURE 53.4 Cytochrome P450 isoforms (CYPs) and uridine-5′-diphosphate glucuronosyl transferase 1 (UGT1) as autoantigenic targets in various types of hepatitis occurring in spontaneous autoimmune hepatitis (AIH) type 2 *(red)*, or associated with drug reactions *(blue)* or hepatitis virus infection *(green)*, or other spontaneous autoimmune conditions *(orange)*. (APS1, autoimmune polyendocrine syndrome type 1.)

From Manns and Obermeyer-Straub (1997) and reproduced with permisstion from *Hepatology*.

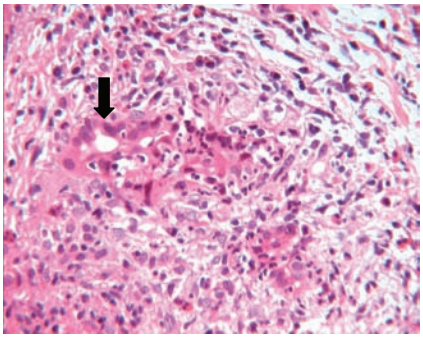


FIGURE 55.2 Portal tract inflammatory cell infiltrate in a case of juvenile sclerosing cholangitis positive for anti-nuclear and anti-smooth muscle antibodies. Plasma cells and lymphocytes cross the limiting plate invading the parenchyma (interface hepatitis). The arrow points to a damaged bile duct.

CHAPTER

56

Pancreatitis

SHIGEYUKI KAWA, HIDEAKI HAMANO, AND KENDO KIYOSAWA

Department of Medicine, Gastroenterology, Shinshu University School of Medicine, Matsumoto, Japan

A unique form of chronic pancreatitis characterized by elderly male preponderance, minimal abdominal pain, irregular narrowing of the pancreatic duct, and swelling of the pancreatic parenchyma has been referred to as autoimmune pancreatitis. Autoimmune pancreatitis, the term used in this chapter, is also characterized by hypergammaglobulinemia, histologic evidence of lymphoplasmacytic inflammation and fibrosis, and a favorable response to glucocorticoid treatment. These findings suggest that autoimmune mechanisms are involved in the pathogenesis of this disease. Another characteristic feature of this disease is high serum IgG4 concentrations, which are closely associated with disease activity. This disease has been frequently misdiagnosed as pancreatic cancer, leading to unnecessary surgery.

HISTORIC BACKGROUND

In 1961, Sarles et al. first reported this disease as "chronic inflammatory sclerosis of the pancreas." They summarized the clinical characteristics as: minimal or no severe abdominal pain, emaciation, jaundice, nonalcoholic background, hyperglobulinemia, diffuse enlargement of the pancreas, no dilatation of the pancreatic duct, and histologically marked lymphocytic infiltration. Waldram et al. (1975) then reported an association in these patients with salivary gland lesions and sclerosing cholangitis. Nakano et al. (1978) reported patients with Sjögren syndrome and pancreatitis who had been successfully treated with corticosteroids. Kawaguchi et al. (1991) then designated this condition as a lymphoplasmacytic sclerosing pancreatitis based on a detailed pathologic study. Toki et al. (1992) focused on the characteristic radioimages of pancreatic duct examination, and reported four patients as having an unusual type of chronic pancreatitis showing diffuse irregular narrowing of the entire main pancreatic duct by endoscopic retrograde cholangiopancreatography (ERCP). They summarized the clinical features and designated this condition as autoimmune pancreatitis based on clinical findings, laboratory evidence of hypergammaglobulinemia, positive autoantibodies and lymphoplasmacytic infiltration in the pancreas, and a favorable response to corticosteroid treatment (Yoshida et al., 1995; Ito et al., 1997). Since then, many patients have been diagnosed based on these characteristic clinical findings. A number of case reports or reports of small series of cases have been published using various other terms such as "nonalcoholic duct-destructive chronic pancreatitis" (Ectors et al., 1997), "chronic sclerosing pancreatitis" (Motoo et al., 1997) and "chronic pancreatitis with diffuse irregular narrowing of the pancreatic duct" (Wakabayashi et al., 1998).

In 2001, a new classification system was proposed for chronic pancreatitis, based on etiologic risk factors, toxic-metabolic causes, idiopathic, genetic, autoimmune, recurrent severe acute pancreatitis-associated chronic pancreatitis, and obstructive chronic pancreatitis (the TIGAR-O risk classification system) (Etemad and Whitcomb, 2001). An autoimmune mechanism was first recognized in this

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

classification system, and isolated autoimmune chronic pancreatitis and syndromic autoimmune pancreatitis were separately identified. "Autoimmune pancreatitis" seems to be equivalent to isolated autoimmune chronic pancreatitis in this system. Hamano et al. (2001) reported that patients with autoimmune pancreatitis frequently and specifically have high serum IgG4 concentrations that correlate well with disease activity, suggesting that this disease is a discrete clinical entity different from ordinary chronic pancreatitis.

In 2002, The Japanese Pancreas Society proposed Japanese diagnostic criteria for autoimmune pancreatitis: 1) diffuse irregular narrowing of the main pancreatic duct and enlargement of the pancreas (essential); 2) serum elevations of γ-globulin and/or IgG, and/or the presence of autoantibodies; and 3) fibrosis with lymphoplasmacytic infiltration in pancreatic tissue. Such diagnostic criteria will allow us to be more stringent in our diagnosis and to differentiate these conditions from pancreatic cancer (Steinberg et al., 2003).

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Clinical Features

We analyzed the clinical findings in over 40 patients with autoimmune pancreatitis who fulfilled the diagnostic criteria proposed by The Japanese Pancreas Society in 2002. These findings are summarized below.

The male-to-female ratio was 34:8 (male 81%), and the mean age of occurrence was 62 ± 9 years (range 38–76), suggesting that this disease shows an elderly male preponderance. Few patients complained of severe attacks of pancreatitis, but many had mild-to-moderate epigastralgia or discomfort (78%). The clinical symptoms were thus different from those of acute or severe pancreatitis. A striking feature was an obstructive jaundice in 68% of patients, which was caused by the stenosis or obstruction of the intrapancreatic common bile duct (Yoshida et al., 1995; Horiuchi et al., 1998; 2001). Diabetes mellitus (DM) was often observed and the majority was thought to have type 2 DM; some patients improved after steroid therapy (Tanaka et al., 2000).

Laboratory Tests

Laboratory tests showed several abnormal findings related to the obstructive jaundice, such as elevated serum levels of bilirubin, biliary enzymes, and transaminase in 70–80% of patients. The tumor-associated antigen, CA19-9, was elevated in 50% of patients, probably due to the cholestasis and not to a malignant process. Serum elevations of pancreatic enzymes were mild or moderate, and were found in 60% of patients. Serum elevations of γ-globulin and IgG were found in 59% and 70%, respectively. Decreased exocrine and endocrine function by the bentiromide test and HbA1c were found in 66% and 51%, respectively (Horiuchi et al., 1998; 2001) (Table 56.1).

Imaging

Abdominal ultrasonography (US) showed characteristic sonolucent swelling—the so-called "sausage-like" appearance (Figure 56.1), and dilatation of the common bile duct. Computed tomography (CT) with contrast material revealed

TABLE 56.1 Normal blood results and abnormal results for patients with autoimmune pancreatitis

	Results for autoimmune pancreatitis patients		
Test (normal values)	**Mean**	**Range**	**Abnormal (%)**
White blood cells (3500–9800/μl)	5929	2130–10.700	2/42 (5)
Eosinophils (%)	5.3	0–20	12/33 (36)
Amylase (44–127 Iμ/L)	231	82–2622	26/42 (62)
Elastase (100–400 ng/dl)	1190	69–5000	16/24 (67)
Total bilirubin (0.3–1.2 mg/dl)	4.0	0.4–20.9	29/42 (69)
Alkaline phosphatase (124–367 μ/L)	1279	132–5562	33/42 (79)
γ-GTP (8–50 IU/L)	645	10–1980	34/42 (81)
AST (12–37 IU/L)	191	13–854	30/42 (71)
ALT (7–45 IU/L)	286	12–1003	32/42 (76)
γ-Globulin (<2 g/dl)	2.23	0.8–4.31	24/41 (59)
IgG (800–1800 mg/dl)	2314	892–4562	28/40 (70)
IgE (0–400 IU/ml)	370	8–1024	10/30 (33)
CA19-9 (<37 IU/ml)	108	0–919	18/34 (53)
HbA1c (4.3–5.8%)	6.3	4.5–11.0	20/39 (51)
Bentiromide test (>70%)	60.6	22–90	19/29 (66)

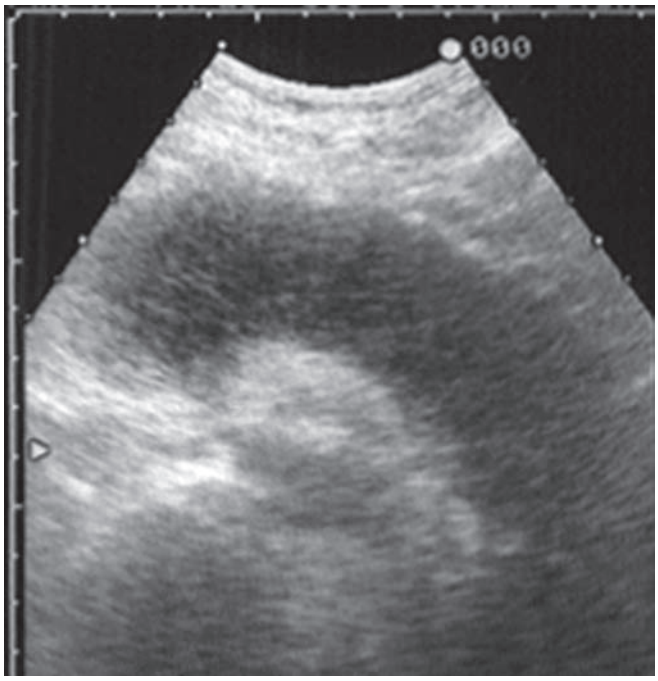


FIGURE 56.1 Abdominal ultrasonography of autoimmune pancreatitis, showing characteristic sonolucent swelling, the so-called "sausage-like" appearance.

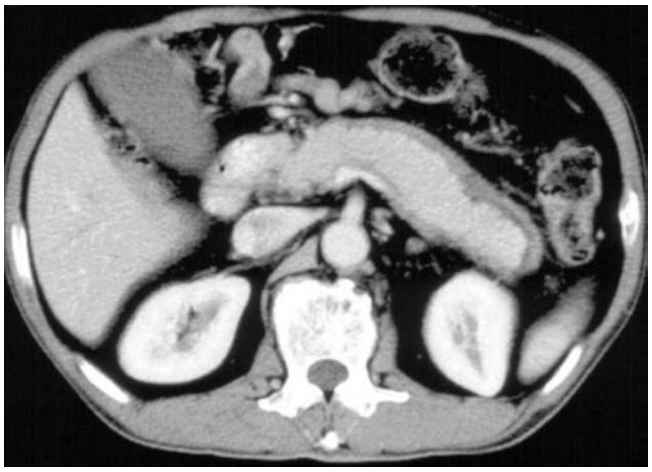


FIGURE 56.2 Computed tomography with contrast material of autoimmune pancreatitis, showing swelling and delayed enhancement of parenchymal tissue, resulting in a peripheral low-density area and a capsule-like rim.

swelling and delayed enhancement of parenchyma, resulting in a peripheral low-density area and a capsule-like rim (Figure 56.2). Absence of pancreatic duct dilatation or pancreatic stones or pseudocysts have been reported to be characteristic features of this disease and are commonly shown by US, CT, and magnetic resonance imaging (MRI) (Yoshida et al., 1995).

ERCP showed a characteristic pancreatogram of diffuse irregular narrowing with the following characteristics: 1) narrow caliber; 2) wall irregularity—both of which can extend for some distance; and (3) the absence of upstream dilatation (Figure 56.3). Approximately 50% of patients showed these changes throughout the main pancreatic duct; however, in other patients, focal changes were restricted to the pancreatic head or tail, which made differentiation from pancreatic cancer difficult in these cases. During follow-up, focal changes often progressed to diffuse disease (Horiuchi et al., 2002a). In patients with severe narrowing of the pancreatic duct, a pancreatogram may show obstruction of the main pancreatic duct (Fernández-del Castillo et al., 2003).

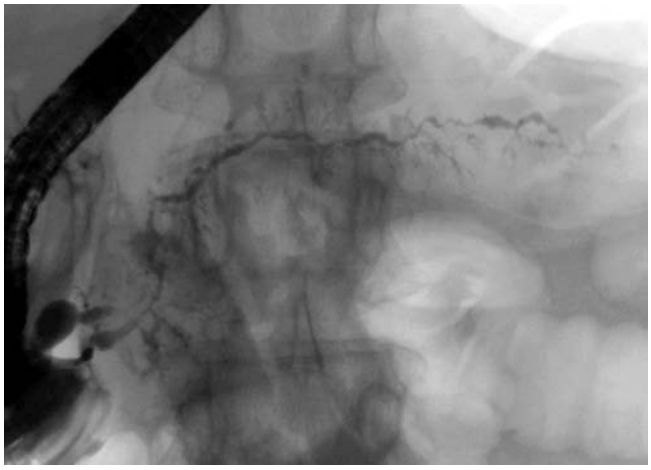


FIGURE 56.3 Endoscopic retrograde cholangiopancreatography in autoimmune pancreatitis showing the characteristic pancreatogram of diffuse irregular narrowing.

Cholangiography showed severe narrowing of the lower bile duct, which was caused by swelling of the head of the pancreas. However, intraductal ultrasonography (IDUS) showed that thickening of the bile duct wall also contributed to narrowing of the bile duct. In some cases, bile duct changes extended to extrapancreatic regions, such as the intrahepatic bile duct system; these cases showed similar findings to primary sclerosing cholangitis (PSC) (Figure 56.4). Gallium-67 scintigraphy showed characteristic hilar and pancreatic accumulation during the active stage of the disease (Saegusa et al., 2003).

Extrapancreatic Lesions

Another characteristic feature of this disease is a variety of complications or extrapancreatic lesions, such as swelling of lacrimal and salivary glands (Kamisawa et al., 2003), sclerosing cholangitis (Erkelens et al., 1999; Horiuchi et al., 2001; Nakazawa et al., 2001), retroperitoneal fibrosis (Hamano 2002), hilar lymphadonopathy (Saegusa, 2003), gastric mucosal disease (Shinji et al., 2004), and hypothyroidism (Komatsu et al., 2005). These extrapancreatic

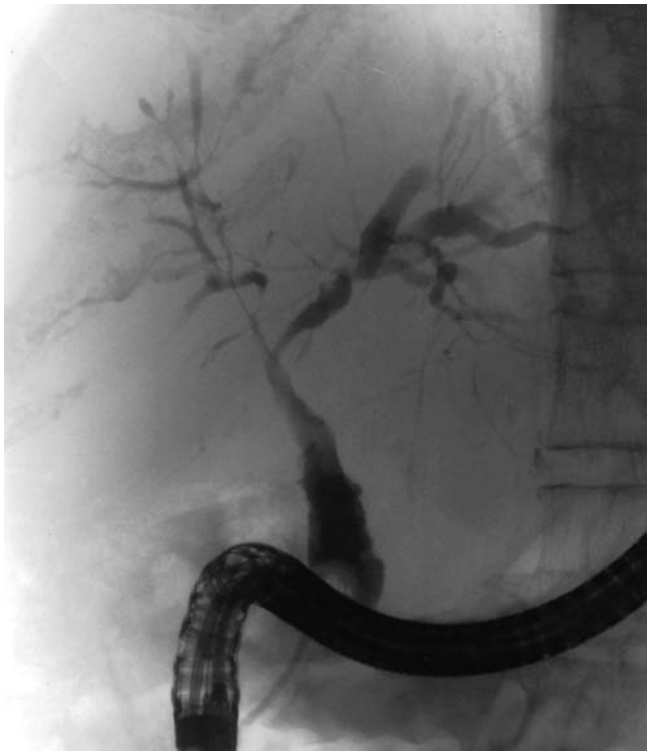


FIGURE 56.4 Endoscopic retrograde cholangiopancreatography in autoimmune pancreatitis showing the characteristic intrahepatic bile duct changes similar to those of primary sclerosing cholangitis.

lesions have often been misdiagnosed as Sjögren syndrome, PSC, urethral tumor, sarcoidosis, or gastric ulcer. It should be stressed that salivary gland or lacrimal gland lesions and sclerosing cholangitis differ from typical Sjögren syndrome and classical PSC, respectively, because they show a favorable response to corticosteroid therapy and characteristic findings on blood tests or pathology. The diversity of extrapancreatic lesions found in this disease may coincide with those of multifocal fibrosclerosis, which is an uncommon fibroproliferative systemic disorder with multiple manifestations, including retroperitoneal fibrosis, sclerosing cholangitis, Riedel thyroiditis, fibrotic pseudotumor of the orbit, and fibrosis of the salivary glands (Comings et al., 1967). It is possible that autoimmune pancreatitis is a pancreatic manifestation of this systemic fibrosing disease (Kamisawa et al., 2003).

Misdiagnosis of Pancreatic Cancer

Patients with autoimmune pancreatitis sometimes receive a diagnosis of pancreatic cancer because of the preponderance of this disease in the elderly, obstructive jaundice, serum elevations of CA19-9, and irregular narrowing or obstruction of the pancreatic duct. Pathologic analysis after pancreaticoduodenectomy (Whipple resection), performed because of a diagnosis of pancreatic cancer, led to a revised diagnosis of autoimmune pancreatitis (lymphoplasmacytic sclerosing pancreatitis) in 2.2% of patients (Hardacre et al., 2003). Due to similar pathologic findings, this disease has also been diagnosed as pancreatic lymphoma (Horiuchi et al., 1996).

Pathologic Features

On gross examination, the involved pancreas appears glistening white, is firm or hard, and may be enlarged or show mass lesions. The lesion may be limited to one portion of the pancreas, most commonly the head, or may involve the body, tail, or entire organ. Lymphoplasmacytic infiltration and fibrosis are characteristic microscopic features of the pancreatic lesions, and in some cases result in the formation of lymphoid follicles. Immunostaining has demonstrated that T-cells predominate over B-cells among the infiltrating lymphocytes. The infiltrating plasma cells characteristically bear IgG4 (Hamano et al., 2002). The cell infiltration is prominent around the pancreatic duct, resulting in stenosis or obstruction of the duct, stasis of the pancreatic secretions, and damage to the pancreatic lobules. Obliterating phlebitis is another characteristic feature, which shows a marked cellular infiltration of the venous wall and venous thrombosis (Kawaguchi et al., 1991). Conspicuous negative features typically include absence of calculi in ducts, absence of pseudocysts, and absence of more than minimal fatty necrosis. The histologic features observed in the extrapancreatic lesions such as in the salivary glands (Kamisawa et al., 2003) or retroperitoneal fibrosis (Hamano et al., 2002) often resemble those seen in the pancreas.

Epidemiologic Features

The incidence of autoimmune pancreatitis in Japan or in the world at large is not known. However, between September 1994 and September 2002, we identified 42 patients with autoimmune pancreatitis among the 2,215,000 population of the Nagano prefecture in Japan (Takayama et al., 2004). Accordingly, autoimmune pancreatitis is a rare disorder, though its exact prevalence remains unknown.

AUTOIMMUNE FEATURES

The positive rates for various autoantibodies are summarized in Table 56.2. Positive rates for antinuclear antibody were 40%, and those for other antibodies were 10–30%. No patient had a positive test for SS-A or SS-B antibodies, or antimitochondrial antibodies, which are the disease-specific antibodies for the diagnosis of Sjögren syndrome and primary biliary cirrhosis (PBC), respectively. These findings suggest that the autoantibodies found in autoimmune pan-

TABLE 56.2 Positive rates for autoantibodies in patients with autoimmune pancreatitis

Antibody	Cut-off value	Number of positive tests	Positive rate (%)	Method	Substrates
Antinuclear antibody (ANA)	<1:40	24/44	54.5	IFL	Hep-2
	<1:80	17/44	38.6		
Anti-dsDNA antibody	<12 IU/ml	2/44	4.5	ELIAS	dsDNA
Anti-SS-A antibody	<10	0/37	0.0	ELISA	SS-A/Ro protein
Anti-SS-B antibody	<15	0/37	0.0	ELISA	SS-B/La protein
Antimitochondrial antibody	<7	2/44	4.5	ELISA	M2 antigen*
Anti-smooth muscle antibody	<1:20	9/44	20.5	IFL	Tissue sections of rat stomach and kidney
Rheumatoid factor (RAPA)	<10 IU/ml	13/44	29.5	TIA	IgG
Antithyroglobulin antibody	<10 IU/ml	7/41	17.1	ELISA	Thyroglobulin
Antithyroid peroxidase antibody	<10 IU/ml	5/41	12.2	ELISA	Thyroid peroxidase
Any autoantibodies	(<1:40 ANA)	35/44	79.5		
	(<1:80 ANA)	31/44	70.5		

*Recombinant PDC-E2, BBCOADE-E2, OGDC-E2.
ELISA, enzyme-linked immunosorbent assay; IFL, indirect immunofluorescence; TIA, turbidimetric immunoassay.

creatitis are not pathogenic, and that patients have a tendency to produce various autoantibodies that are not disease specific. The occasional coexistence of pancreatitis with salivary gland lesions and cholangitis suggests shared target antigens. Carbonic anhydrase II and lactoferrin are distributed in the cells of several exocrine organs, including the pancreas, salivary glands, and biliary duct. In this context, carbonic anhydrase II or lactoferrin have been proposed as candidate target antigens, but the presence of autoantibodies for these antigens is not sufficiently specific or sensitive (Kino-Ohsaki et al., 1996; Okazaki and Chiba, 2002). Autoantibody for α-fodrin, which is a candidate autoantigen for the development of Sjögren syndrome, is useful for discriminating cases with extrapancreatic lesions, such as sclerosing cholangitis or swelling of the salivary and lacrimal glands (Horiuchi et al., 2002b).

In peripheral blood or pancreatic tissue, activated $CD4^+$ or $CD8^+$ T-cells bearing HLA-DR molecules are aggregated mainly around the pancreatic duct, and pancreatic ducts express HLA-DR molecules (Okazaki et al., 2000). $CD4^+$ T-cells are subdivided into type 1 helper T (Th1) and Th2 cells based on profiles of cytokine production (see Chapter 7). In some cases of autoimmune pancreatitis, $CD4^+$ Th1 cells predominate over Th2 cells, suggesting that Th1 cytokines are essential for the induction and maintenance of this disease, whereas Th2 cytokines are involved in disease progression (Okazaki and Chiba, 2002).

Protein electrophoresis of patient's sera showed a polyclonal band in the rapidly migrating fraction of γ-globulins. Immunoelectrophoresis confirmed that this polyclonal band was caused by a high serum concentration of IgG4. We found serum IgG4 elevation in 90% of patients with autoimmune pancreatitis, but in very few patients with other conditions, including pancreatic cancer, chronic pancreatitis, and Sjögren syndrome, suggesting that IgG4 is a sensitive and specific marker for this disease. In addition, IgG4 correlates well with disease activity. Similar to IgG4, IgG4-type immune complexes are also elevated in the serum and correlate well with disease activity (Hamano et al., 2001).

GENETIC FEATURES

HLA-DR4 and -DQ4 are the markers most frequently associated with autoimmune pancreatitis among MHC class I and class II molecules. Among DR4 and DQ4 subtypes, the frequencies of DRB1*0405 and DQB1*0401 are significantly higher in patients with autoimmune pancreatitis than in normal subjects and those with chronic pancreatitis. In the Japanese population, DRB1*0405 is known to be in strong linkage disequilibrium with DQB1*0401, resulting in formation of the DRB1*0405 DQB1*0401 haplotype. The DRB1*0405 DQB1*0401 haplotype in autoimmune pancreatitis shows no significant association with any HLA class I molecules, in contrast to the B54 DRB1*0405 DQB1*0401 haplotype reported in autoimmune hepatitis (Seki et al., 1990; 1992), suggesting that autoimmune pancreatitis has a different immunogenetic background from autoimmune hepatitis. Accordingly, the DRB1*0405 DQB1*0401 haplotype is specifically associated with susceptibility to autoimmune pancreatitis, and may play a functional role in antigen presentation and induction of an autoimmune response (Kawa et al., 2002). The relative linkage disequilibrium value for both risk alleles is 100% in the Japanese population. Thus, it is difficult to determine which allele is actually associated with susceptibility to autoimmune pancreatitis.

ANIMAL MODELS

A putative animal model of autoimmune pancreatitis was created by immunizing neonatally thymectomized mice with carbonic anhydrase II or lactoferrin, and also by transferring immunized spleen cells to nude mice (Uchida et al., 2002). Inflammation was found to be present in the pancreas of immunized neonatally thymectomized mice and of all mice receiving whole spleen cells or $CD4^+$ cells. Carbonic anhydrase II- or lactoferrin-immunized mice developed apoptotic duct cells or acinar cells, respectively. These findings suggest that an immunologic mechanism against carbonic anhydrase II or lactoferrin is involved in the pathogenesis of this pancreatitis model, in which the effector cells appear to be Th1-type $CD4^+$ T-cells, although this requires confirmation.

PATHOLOGIC MECHANISMS

Because serum IgG4 and IgG4-type immune complexes are closely associated with disease activity, they may play a major role in the pathogenesis of autoimmune pancreatitis. To date, the pathologic effects of IgG4 and IgG4-type immune complexes have been reported in limited settings, such as in pemphigus and membranous nephropathy. Pemphigus vulgaris and pemphigus foliaceus are autoimmune skin diseases (see Chapter 57) characterized by the presence of IgG4-type autoantibodies against the cell-adhesion molecules desmoglein-3 and -1; the IgG4-type antidesmoglein antibodies may even cause the characteristic skin lesions of acantholysis. In autoimmune pancreatitis, IgG4-type autoantibody may induce an inflammatory response after interaction with an undefined target antigen. IgG4 is unique among the IgG subclasses in its inability to fix C1q complement and its low affinity for target antigens, resulting in the formation of a unique type of immune complex. In the pathogenesis of membranous nephropathy, these characteristics of IgG4 may account for the slow rate of clearance of IgG4-type immune complexes from the circulation, and for the formation of membrane-attack complexes in tissues by an alternative pathway, or the mannose-binding lectin pathway. As we found high serum concentrations of IgG4-type immune complexes in most patients with autoimmune pancreatitis who had active inflammation, the same mechanism may be operating in the pathogenesis of this disease.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

Positive rates for various immunologic markers are as follows: raised γ-globulin 60%, raised IgG 70%, any of the described autoantibodies 80%, and raised IgG4 90%. Serum IgG4 elevation is observed in very few patients with other conditions, i.e., pancreatic cancer, chronic pancreatitis, Sjögren syndrome, PBC, and PSC, suggesting that IgG4 is the most sensitive and specific immunologic marker for diagnosis (Hamano et al., 2001). IgG4-type immune complexes are another useful marker, but we do not yet have a reliable assay system. Although autoantibodies to carbonic anhydrase II and lactoferrin have been reported to be useful markers, their diagnostic value for autoimmune pancreatitis has not been fully accepted because of their insufficient sensitivity and specificity (Kino-Ohsaki et al., 1996, Okazaki and Chiba, 2002).

TREATMENT AND OUTCOME

For autoimmune pancreatitis, intensive treatment for acute pancreatitis is not required. Most patients with autoimmune pancreatitis respond favorably to corticosteroid treatment, resulting in an improvement in symptoms, laboratory tests, and imaging findings. However, no general agreement has been reached regarding the general usage of corticosteroids for all patients, as we have found spontaneous remission in some patients who needed a biliary drainage procedure for obstructive jaundice. It is possible, however, that early corticosteroid therapy could prevent the residual structural changes of pancreatic parenchyma, such as duct change or fibrosis. Corticosteroid treatment is mandatory for patients who are in a highly active state with systemic manifestations, such as sclerosing cholangitis or retroperitoneal fibrosis. For other cases, corticosteroid therapy remains optional.

As autoimmune pancreatitis is a new disease entity and there are no therapeutic guidelines for this condition, the regimen of steroid therapy was initially based on that for other autoimmune diseases. Patients who showed obstructive jaundice and diffuse pancreatic swelling were treated with 40 mg/day of prednisolone for 4 weeks, after which the dose was reduced by 5 mg/week over 7 weeks until a dose of 5 mg/day was reached. Maintenance therapy was continued, at 2.5–5 mg/day, if the autoimmune process did not resolve.

Surgery is often necessary for differentiation from malignancy.

To clarify the long-term outcome of autoimmune pancreatitis, we performed a follow-up study for periods longer than 12 months (median follow-up: 54.5 months; range: 13–111 months). Twenty-six percent of patients who had been treated with prednisolone had recurrent attacks and 19% developed pancreatic calculi during a median follow-up period of 22 months. Because 55% of patients who suffered relapse showed pancreatic calculi, this appears to be significantly associated with relapsing compared with non-relapsing cases. These findings suggest that autoimmune

pancreatitis has the potential to be a progressive disease with pancreatic lithiasis (Takayama et al., 2004), contrary to a previous report (Yoshida et al., 1995). As few patients suffered from acute attacks, acute inflammation would only play a minor role in pancreatic stone formation. Incomplete obstruction of the pancreatic duct system and stasis of pancreatic secretions provide another possible explanation for the formation of pancreatic calculi.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Autoimmune pancreatitis is clearly distinguishable from ordinary chronic pancreatitis, and shows a distinctive disease profile with characteristic high serum IgG4 concentrations. However, the following issues remain to be clarified: 1) the target antigen recognized by IgG4 autoantibody and the role of IgG4 elevation; 2) details of extrapancreatic lesions with respect to systemic disease, such as multifocal fibrosclerosis; 3) therapeutic strategies, including corticosteroid therapy; and 4) long-term outcome with respect to the pancreatic structure and function, and predisposition to malignancy.

Autoimmune pancreatitis seems to receive particular attention in Japan. Articles on this disease have been mainly published from Japan, with some from Europe and the USA. The major reason for this bias is probably the special interest in this disease in Japan during the past 10 years, and many patients in other countries have been overlooked or given another diagnosis (Kim et al., 2004). Recent increasing awareness of the entity will result in an increase in the diagnosis of autoimmune pancreatitis worldwide.

References

Comings, D.E., Skubi, K.B., Van Eyes, J., and Motulsky A.G. (1967). Familial multifocal fibrosclerosis. Findings suggesting that retroperitoneal fibrosis, mediastinal fibrosis, sclerosing cholangitis, Riedel's thyroiditis, and pseudotumor of the orbit may be different manifestations of a single disease. *Ann. Intern. Med. 66*, 884–892

Ectors, N., Maillet, B., Aerts, R., Geboes, K., Donner, A., Borchard, F., Lankisch, P., Stolte, M., Luttges, J., Kremer, B., and Kloppel, G. (1997). Non-alcoholic duct destructive chronic pancreatitis. *Gut 41*, 263–268.

Erkelens, G.W., Vleggaar, F.P., Lesterhuis, W., van Buuren, H.R., and van der Werf, S.D. (1999). Sclerosing pancreato-cholangitis responsive to steroid therapy. *Lancet 354*, 43–44.

Etemad, B. and Whitcomb, D.C. (2001). Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology 120*, 682–707.

Fernández-del Castillo, C.F., Sahani, D.V., and Lauwers, G.Y. (2003). Case 27–2003 A 36-year-old man with recurrent epigastric pain and elevated amylase levels. *N. Engl. J. Med. 349*, 893–901.

Hamano, H., Kawa, S., Horiuchi, A., Unno, H., Furuya, N., Akamatsu, T., Fukushima, M., Nikaido, T., Nakayama, K., Usuda, N., and Kiyosawa, K. (2001). High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N. Engl. J. Med. 344*, 732–738.

Hamano, H., Kawa, S., Ochi, Y., Unno, H., Shiba, N., Wajiki, M., Nakazawa, K., Shimojo, H., and Kiyosawa, K. (2002). Hydronephrosisi associated with retroperitoneal fibrosis and sclerosing pancreatitis. *Lancet 359*,1403–1404.

Hardacre, J.M, Lacobuzio-Donahue, C.A., Sohn, T. A., Abraham, S.C., Yeo, C.J., Lillemoe, K.D., Choti, M.A., Campbell, K.A., Schulick, R.D., Hruban, R.H., Cameron, J.L., and Leach, S.D. (2003). Results of pancreaticoduodenectomy for lymphoplasmacytic sclerosing pancreatitis. *Ann. Surg. 237*, 853–859.

Horiuchi, A., Kaneko, T., Yamamura, S., Nagata, A., Nakamura, T., Akamatsu, T., Mukawa, K., Kawa, S., and Kiyosawa, K. (1996). Autoimmune chronic pancreatitis simulating pancreatic lymphoma. *Am. J. Gastroenterol. 91*, 2607–2609.

Horiuchi, A., Kawa, S., Aoli, Y., Mukawa, K., Furuya, N., Ochi, Y., and Kiyosawa, K. (1998). Characteristic pancreatic duct appearance in autoimmune chronic pancreatitis: a case report and review of the Japanese literature. *Am. J. Gastroenterol. 93*, 260–263.

Horiuchi, A., Kawa, S., Hamano, H., Ochi, Y., and Kiyosawa, K. (2001). Sclerosing pancreato-cholangitis responsive to corticosteroid therapy: report of 2 case reports and review. *Gastrointest. Endosc. 53*, 518–522.

Horiuchi, A., Kawa, S., Hamano, H., Hayama, M., Ota, H., and Kiyosawa, K. (2002a). ERCP features in 27 patients with autoimmune pancreatitis. *Gastrointest. Endosc. 55*, 494–499.

Horiuchi, A., Kawa, S., Hamano, H., and Kiyosawa, K. (2002b). Does a lack of reactivity to α-fodrin indicate the existence of primary autoimmune pancreatitis? *Am. J. Gastroenterol. 97*, 1275–1277.

Ito, T., Nakano, I., Koyanagi, S., Miyahara, T., Migita, Y., Ogoshi, K., Sakai, H., Matsunaga, S., Yasuda, O., Sumii, T., and Nawata, H. (1997). Autoimmune pancreatitis as a new clinical entity. Three cases of autoimmune pancreatitis with effective steroid therapy. *Dig. Dis. Sci. 42*, 1458–1468.

Kamisawa, T., Funata, N., Hayashi, Y., Tsuruta, K., Okamoto, A., Amemiya, K., Egawa, N., and Nakajima, H. (2003). Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis. *Gut 52*, 683–687.

Kawa, S., Ota, M., Yoshizawa, K., Horiuchi, A., Hamano, H., Ochi, Y., Nakayama, K., Tokutake, Y., Katsuyama, Y., Saito, S., Hasebe, O., and Kiyosawa, K. (2002). HLA DRB1*0405-DQB1*0401 haplotype is associated with autoimmune pancreatitis in the Japanese population. *Gastroenterology 122*, 1264–1269.

Kawaguchi, K., Koike, M., Tsuruta, K., Okamoto, A., Tobata, I., and Fujita, N. (1991). Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas. *Hum. Pathol. 22*, 387–395.

Kim, K.P., Kim, M.H., Song, M.H., Lee, S.S., Seo, D.W., and Lee, S.K. (2004). Autoimmune chronic pancreatitis. *Am. J. Gastroenterol. 99*, 1605–1616.

Kino-Ohsaki, J., Nishimori, I., Morita, M., Okazaki, K., Yamamoto, Y., Onishi, S., and Hollingsworth, M.A. (1996). Serum antibodies to carbonic anhydrase I and II in patients with idiopathic pancreatitis and SjS. *Gastroenterology 110*, 1579–1586.

Komatsu, K., Hamano, H., Ochi, Y., Takayama, M., Muraki, T., Yoshizawa, K., Sakurai, A., Ota, M., and Kawa, S. (2005). High prevalence of hypothyroidism in patients with autoimmune pancreatitis. *Dig. Dis. Sci. 50*, 1052–1057.

Motoo, Y., Minamoto, T., Watanabe, H., Sakai, J., Okai, T., and Sawabu, N. (1997). Sclerosing pancreatitis showing rapidly progressive changes with recurrent mass formation. *Int. J. Pancreatol. 21*, 85–90.

Nakano, S., Takeda, I., and Kitamura, K. (1978). Vanishing tumor of the abdomen in patients with Sjögren's syndrome. *Dig. Dis. Sci. 23*, 75–79.

Nakazawa, T., Ohara, H., Yamada, T., Ando, H., Sano, H., Kajino, S., Hashimoto, T., Nakamura, S, Ando, T., Nomura, T., Joh, T., and Itoh, M. (2001). Atypical primary sclerosing cholangitis cases associated with unusual pancreatitis. *Hepatogastroenterology 48*, 625–630.

Okazaki, K., Uchida, K., Ohana, M., Nakase, H., Uose, S., Inai, M., Matsushima, Y., Katamura, K., Ohmori, K., and Chiba, T. (2000). Autoimmune-related pancreatitis is associated with auto antibodies and Th1/Th2-type cellular immune response. *Gastroenterology 118*, 573–581.

Okazaki, K. and Chiba, T. (2002). Autoimmune related pancreatitis. *Gut 51*, 1–4.

Saegusa, H., Momose, M., Kawa, S., Hamano, H., Ochi, Y., Takayama, M., Kiyosawa, K., and Kadoya, M. (2003). Hilar and pancreatic gallium-67 accumulation is characteristic feature of autoimmune pancreatitis. *Pancreas 27*, 20–25.

Sarles, H., Sarles, J.C., Muratore, R., and Gulen, C. (1961). Chronic inflammatory sclerosis of the pancreas—an autonomous pancreatitis disease? *Am. J. Dig. Dis. 6*, 688–698.

Seki, T., Kiyosawa, K., Inoko, H., and Ota, M. (1990). Association of autoimmune hepatitis with HLA Bw54 and DR4 in Japanese patients. *Hepatology 12*, 1300–1304.

Seki, T., Ota, M., Furuta, S., Fukushima, H., Kondo, T., Hino, K., Mizuki, N., Ando, A., Tsuji, K., Inoko, H., and Kiyosawa, K. (1992) HLA class II molecules and autoimmune hepatitis susceptibility in Japanese patients. *Gastroenterology 103*, 1041–1047.

Shinji, A., Sano, K., Hamano, H., Unno, H., Fukushima, M. Nakamura, N., Akamatsu, T., Kawa, S., and Kiyosawa, K. (2004) Autoimmune pancreatitis is closely associated with gastric ulcer presenting with abundant IgG4-bearing plasma cell infiltration. *Gastrointest. Endoscopy 59*, 506–511.

Steinberg, W.M., Barkin, J.S., Bradley, E.L., 3rd, DiMagno, E., and Layer, T. (2003). Controversies in clinical pancreatology: autoimmune pancreatitis: does it exist? *Pancreas 27*, 1–13.

Takayama, M., Hamano, H., Ochi, Y., Saegusa, H., Komatsu, K., Muraki, T., Arakura, N., Imai, Y., Hasebe, O., and Kawa, S. (2004). Recurrent attacks of autoimmune pancreatitis result in pancreatic stone formation. *Am. J. Gastroenterol. 99*, 932–937.

Tanaka, S., Kobayashi, T., Nakanishi, K., Okubo, M., Murase, T., Hashimoto, M., and Takeuchi, K. (2000). Corticosteroid-responsive diabetes mellitus associated with autoimmune pancreatitis. *Lancet 356*, 910–911.

Toki, F., Kozu, T., and Oi, I. (1992). An unusual type of chronic pancreatitis showing diffuse irregular narrowing of the entire main pancreatic duct on ERCP-A report of four cases. *Endoscopy 24*, 640.

Uchida, K., Okazaki, K., Nishi, T., Uose, S., Nakase, H., Ohana, M., Matsushima, Y., Omori, K., and Chiba, T. (2002). Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydrase II and lactoferrin. *Lab. Invest. 82*, 411–424.

Wakabayashi, T., Motoo, Y., Kojima, Y., Makino, H., and Sawabu, N. (1998). Chronic pancreatitis with diffuse irregular narrowing of the main pancreatic duct. *Dig. Dis. Sci. 43*, 2415–2425.

Waldram, R., Kopelman, H., Tsantoulas, D., and Williama, R. (1975). Chronic pancreatitis, sclerosing cholangitis, and sicca complex in two siblings. *Lancet 1*, 550–552.

Yoshida, K., Toki, F., Takeuchi, T., Watanabe, S., Shiratori, K., and Hayashi, N. (1995). Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig. Dis. Sci. 40*, 1561–1568.

CHAPTER

57

Bullous Skin Diseases: Pemphigus Pemphigoid

NING LI, ZHI LIU, JULIO HILARIO-VARGAS, AND LUIS A. DIAZ

Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Autoimmune bullous diseases are rare disorders affecting skin and mucous membranes that are mediated by pathogenic autoantibodies against desmosomal or hemidesmosomal antigens of squamous epithelium (Diaz and Giudice, 2000). In the epidermis, neighboring keratinocytes adhere to each other through organelles known as desmosomes, whereas at the dermoepidermal junction hemidesmosomes anchor the epidermis to the dermis. The majority of these antigens recognized by these autoantibodies is desmosomal and hemidesmosomal transmembrane glycoproteins involved in epidermal cell–cell and epidermal–dermal adherence.

The desmosome contains two parallel intracellular plaques, which are located just underneath the cell membranes of neighboring cells (Figure 57.1A). The narrow extracellular space shared by two cells, at the level of the desmosome, constitutes the desmosomal core. The desmosomal plaques serve as insertion sites for intracellular cytokeratins and are composed of large molecular weight proteins, whereas the core contains a group of transmembrane calcium-dependent cell adhesion molecules known as desmosomal cadherins. They include desmogleins (Dsg1–4) and desmocollins (Dsc1–3) (Getsios et al., 2004). The isoforms of these desmosomal cadherins vary in different cell types. While Dsg1 is expressed throughout the epidermis with predominance in the upper layers of this tissue, Dsg3 is expressed mainly in the suprabasal layers of the epidermis.

The hemidesmosomes, located on the dermal pole of the epidermal basal cells, also contain an intracellular plaque and a core structure (Figure 57.1B). The extracellular space, called the lamina lucida (corresponding to the desmosomal core), separates these cells from the underlying basal lamina (composed of collagen IV) (Diaz and Giudice, 2000). The

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

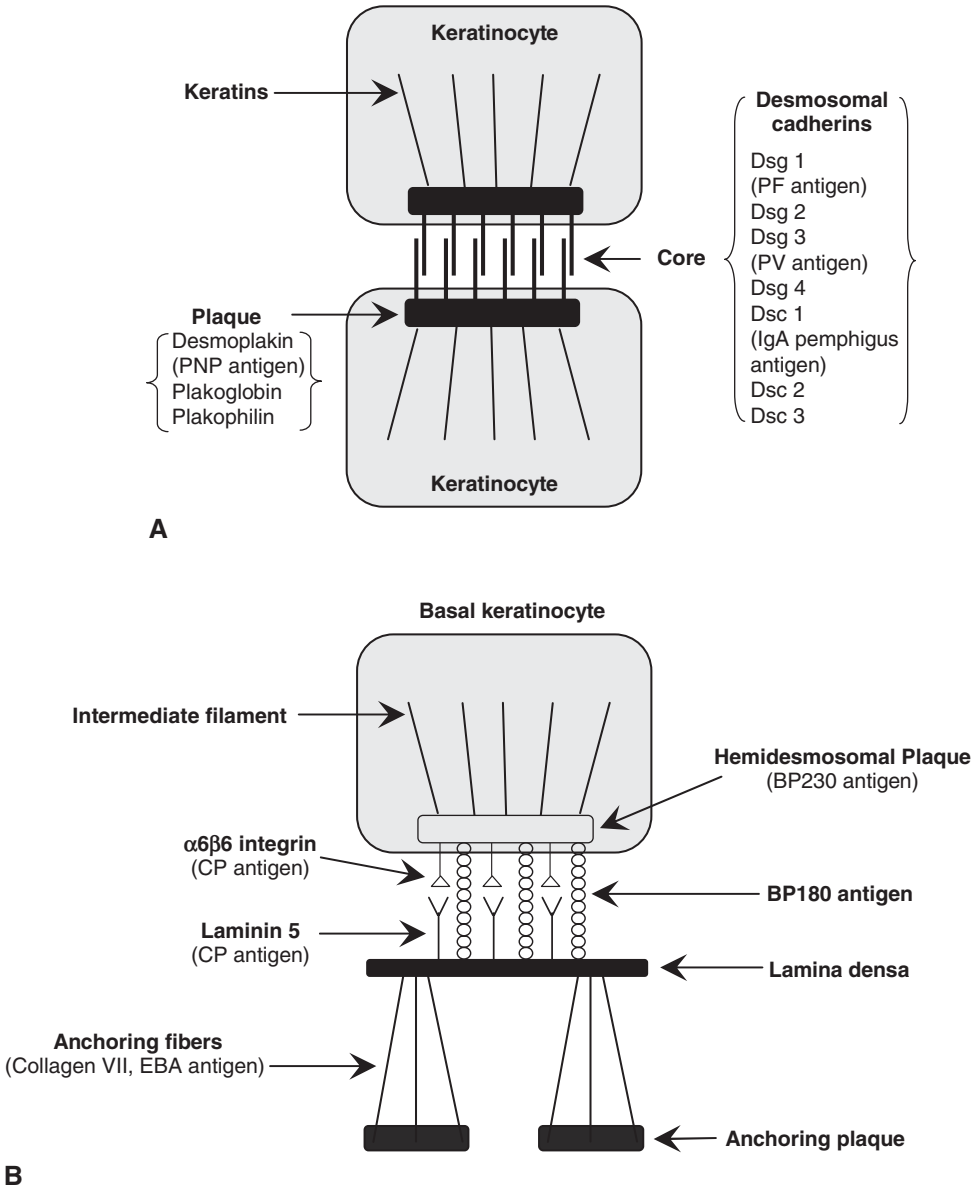


FIGURE 57.1 *A*, Schematic diagram of the desmosome and *B*, hemidesmosome. BP, bullous pemphigoid; CP, cicatricial pemphigoid; EBA, epidermolysis bullosa acquisita; PF, pemphigus foliaceus; PNP, paraneoplastic pemphigus; PV, pemphigus vulgaris.

sub-basal lamina region contains other matrix molecules and the anchoring fibers (collagen VII). The hemidesmosomal plaque, linked to the cytokeratin network, contains two intracellular proteins, BP230 and plectin. The lamina lucida contains the ectodomains of two transmembrane proteins, BP180 and α6β4 integrin, and laminin 5.

The pemphigus syndromes include diseases that are characterized by loss of epidermal cell–cell cohesion (named acantholysis) (Civatte, 1943) and autoantibodies against desmosomal cadherins (Beutner and Jordon, 1964; Anhalt and Diaz, 2001). This group comprises the two classical forms of pemphigus: pemphigus vulgaris (PV) and pemphigus foliaceus (PF). PV is characterized by suprabasilar acantholysis and anti-Dsg3 IgG autoantibodies, whereas PF is characterized by subcorneal acantholysis and anti-Dsg1 IgG autoantibodies. Other infrequent forms of pemphigus include paraneoplastic pemphigus (PNP), drug-induced pemphigus, and IgA pemphigus (Table 57.1).

The pemphigoid syndromes and other rare subepidermal autoimmune blistering diseases (see Table 57.1) are charac-

TABLE 57.1 Autoimmune blistering diseases of the skin

Disease	Cleavage site	Skin organelle	Autoantigens	Pathogenic autoantibodies
Pemphigus vulgaris	Suprabasilar acantoholysis	Desmosome	Dsg3	Passive transfer
Pemphigus foliaceus	Subcorneal acatoholysis	Desmosome	Dsg1	Passive transfer
Paraneoplastic pemphigus	Suprabasilar acantoholysis	Desmosome, hemidesmosome	Dsg3, Dsg1 and plakin family	Passive transfer (Dsg3)
Drug-induced pemphigus	Suprabasilar acantoholysis (commonly)	Desmosome	Dsg1	?
IgA pemphigus	Subcorneal/intraepidermal pustules	Desmosome	Dsg1	?
Bullous pemphigoid	Subepidermal	Hemidesmosome	BP180 and BP230 antigens	Passive transfer (BP180)
Herpes gestationis	Subepidermal	Hemidesmosome, lamina lucida	BP180 antigen	Passive transfer
Cicatricial pemphigoid	Subepidermal	Hemidesmosome	BP180 antigen, laminin 5, $\alpha 6\beta 4$ integrin	Passive transfer (laminin 5)
Lichen planus pemphigoid	Subepidermal	Hemidesmosome	BP180 antigen	?
Linear IgA dermatoses	Subepidermal	Hemidesmosome	BP180 fragments	?
Epidermolysis bullosa acquisita	Subepidermal	Anchoring fibers	Type VII collagen	Passive transfer
Dermatitis herpetiformis	Subepidermal	Dermal papilla	Transglutaminase	?

Dsg, desmoglein; DSC, desmollin.

terized by separation of the epidermis from the dermis. Bullous pemphigoid (BP), the most common autoimmune bullous disease seen in the elderly (Lever, 1965), is characterized by subepidermal blisters and autoantibodies against the hemidesmosomal BP180 and BP230 antigens (Stanley et al., 1981; Mutasim et al., 1985; Labib et al., 1986). Other subepidermal blistering diseases, such as cicatricial pemphigoid (CP), herpes gestationis (HG), lichen planus pemphigoides (LPP), and linear IgA dermatoses (LAD) exhibit distinctive clinical, histologic, and immunogenetic features, and share a humoral autoimmune response to the BP180 antigen. The remaining acquired subepidermal blistering diseases show autoantibody responses to structural molecules of the dermal extracellular matrix; they include epidermolysis bullosa acquisita (EBA) and dermatitis herpetiformis (DH). The antigen in EBA is collagen VII and in DH is a tissue transglutaminase. The autoantibody response in all these autoimmune skin diseases belongs to the IgG class, except in IgA pemphigus, LAD, and DH, where the immunoglobulin class is IgA.

Passive transfer of IgG from patients with PV, PF, PNP, and CP, or rabbit antiserum against BP and EBA autoantigens, into experimental animals duplicated the main skin features of the human disease in these animals, suggesting that these autoantibodies are indeed pathogenic (Anhalt et al., 1982; Roscoe et al., 1985; Liu et al. 1993; Amagai et al., 1998; Chen et al., 2004). Numerous studies have demonstrated that unique epitopes within the respective antigens are bound by pathogenic autoantibodies in these patients (Guidice et al., 1993; Lapiere et al., 1993; Mahoney et al., 1998; Zillikens et al., 1998; Christophoridis et al., 2000; Sekiguch et al., 2001; Sardy et al., 2002; Li et al., 2003; Zone et al., 2004).

Little is known about the etiology and how the autoimmune response is triggered in these disorders except in the endemic form of PF (also known as fogo selvagem), where an environmental etiology is strongly suggested. In this chapter we shall review the current clinical, histologic, and immunologic features of the most common forms of autoimmune bullous diseases (PV, PF, and BP), and briefly discuss other infrequent forms of pemphigus and other subepidermal autoimmune bullous diseases.

PEMPHIGUS VULGARIS

Clinical and Histopathologic Features

PV is the most severe and common form of pemphigus (Lever, 1965). It affects skin and mucous membranes. Although found in all ethnic and racial groups, the disease is more prevalent in Jewish patients exhibiting the HLA DRB1*0402 allele. Patients affected are usually in their fourth to sixth decade of life. The disease usually begins with painful ulcerations or erosions of the oral mucosa (mucosal PV), which may last for several months. This

process is gradually followed by involvement of the skin (mucocutaneous PV), where the disease produces flaccid blisters and erosions. Other squamous epithelial tissues (e.g., nasal, esophageal, larynx, pharynx, conjunctival, vaginal, and rectal) may also be involved.

Histologically, the epithelial lesions of PV show intraepidermal blisters that are located above the basal cell layer of the epidermis (superbasilar acantholysis) (Figure 57.2, left panel). The basal cells remain attached to the dermis but laterally detached from each other, producing the histologic sign known as "row of tombstones" (Lever, 1965). Inflammatory infiltration is usually negligible, but early lesions may show eosinophilic spongiosis.

Autoimmune Features

Autoantibodies

The serum of PV patients contains IgG autoantibodies that stain the epidermal intercellular spaces (ICSs) by indirect immunofluoresence (IF), producing titers that roughly correlate with disease activity (Beutner and Jordon, 1964). Direct IF of perilesional epidermis reveals IgG bound to keratinocyte cell surfaces. These autoantibodies are predominantly of the IgG4 subclass (Jones et al., 1988).

The target antigen recognized by PV autoantibodies is Dsg3, a 130-kDa desmosomal core glycoprotein (Amagai et al., 1991). While patients with limited mucosal lesions have

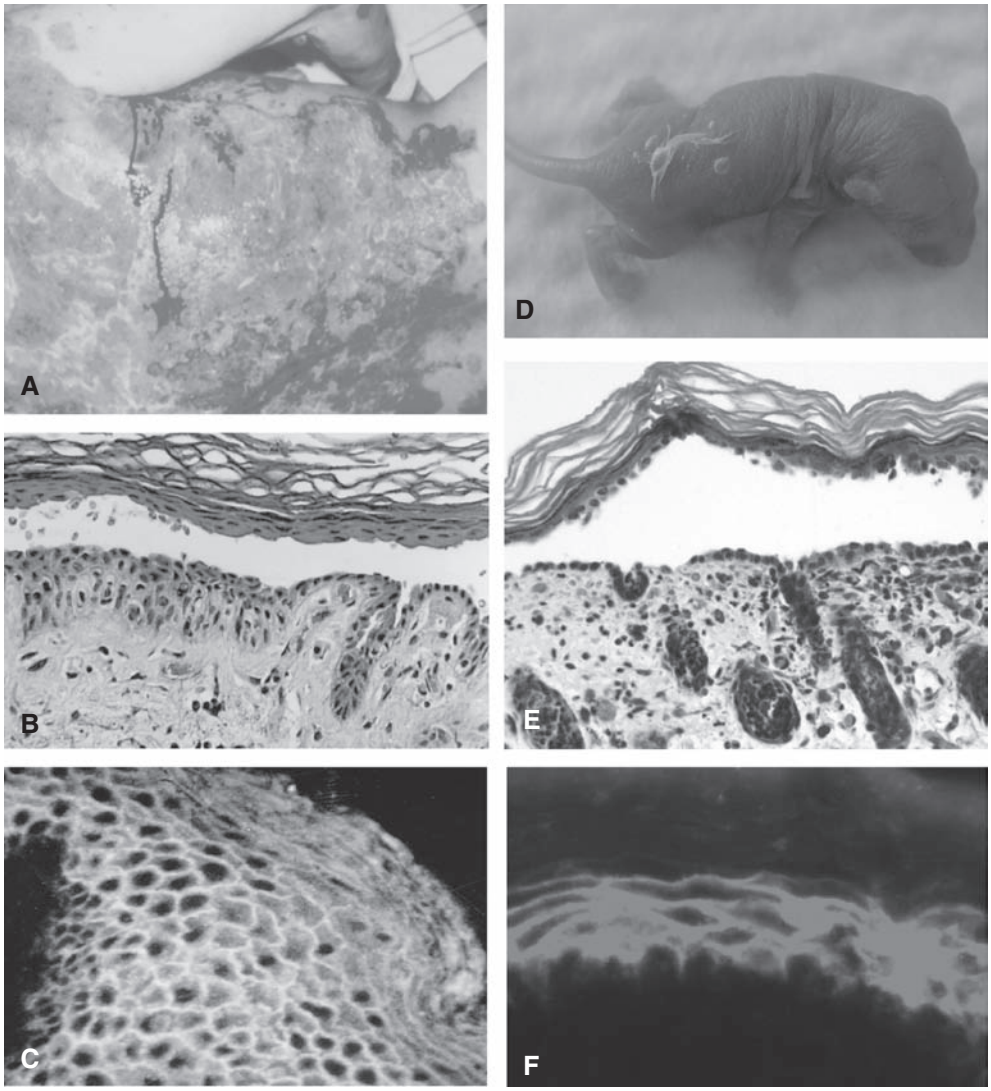


FIGURE 57.2 Left panel, clinical (A), histologic (B), and immunofluorescent (C) features of pemphigus vulgaris (PV). Right panel, mouse model of PV. The key features of the human disease are reproduced in these animals by passive transfer of PV autoantibodies (D–F). See color plate section.

autoantibodies to Dsg3 exclusively, patients who develop skin lesions may possess another population of autoantibodies against Dsg1, the autoantigen of PF (Ding et al., 1997). It is well established that about 50% of PV patients show autoantibodies against Dgs3 and Dsg1. Mucosal epithelium expresses Dsg3 predominantely, whereas the lower epidermis coexpresses both Dsg1 and Dsg3, compensating their function in pathologic states (compensation theory) (Mahoney et al., 1999a). Thus, anti-Dsg3 autoantibodies alone are not sufficient to induce spontaneous blisters in the skin, but they are pathogenic to mucosal epithelium.

Dsg1 and Dsg3 belong to the cadherin superfamily of calcium-dependent cell adhesion molecules and share high sequence homology (Getsios et al., 2004). The ectodomain of these glycoproteins are composed of four cadherin repeats (EC1–4) and a variable extracellular anchor (EC5) (Figure 57.3). The six putative calcium-binding motifs residing on the ectodomain are believed to be involved in maintaining the conformation and adhesive function of Dsg3. A putative adhesion site (RAL) is located on the EC1 domain. The ectodomain of Dsg3 has been expressed in the baculovirus system and employed to adsorb pathogenic autoantibodies from PV serum. The affinity-purified autoantibodies against Dsg3 induced suprabasilar acantholysis in neonatal mice (Amagai et al., 1992; Ding et al., 1999). These pathogenic autoantibodies recognize conformational and calcium-dependent epitopes located on the EC1 domain of Dsg3 (Sekiguch et al., 2001), which is believed to be involved in the adhesive function of the molecule.

Pathogenic Role

Several studies have demonstrated the correlation of PV autoantibody titers and disease extent and activity. Further, it was observed that these autoantibodies are present in the serum of babies born to mothers with active disease. This PV neonatorum was transient, i.e., the skin lesions healed spontaneously and the autoantibodies disappeared from the serum of the babies shortly after birth. *In vitro* studies also showed that PV IgG can induce acantholysis in skin organ cultures and cell detachment in primary epidermal cell cultures. The *in vivo* passive transfer studies, demonstrating that PV IgG is able to faithfully reproduce the disease in neonatal mice, finally attributed a pathogenic role to PV autoantibodies (see Figure 57.2, right panel) (Anhalt et al., 1982). These pioneer studies were extended to demonstrate that PV autoantibodies are able to induce disease by passive transfer in a process that is independent of activation of complement or plaminogen activator (Anhalt et al., 1986; Mascaro et al., 1997; Mahoney et al., 1999b).

Another area of intense research is addressing the molecular mechanisms of acantholysis, i.e., how PV autoantibodies trigger cell detachment. Earlier ultrastructural studies showed that PV autoantibodies bind keratinocyte cell surfaces, forming clusters of immune complexes that are internalized and fused with lysosomes (Patel et al., 1984). Mice passively transferred with PV IgG showed binding of these autoantibodies to the epidermal ICSs and initiation of the process of cell detachment in the spaces between desmosomes. This process was followed by desmosome splitting and completes cell detachment (Takahashi et al., 1985). Similar findings were also reported in an active mouse model for PV (Shimizu et al., 2004).

Two hypotheses on the molecular mechanisms of acantholysis are currently under investigation: impairment of the adhesive function of Dsg3 by steric hindrance (Diaz and Marcelo, 1978) and the activation of intracellular signaling pathways by binding of PV autoantibodies to Dsg3 (Caldelari et al., 2001; Kitajima, 2003; Rubenstein et al., 2004). Results of these studies are promising.

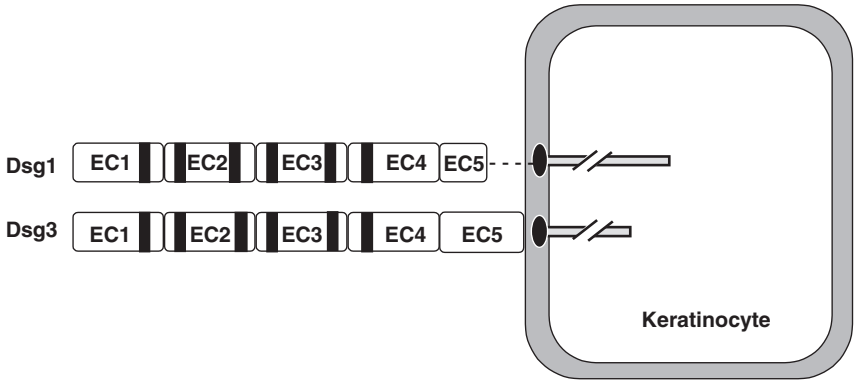


FIGURE 57.3 Molecular structure of desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3). Dsg1 and Dsg3 are structurally similar desmosomal transmembrane glycoproteins. The extracellular domain of the two molecules contains four cadherin-like domains (EC1–EC4) and a membrane-proximal domain (EC5). The black stripes represent six putative Ca^{2+}-binding motifs found in the cadherin repeats.

T-Cell Activation

Like for most other antibodies, induction of anti-Dsg3 autoantibodies is also T-cell dependent. Indeed, *in vitro* anti-Dsg3 antibody production by autoreactive B cells was abolished upon depletion of $CD4^+$ T cells (Nishifuji et al., 2000). Other investigators have successfully derived T-cell clones from PV patients that proliferated when stimulated with various Dsg3 peptides (Wucherpfennig et al., 1995; Lin et al., 1997; Veldman et al., 2004). Most of the T-cell epitopes identified are located in the first three ectodomains of Dsg3. The HLA restrictions and cytokine profiles of the T-cell clones have been characterized. It was shown that the reactivity of one of these T-cell clones with the Dsg3 peptide (residues 190–204) was restricted to the PV-associated DRB1*0402 allele, and secreted high levels of type 2 helper T (Th2)-cell cytokines (Wucherpfennig et al., 1995). Similarly, $CD4^+$ T cells from PV patients responsive to three polypeptides (residues 145–192, 240–303, and 570–614) were also restricted to HLA-DR and exhibited a Th2-like cytokine profile (Lin et al., 1997). These studies suggest that Th2 cells are relevant in the induction of Dsg3-specific autoantibodies. However, a recent study by Veldman et al. (2004) showed that not only autoreactive Th2 cells are present in PV patients, but also Th1 cells. It was suggested that both Th1 and Th2 cells may be involved in the production of PV autoantibodies, since the ratio of Dsg3-specific Th1:Th2 cells in PV patients correlated well with the serum autoantibody titers of these patients.

Interestingly, Dsg3-specific T cells are not only detected in PV patients, but also detected in healthy individuals who carry PV-susceptible HLA alleles (Veldman et al., 2004). However, in contrast to PV patients, Dsg3-responsive T-cell clones from healthy donors exhibit exclusively Th1 [interferon (IFN)-γ] cytokine profiles. Of note, nonpathogenic anti-Dsg3 autoantibodies have been detected in first-degree relatives of PV patients, which are mainly IgG1 subclass (Bhol et al., 1995; Kricheli et al., 2000).

Genetic Features

HLA alleles may play important roles in the development and progression of PV (Sinha et al., 1988; Delgado et al., 1997). Two haplotypes, DR4 (DRB1*0402 or 0406)/DQB1*0302 and DR14 (DRB1*1401, 1404, or 1405)/DQB1*0503) are strongly associated with PV in different ethnic groups. It is suggested that the association of DRB1*0402 and DQB1*0503 is primary, whereas the association of DRB1*1401 and DQB1*0302 is secondary due to linkage disequilibrium. While DRB1*0402 is highly associated with PV in Jewish and white patients, DQB1*0503 is found in non-Jewish patients.

Animal Models

Autoantibody Passive Transfer Model

As described above, neonatal mice have been used as targets for passive transfer experiments of PV IgG (Anhalt et al., 1982). The small size of these animals and their lack of hair allow the use of smaller amounts of IgG and the lesions are easily visible on the hairless skin. PV IgG transferred into neonatal mice reproduces the clinical, histologic, and immunologic features of human disease (see Figure 57.2, right panel). The disease induced in these animals is dose-dependent and also correlates with the titers of PV autoantibodies detected in the sera of the injected mice.

Active Immunization Model

Efforts to induce PV phenotype in adult mice by conventional active immunization with human Dsg3 have been largely unsuccessful. The immunized animals, however, do produce antibodies that were able to induce skin blisters when passively transferred into neonatal mice (Fan et al., 1999). Amagai et al. (2000) have developed an active mouse model of PV using a novel strategy to overcome the barrier of self-tolerance. They immunized Dsg3 knock-out mice (Koch et al., 1997) with human Dsg3, and adoptively transferred the splenocytes from these immunized animals to immunodeficient mice, Rag-$2^{-/-}$, which express Dsg3 in their epidermis. The recipient mice produced anti-Dsg3 antibodies and showed suprabasilar acantholysis histologically. Some of the animals developed spontaneous crusted erosions on the skin around the snout.

PEMPHIGUS FOLIACEUS

Clinical and Histopathologic Features

Unlike PV, PF affects skin only, where the disease manifests as superficial blisters and erosions that may lead to crusting and formation of keratotic plaques (Figure 57.4, left panel). The skin lesions initially involve the central areas of the face, scalp, the mid chest, and the upper back. From these areas the disease may spread to involve the entire body, producing an exfoliative erythroderma. Histologic examination of these lesions reveals subcorneal vesicles and acantholysis, predominantly in the upper layers of the stratum spinosum (Lever, 1965).

The classical form of PF is nonendemic and is seen sporadically in different parts of the world. A second form of PF is endemic and was originally described in certain states of Brazil under the name of fogo selvagem (FS) (Diaz et al., 1989). Endemic forms of PF have also been reported in

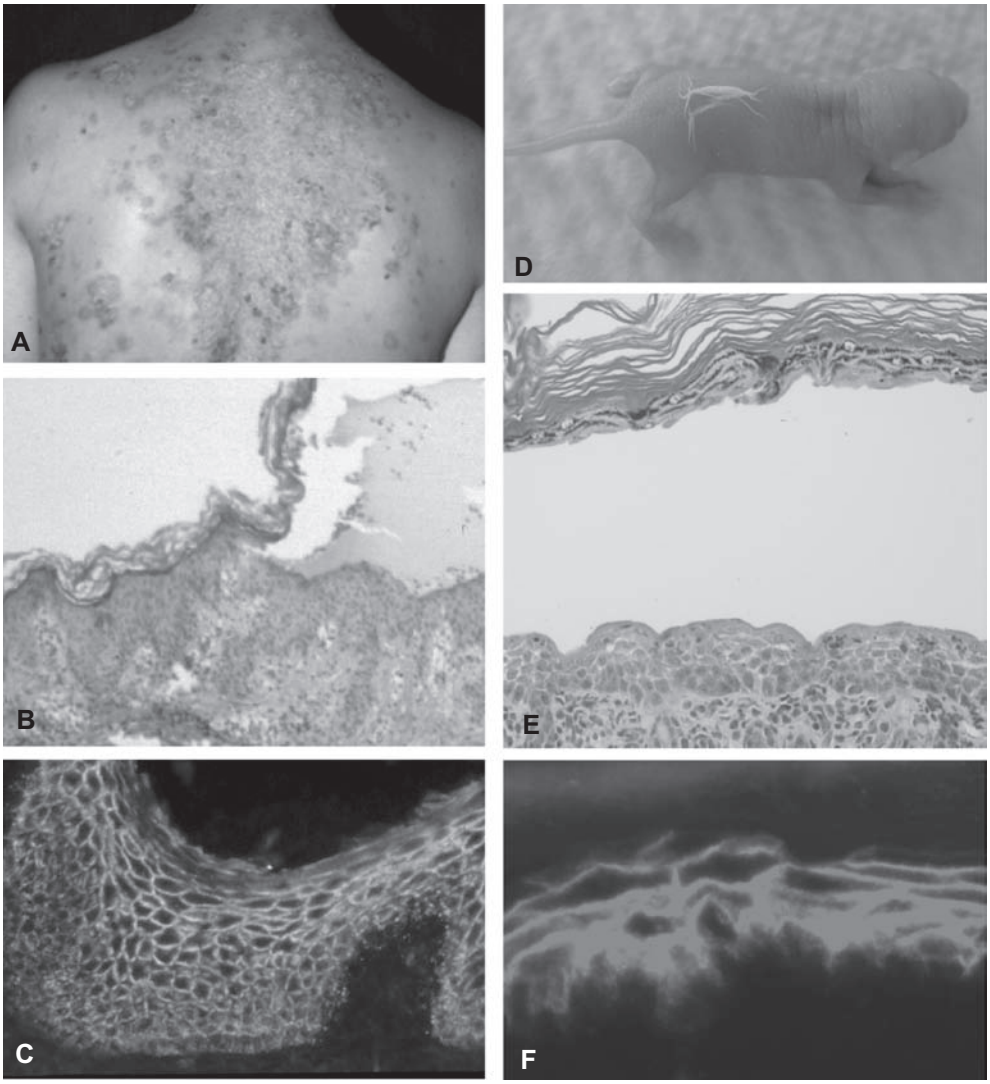


FIGURE 57.4 Left panel, clinical (A), histologic (B), and immunofluorescent (C) features of endemic pemphigus foliaceus (PF) (fogo selvagem). Right panel, mouse model of PF. The animals have been passively transferred with human PF IgG (and IgG4) and develop skin lesions (D), which histologically (E) are similar to the human disease. The human anti-Dsg1 autoantibodies are detected bound to the lesional epidermis (F) and circulating in the mouse serum. See color plate section.

Colombia and Tunisia. Epidemiologic studies suggest environmental triggers for FS development (Aoki et al., 2004).

Autoimmune Features

Autoantibodies

Similar to PV, PF patients are also characterized by anti-epidermal ICS autoantibodies, predominantly of the IgG4 subclass (Rock et al., 1989). These autoantibodies are detected bound to diseased epidermis and circulating in the serum of the patients. These titers roughly correlate with disease extent and activity. The IgG4 autoantibodies are pathogenic as demonstrated by passive transfer studies (Figure 57.4, right panel) (Rock et al., 1989). Activation of the complement cascade or plasminogen activator is not required for the induction of acantholysis by these autoantibodies in the mouse model (España et al., 1997; Mahoney et al., 1999b).

Dsg1, expressed mostly in the superficial layers of the epidermis, is the target antigen of PF autoantibodies. Affinity-purified anti-Dsg1 antibodies from PF serum are able to induce disease in neonatal mice (Amagai et al., 1995). The majority of PF autoantibodies recognize

conformational and calcium-dependent epitopes residing in the N-terminal amino acids 1–161 of Dsg1 (Sekiguch et al., 2001). We have recently shown that the anti-Dsg1 autoantibodies in FS recognize different domains of the ectodomain of the molecule. In the preclinical stage of FS or during disease remission and in normal individuals not exhibiting skin disease, the anti-Dsg1 autoantibodies recognize the EC5 domain only. On the contrary, FS anti-Dsg1 autoantibodies from patients showing active disease or during disease relapses recognize the EC1/EC2 domains (Li et al., 2003). Although the majority of PF/FS autoantibodies are of the IgG4 subclass, it appears that epitope specificity, rather than the subclass of IgG, is the driver of the pathogenicity of the autoantibodies (Li et al., 2002; Hacker-Foegen et al., 2003). Considering these findings, we hypothesized that an environmental agent(s) cross-reacts with the EC5 domain of Dsg1 and triggers an initial nonpathogenic anti-EC5 autoimmune response (Figure 57.5) (Diaz et al., 2004). In genetically predisposed individuals, the autoimmune response may undergo intramolecular epitope spreading toward pathogenic epitopes on the EC1/EC2 domains of Dsg1, which leads to disease onset.

T-Cell Activation

It has been demonstrated that $CD4^+$ T-cell lines and clones derived from peripheral blood of FS patients show a proliferative response when incubated with the Dsg1 ectodomain (Lin et al., 2000a). The stimulation of these $CD4^+$ T cells is HLA-DR restricted. Moreover, these cells secrete Th2-like cytokines. The Dsg1 epitopes recognized by these T cells have not yet been characterized.

Genetic Features

The HLA alleles strongly associated with FS are DRB1*0404, *1406, *1402 (relative risk = 14) and *0102 (relative risk = 7.3) (Moraes et al., 1997). A common epitope of LLEQRRAA, the residues 67–74 of the third hypervariable region of the DRB1 molecule, is shared by these susceptibility alleles. Similarly, a strong association of DRB1*0102 and DRB1*0404 is also found in nonendemic PF patients in France (Loiseau et al., 2000). Interestingly, two susceptible alleles in PV, DRB1*1401 and DQB1*0503, have been reported also with high frequencies in Italian and Japanese PF patients (Lombardi et al., 1999; Miyagawa et al., 1999).

Animal Models

The classic animal model of PF was developed by passively transferring IgG from PF patients into neonatal mice (Roscoe et al., 1985). These animals develop skin blisters, which show the typical histologic features of the human disease, i.e., subcorneal vesicles (Figure 57.4, right panel). The extent of the disease correlates well with the indirect IF titers of human autoantibodies detected in the mouse model. These animals develop classical ultrastructural subcorneal acantholysis (Futamura et al., 1989). The disease was induced as a complement-independent process and by monovalent PF IgG fragments as well (España et al., 1997). Despite the availability of recombinant human and murine Dsg1, studies on inducing disease by active immunization have been unsuccessful.

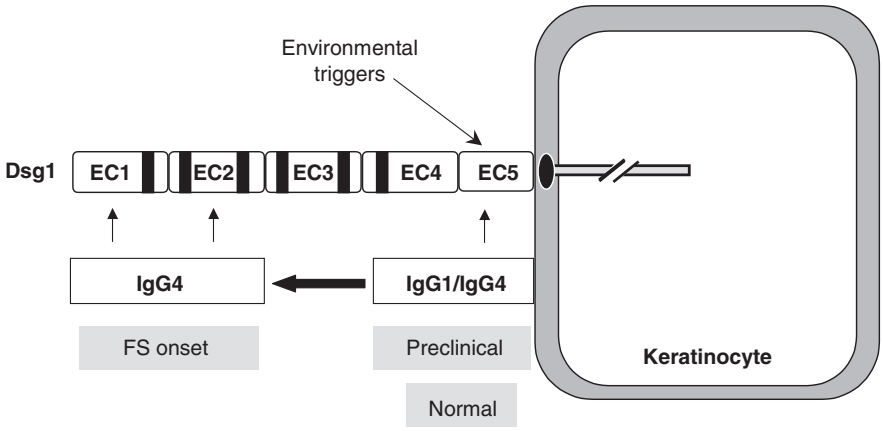


FIGURE 57.5 Proposed intramolecular epitope spreading in fogo selvagem (FS). An environmental factor(s) may initiate the production of nonpathogenic IgG1 and IgG4 antibodies against the EC5 domain of Dsg1. In certain genetically predisposed individuals, intramolecular epitope spreading results in the generation of predominantly IgG4 antibodies against the EC1 and EC2 domains of the molecule, resulting in disease.

Environmental Factors Involved in Fogo Selvagem

A remarkable characteristic of FS is its epidemiology. Several independent lines of evidence indicate that FS is precipitated by exposure to an environmental factor(s) (Aoki et al., 2004). Anti-Dsg1 autoantibodies have been detected in 55% of normal individuals living in an area exhibiting a high prevalence (3.4%) of FS (Warren et al., 2000). Remarkably, anti-Dsg1 autoantibodies are also detected in FS patients before the clinical onset of disease. These antibodies are a mixture of IgG1 and IgG4 subclasses and recognize the EC5 domain of Dsg1 (Warren et al., 2003; Li et al., 2003). A case–control epidemiologic study points to a hematophagous insect as a prime etiologic agent of FS (Diaz et al., 1989). For example, we have recently detected anti-Dsg1 EC5 antibodies in the sera of patients with onchocerciasis, leishmaniasis, and Chagas disease, exhibiting no skin lesions of FS (Diaz et al., 2004). It is known that hematophagous vectors (black flies, sand flies, and kissing bugs, respectively) are involved in the transmission of the respective tropical disease. It is postulated that the saliva of these vectors may contain Dsg1 cross-reactive antigens showing certain degrees of sequence homology with the EC5 domain of the molecule, thus inducing a nonpathogenic anti-EC5 domain autoantibody response. This finding may be an example of molecular mimicry. Identification of environmental triggers for FS would shed some light on the pathogenetic mechanisms involved in a human organ-specific autoimmune disease.

OTHER TYPES OF PEMPHIGUS

Paraneoplastic Pemphigus

PNP is a severe mucocutaneous disease that runs a usually lethal course in patients with underlying lymphoproliferative malignancy, i.e., non-Hodgkin lymphoma, chronic lymphocytic leukemia, and Castleman diseases (Anhalt et al., 1990; Anhalt, 2004). Patients exhibit severe stomatitis and skin lesions that may be vesiculobullous, or show erythema multiforme or lichen planus features. In approximately 30% of patients there is a bronchiolitis obliterans syndrome with severe respiratory insufficiency.

Histologic examination of the skin lesions reveals keratinocyte necrosis, basal cell vacuolization, and suprabasilar acantholysis. Direct IF shows deposition of IgG and C3 in the epidermal intercellular spaces (as in pemphigus) and along the basement membrane zone (BMZ). The unique and characteristic immunologic finding in PNP patients is the polyclonal autoantibody response against structural antigens of the desmosome and hemidesmosome. The antigens that have been characterized, besides Dsg3 and Dsg1, are members of the plakin family of proteins, including desmoplakin I and II, BP230, envoplakin, periplakin, and plectin, which are components of the desmosomal and hemidesmosomal plaques. Removal of anti-Dsg3 autoantibodies from PNP sera abrogates the pathogenicity of the IgG fraction (Amagai et al., 1998). Affinity-purified anti-Dsg3 antibodies are able to induce skin lesions in neonatal mice. Autoantibody-mediated disease may explain only part of the complex epithelial injury found in these patients. Other mechanisms of tissue injury that has been proposed are those of cell-mediated cytotoxicity.

It has been proposed that the autoantibody response in PNP is primarily directed against tumor antigens that cross-react with epithelial structural proteins. Additionally, the tumor may produce cytokines that modulate the autoimmune response. Interestingly, Wang et al. (2004) have demonstrated that tumor B cells are able to produce anti-epidermal antibodies.

The diagnosis of PNP is made clinically, histologically, and immunologically. By indirect IF, the serum of PNP patients typically stains rat bladder epithelium, which produces negative results when stained with the serum of PV or PF. Immunoprecipitation techniques using radiolabeled keratinocyte extracts reveal reactivity with the plakin proteins.

Drug-Induced Pemphigus

Certain drugs, particularly thiol-containing drugs, such as penicillamine and captopril, may induce clinical and histologic features of PF, and less commonly PV. The majority of drug-induced pemphigus patients exhibit circulating autoantibodies to the epidermal ICSs and epidermal-bound IgG. In cases of drug-induced PF, the autoantibodies recognize Dsg1. Some of these drugs have been shown to cause acantholysis directly *in vitro*. Drug-induced pemphigus may be transient and usually resolves upon withdrawal of the medication, although in rare cases the disease may run a chronic course. The mechanisms involved in the induction of the autoimmune response by drugs in pemphigus remain obscure.

IgA Pemphigus

This clinical variant of pemphigus is unique because of its clinical and histologic phenotype. Clinically, patients show superficial clusters of small vesicles and pustules, in some cases producing annular patterns. The Nikolski sign is positive and the great majority of patients show no mucosal lesions. The histologic features show, in addition to the acantholysis, an intense neutrophilic infiltrate in the epidermis. This infiltrate may be subcorneal or located in the mid-epidermis. The immunologic hallmark of this form of pemphigus is the presence of IgA class autoantibodies directed

to the epidermal ICSs (like PV or PF). The antigen recognized by some of these antibodies has been shown to be another desmosomal core cadherin, named desmocollin 1 (Hashimoto, 2001). The pathogenic role of these IgA autoantibodies has not been demonstrated. Further, the intense neutrophilic infiltrate of the epidermis and a rapid response of the disease to dapsone (a neutrophilic targeted drug) might indicate a unique IgA-mediated pathway of tissue damage.

BULLOUS PEMPHIGOID

Clinical and Histopathologic Features

BP is the most common autoimmune bullous disease affecting primarily the skin (Lever, 1965). It is estimated that about 5–10 new cases are seen yearly in a large hospital referral center in the USA. BP occurs most frequently in the elderly (60–80 years of age) and affects men and women equally. The skin lesions usually begin as urticarial plaques or erythematous papules, which evolve into large, tense bullae filled with clear fluid (Figure 57.6, left panel). Histologic examination of these lesions shows detachment of the epidermis from the dermis producing subepidermal blisters. The upper dermis exhibits an inflammatory infiltrate, including eosinophils, neutrophils, lymphocytes, and monocytes/macrophages. The predominant inflammatory cells in early lesions are usually eosinophils. Histologic evidence of mast cell degranulation has also been reported (Wintroub et al., 1978).

Autoimmune Features

Autoantibodies

BP patients have circulating and tissue-bound IgG autoantibodies directed against two hemidesmosomal proteins (Mutasim et al., 1985) known as the BP230 (BPAG1) and the BP180 (BPAG2, type XVII collagen) antigens (Jordon et al., 1967; Stanley et al., 1981; Labib et al., 1986). The autoantibodies to BP180 are predominantly of the IgG1 and IgG4 subclasses (Laffitte et al., 2001). In addition to IgG autoantibodies, IgE autoantibodies to BP180 are found in the majority of untreated BP patients (Dimson et al., 2003). Only antibodies to BP180 have been demonstrated to be pathogenic in neonatal mice (Figure 57.6, right panel) (Liu et al., 1993).

The BP230 antigen is an intracellular hemidesmosmal plaque protein belonging to the plakin family (Tanaka et al., 1991), whereas the BP180 antigen is a hemidesmosomal transmembrane protein belonging to the collagen family (Giudice et al., 1992). The BP180 protein shows a type II orientation, with its N-terminal region toward the intracellular hemidesmosomal plaque and its C-terminal half projecting into the extracellular milieu of the BMZ. The anti-BP180 autoantibodies from BP patients recognize multiple epitopes that cluster within the noncollagen 16A (NC16A) domain of the BP180 ectodomain (Giudice et al., 1993) (see Figure 57.8A). It has been reported that the serum levels of autoantibodies to BP180 NC16A in patients are directly correlated to disease severity (Haase et al., 1998).

T-Cell Activation

It has been reported that BP180-specific autoreactive T cells recognize epitopes located predominantly on the NC16A domain of the molecule (Budinger et al., 1998; Lin et al., 2000b). These T lymphocytes express $CD4^+$ memory T-cell surface markers and exhibit a Th1/Th2 mixed cytokine profile.

Genetic Features

HLA studies involving American, Japanese, and British BP patients showed no significant association between the disease and HLA-A, -B, -C, and -DR loci, while a marked increase in the HLA-DR5 allele was found in BP patients from France (Seignate et al., 1987; Venning et al., 1989). More recently, studies have demonstrated that HLA-DQB1*0301 is associated with BP in white patients (Delgado et al., 1996), whereas DRB1*0403, 0406, or DRB1*1101 have high frequency in Japanese patients (Okazaki et al., 2000).

Animal Models

IgG Passive Transfer Model

Early studies on passive transfer of human BP IgG containing anti-BP180 and anti-BP230 autoantibodies to neonatal mice were unsuccessful. It was found later that the murine BP180 protein did not react with human anti-BP180 antibodies largely due to differences at the amino acid level of the NC16A of the molecule. This problem was overcome by raising rabbit antibodies against a segment of murine BP180 homologous to the human epitope. It was demonstrated that these anti-BP180 antibodies were pathogenic if passively transferred into neonatal mice. The animals recapitulate the key clinical and histologic features of the human disease (see Figure 57.6, right panel) (Liu et al., 1993).

In contrast to the PV and PF mouse models, the subepidermal blistering in mice induced by anti-BP180 antibodies depends on complement activation and a subsequent cascade of inflammatory events, including mast cell degranulation and neutrophil infiltration (Figure 57.7). Proteolytic enzymes (neutrophil elastase and gelatinase B) released from recruited neutrophils are the final effector molecules

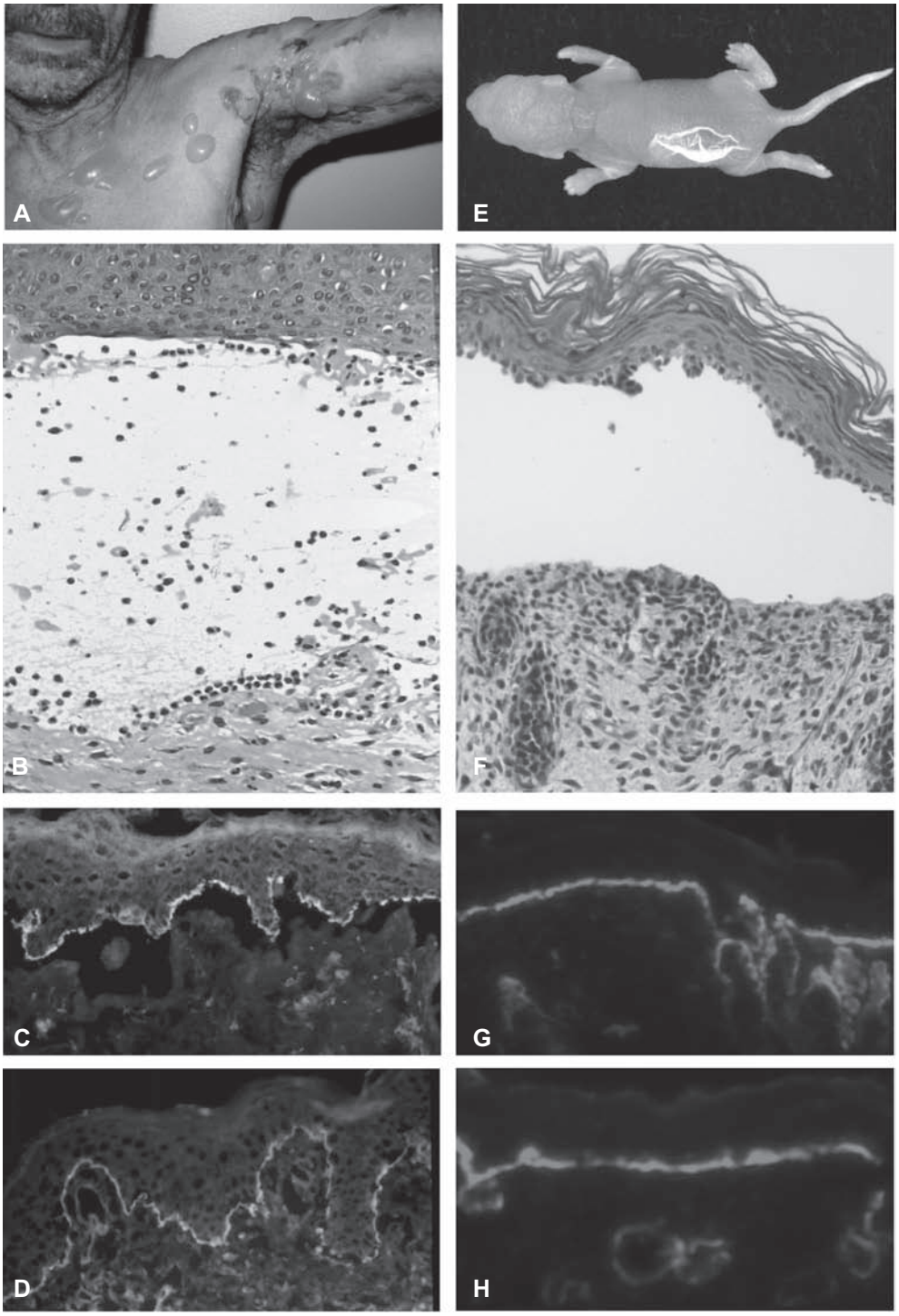


FIGURE 57.6 Bullous pemphigoid (BP). Left panel, key clinical, histologic and immunofluorescent features of BP. Large, tense blisters and erosions are seen in flexural areas (A). The histology reveals subepidermal blisters (B). Direct immunofluorescence (IF) shows *in situ* deposition of IgG (C) and C3 (D) at the cutaneous basement membrane zone (BMZ). Right panel, mouse injected with rabbit antimurine BP180 antibodies. The animals develop skin blisters (E). The histology of these lesions shows dermal–epidermal separation with an inflammatory infiltration (F). Direct IF shows *in situ* deposition of rabbit IgG (G) and mouse C3 (H) at the BMZ. See color plate section.

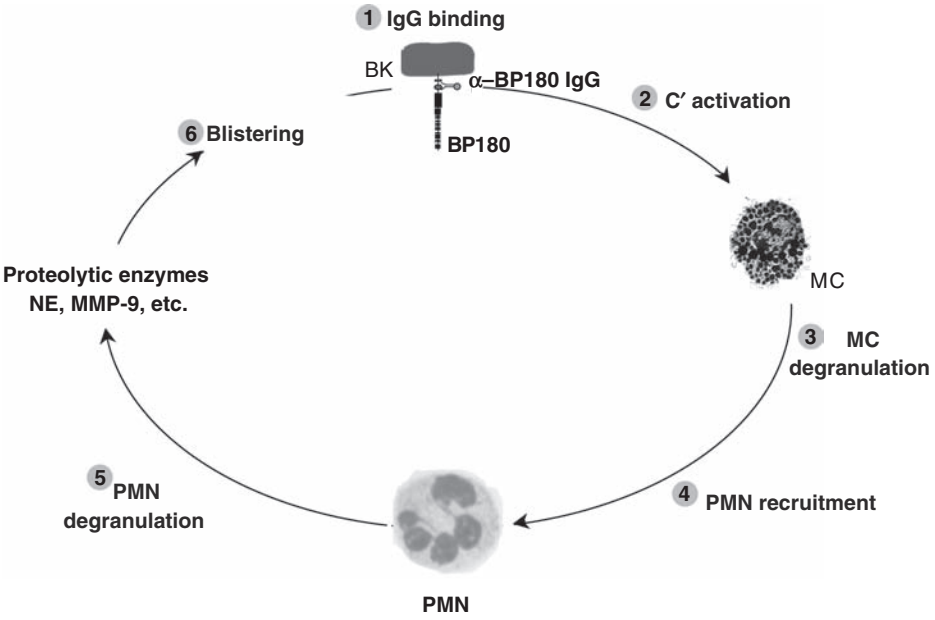


FIGURE 57.7 Proposed mechanism of subepidermal blister formation in experimental bullous pemphigoid. Subepidermal blistering is an inflammatory process that develops in the following steps: 1, anti-BP180 IgG binds to the pathogenic epitope of BP180 antigen in basal keratinocytes (BK); 2, the molecular interaction between BP180 antigen and anti-BP180 IgG activates the complement system (C′); 3, C′ activation products C3a and C5a cause mast cells (MC) to degranulate; 4, proinflammatory mediators released by MC recruit neutrophils (PMN); 5, infiltrating PMNs release proteolytic enzymes, including neutrophil elastase (NE) and gelatinase B (MMP-9); 6, proteolytic enzymes degrade BP180 and other extracellular matrix proteins, leading to subepidermal blistering.

that cause the epidermal–dermal separation seen in skin lesions (Liu, 2004).

Active Immunization Model

Recently, we have developed a new BP animal model by active immunization of adult C57BL/6J mice with a recombinant murine BP180 antigen. Figure 57.8A and B shows the ectodomains of the human and murine BP180 antigen, respectively, and the selected region of the murine protein used to immunize the animals. Three weeks after the boosting immunization, some of the immunized mice developed subepidermal blisters and a dermal inflammatory neutrophilic infiltrate. Perilesional skin of these animals showed *in vivo* deposition of IgG and C3 at the BMZ (Figure 57.8C). The autoimmune response in these animals is an MHC class II-restricted T-cell response (Liu, 2004). Remarkably, BALB/c and SJL mice were unable to mount a pathogenic autoantibody response upon immunization with the murine BP180 antigen.

OTHER SUBEPIDERMAL BULLOUS DISEASES

Herpes Gestationis (Pemphigoid Gestationis)

HG, also known as pemphigoid gestationis (PG), is a variant of BP affecting pregnant women primarily. The incidence is about 1 in 50,000 pregnancies. The disease usually develops during the second or third trimester of pregnancy or in the immediate postpartum period. HG has rarely been associated with underlying trophoblastic tumors. Similar to BP, HG patients develop subepidermal blisters and linear deposition of C3 at the BMZ (Provost and Tomasi, 1973). The sera of these patients is usually negative by conventional indirect IF assays but become positive when complement fixation to the skin is assayed (HG factor) (Provost and Tomasi, 1973). The HG factor was found to be a complement-fixing autoantibody against the BP180 antigen (Jordon et al., 1976; Katz et al., 1976; Morrison et al., 1988; Giudice et al., 1993). This autoantibody is predominantly IgG1 and recognizes the same epitope (within the BP180 NC16A domain) as BP autoantibodies. BP180-specific T cells also recognize the BP180 NC16A and express a $CD4^+$ Th1 memory phenotype (Lin et al., 1999). Like BP, HG is also associated with DQB1*0301. Interestingly, less than 5% of the neonates of mothers with HG develop transient subepidermal vesicles with maternal IgG bound to perilesional skin. The mechanism of autoimmunity in HG and the role of pregnancy are not fully understood.

Cicatricial Pemphigoid

CP, also known as mucous membrane pemphigoid, is a group of heterogeneous diseases characterized by subepithelial blistering involving mucous membranes exclusively

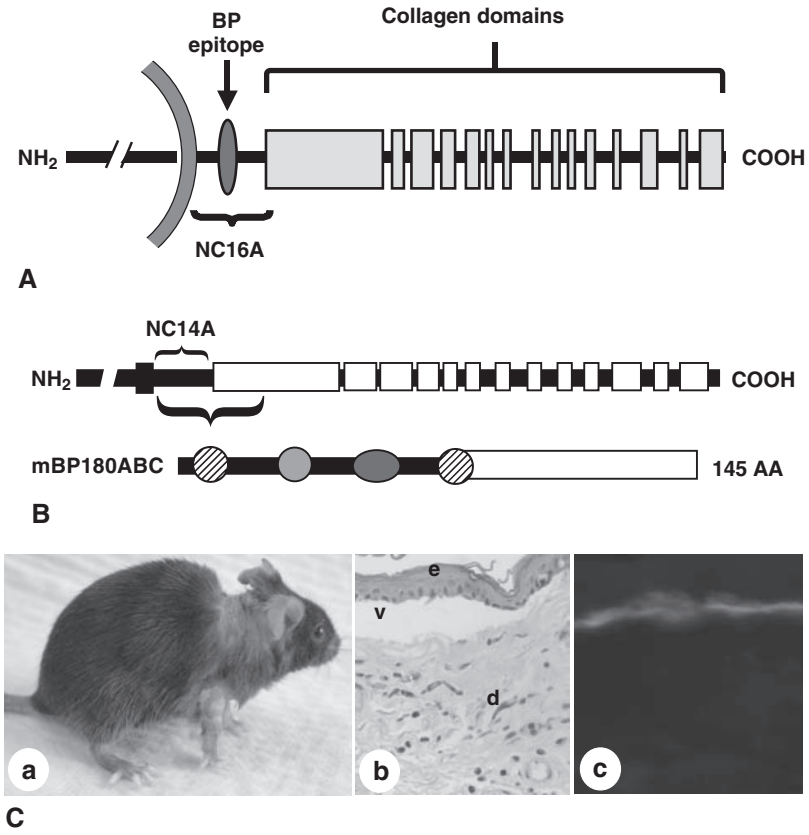


FIGURE 57.8 Active model of bullous pemphigoid (BP). *A*, Schematic diagram of the human BP180 antigen. The C-terminal extracellular region is made up of a series of collagen-like domains (yellow rectangles) interrupted by non-collagen domains (black bars). The major epitope (NC16A) recognized by BP autoantibodies is depicted by the red oval. *B*, Mouse BP180 antigen used to immunize mice. This antigen (referred to as mBP180ABC) contains the largest non-collagen domain (NC14A) and part of the largest collagen-like domain. mBP180ABC harbors four epitopes, one of which (red) is pathogenic. *C*, 8–10-week-old C57BL/6J mouse immunized with mBP180ABC. Some of these mice developed BP-like skin lesions, starting the third week after the boosting immunization (a). Histologic examination shows subepidermal blistering (b). Direct immunofluorescence shows *in vivo* deposition of autoantibodies at the basement membrane zone (c). d, dermis; e, epidermis; v, vesicle. See color plate section.

(Korman and Cooper, 2000). The oral and ocular surfaces are most commonly involved, while skin lesions occur only in one-third of CP patients. A striking clinical feature of CP is that healing of the lesions leads to scarring and dysfunction of the affected organs. For example, ocular involvement may cause blindness due to corneal scarring and fibrosis. Direct IF reveals linear deposition of IgG, IgA or C3 along the epithelial BMZ (Egan et al., 2003). Similarly esophageal involvement may lead to strictures of this organ (Egan et al., 1999). CP is also associated with DQB1*0301 (Delgado et al., 1996).

The heterogeneity of CP is reflected in the sites involved by the autoimmune disease and the syndromes it may produce (ocular only, oral mucosal only, mucocutaneous, and multiple sites). These syndromes may reflect the tissue specificity of targeted autoantigens. The majority of CP patients show circulating IgG autoantibodies against BP180 antigen (Balding et al., 1996). Some of these CP autoantibodies recognize the C-terminal domain of BP180, which is different from BP. A subset of CP patients exhibits circulating autoantibodies against laminin 5, also known as epilagrin (Domloge-Hultsch et al., 1994; Egan et al., 1999). Experimentally, it was shown that antilaminin 5 antibodies are able to induce subepidermal blisters in neonatal mice (Lazarova et al, 1996). Many patients with ocular involvement have autoantibodies against $\alpha 6\beta 4$ integrin (Tyagi et al., 1996). The pathogenic role of the various autoantibodies found in CP and their link to scarring remain to be elucidated. An association of cancer with some CP cases with antilaminin 5 autoantibodies has been reported, but it is not known why these CP patients have an increased relative risk for cancer (Egan et al., 2003).

Lichen Planus Pemphigoides

LPP is characterized by bullae arising on lichen planus papules. Some authors suggest LPP is the concurrence of lichen planus (LP) and BP, while others consider LPP as an

entity in its own right. In contrast to BP, LPP is seen in younger patients and the blisters are often distributed on the distal extremities. Like BP, linear deposition of IgG and C3 is found along the BMZ. LPP IgG autoantibodies recognize an epitope in NC16A of the BP180 antigen that is not reactive with BP autoantibodies (Zillikens et al., 1999).

Linear IgA Disease

LAD is a subepidermal blistering disorder characterized by pruritic lesions and linear deposition of IgA autoantibodies at the BMZ (Nemzer et al., 2000). Mucosal membrane involvement is common (60–80%). LAD can be subdivided into adult-onset, childhood-onset, and drug-induced LAD (Zone et al., 2004). The histology of lesional and perilesional skin reveals subepidermal vesicles with neutrophilic infiltration along the BMZ and in the superficial dermis.

IgA autoantibodies in LAD serum react with multiple antigenic peptides derived from the BP180 ectodomain. The most common antigenic peptides for the major type of LAD (the lamina lucida type), detected by immunoblotting using epidermal extracts, are the 97-kDa protein (LABD97) and the 120-kDa antigen (LAD-1), which are considered to be the proteolytic fragments of the BP180 polypeptide (Zone et al., 2004). These findings suggest that the majority of LAD autoantibodies recognize epitopes on LABD97 and LAD-1, which are different from those bound by BP autoantibodies on the intact BP180 molecule.

Epidermolysis Bullosa Acquisita

EBA is an acquired subepithelial blistering disease of the skin and mucous membranes mediated by IgG autoantibodies against type VII collagen (O'Toole and Woodley, 2000). Lesions occur predominantly on areas of trauma and often heal with scarring, like CP. Subepidermal blister are formed as a result of detachment of the epidermis at the level of the sub-basal lamina densa, as demonstrated by ultrastuctural studies (see Figure 57.1). EBA is associated with HLA-DR2 (Gammon et al., 1988).

The target antigen of EBA is type VII collagen (C-VII), which is confined to anchoring fibers of the sub-basal lamina densa region of the skin. EBA autoantibodies react with four major epitopes within the N-terminal noncollagenous NC1 domain of C-VII. It is hypothesized that binding of these autoantibodies may interfere with dimer formation of the C-VII molecule or may impair the association of C-VII with its ligands, laminin 5 and fibronectin. It has also been suggested that complement activation by the autoantibodies induces inflammation and blistering. EBA IgG autoantibodies, when injected into neonatal mice, induced microscopic subepidermal blisters below the lamina densa similar to the human disease. Furthermore, rabbit antihuman type VII collagen NC1 domain IgG also induced EBA-like lesions in adult hairless mice and nude mice bearing human skin grafts (Chen et al., 2004).

Dermatitis Herpetiformis

DH is an IgA-mediated skin blistering disease characterized by intensive pruritic erythematous papules and vesicles symmetrically distributed over extensor surfaces (Bagheri and Hall, 2000). The skin rash is gluten dependent and responds to a gluten-free diet. Skin biopsies from DH lesions show a characteristic neutrophilic infiltration in the upper dermis involving the dermal papillae. Perilesional and normal skin in DH shows a granular deposition of IgA along the BMZ. It has been shown that DH patients possess in their sera IgA autoantibodies that recognize epidermal transglutaminase (Sardy et al., 2002). It is unknown if these antitransglutaminase autoantibodies are pathogenic or an epiphenomenon, but the autoantibodies can be used as a sensitive serologic marker for DH. A remarkable feature of DH is its high association with celiac disease, another IgA related gluten-sensitive (GSE) disorder involving the small intestine. Patients with DH and GSE share an increased expression of the HLA-A1, HLA-B8, and HLA-DR3, and HLA-DQA1*0505 and DQB1*02 genes (Sollid, 2000) and a humoral response to transglutaminases. Both diseases are exacerbated by the intake of gluten-containing food. The mechanisms of gluten-induced autoimmunity and the relationship of the IgA autoantibody response to transglutaminase in DH remain unclear (see Chapter 51).

TREATMENT

The aim of therapy in all autoimmune blistering diseases of the skin is to abrogate the pathogenic autoantibodies and to decrease the tissue inflammatory response triggered by some of these antibodies. Elimination of pathogenic autoantibodies from the patient is accomplished by immunosuppression or plasmapheresis. The inflammatory response in the skin may be modulated by using topical or systemic steroids or drugs that are known to impair the effector function of neutrophils such as dapsone. It is understood that systemic steroids may modulate the antibody production and also benefit the local inflammatory response in the skin.

Systemic corticosteroids are the first line of therapy for patients with all clinical forms of pemphigus and pemphigoid. Doses of prednisone in the range of 1.0 mg/kg every morning are used initially. If patients continue to develop new lesions, the dose of prednisone can be increased incrementally to 2.0 mg/kg; however, in our practice we rarely exceed a total dose of 100 mg/day. The use of azathioprine, cyclophosphamide, methotrexate or mycophenolate mofetil as adjunctive therapy is beneficial in controlling

the disease and enhancing the chances of inducing a prolonged remission. They are excellent steroid-sparing agents. Patients must be fully evaluated clinically and tested for hematologic, renal and liver function parameters before starting treatment. The doses and side effects for any of these drugs are described in detail in dermatologic textbooks.

The mortality rate for patients with pemphigus has been reduced to less than 10%; however, these patients are prone to develop severe complications of the therapy, i.e., osteoporosis, diabetes, hypertension, and obesity.

It is well documented that some patients are "resistant" to all these therapeutic modalities. These rare patients may benefit from plasmapheresis or parenteral infusions of human immunoglobulin. Recently, it has been reported that therapy with anti-CD20 humanized monoclonal antibodies may induce clinical and serologic remissions in severe cases of pemphigus (Salopek et al., 2002; Dupuy et al., 2004).

Dapsone is the drug of choice in DH. It exerts its anti-inflammatory effects through direct action on the neutrophil by interfering with the myeloperoxidase–hydrogen peroxide–halide-mediated cytotoxic system in neutrophils. Dapsone can cause hemolysis and methemoglobinemia and therefore, it is mandatory to assay the levels of the enzyme glucose 6-phosphate dehydrogenase (G6PD) prior to beginning dapsone therapy as G6PD-deficient patients may experience severe hemolysis. Other side effects of dapsone, such as hepatitis and thrombocytopenia, may need to be monitored. Patients with LAD or IgA pemphigus also show a remarkable clinical response to dapsone. These diseases may be kept in remission with small doses of dapsone. Additionally, CP and certain cases of BP can respond favorably to dapsone.

Therapy for patients with EBA is limited since most of the drugs used to control other autoimmune blistering diseases do not change the course of this disease in most patients. The use of systemic steroids, immunosuppressive drugs or dapsone may be individualized in each patient. Removal of underlying malignancy, steroids, immunosuppressive drugs and supportive therapy has been attempted in PNP patients. The prognosis for these patients, however, is poor.

Acknowledgments

This work was supported in part by U.S. Public Health Service Grants R01-AI40768, R01 AI061430 (Z.L.), R37-AR32081, R01-AR32599 (L.A.D.), and training grant T32-AR07577 from the National Institutes of Health (L.A.D.).

References

Amagai, M., Klaus-Kovtun, V., and Stanley, J.R. (1991). Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell. 67*, 869–877.

Amagai, M., Karpati, S., Prussick, R., Klaus-Kovtun, V., and Stanley, J.R. (1992). Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. *J. Clin. Invest. 90*, 919–926.

Amagai, M., Hashimoto, T., Green, K.J., Shimizu, S., and Nishikawa, T. (1995). Antigen-specific immunoabsorption of pathogenic autoantibodies in pemphigus foliaceous. *J. Invest. Dermatol. 104*, 895–901.

Amagai, M., Nishikawa, T., Nousari, H.C., Anhalt, G.J., and Hashimoto, T. (1998). Antibodies against desmoglein 3 (pemphigus vulgaris antigen) are present in sera from patients with paraneoplastic pemphigus and cause acantholysis *in vivo* in neonatal mice. *J. Clin. Invest. 102*, 775–782.

Amagai, M., Kazuyuki, T., Suzuki, H., Nishifuji, K., Koyasu, S., and Nishikawa, T. (2000). Use of autoantigen-knockout mice in development and active autoimmune disease model for pemphigus. *J. Clin. Invest. 105*, 625–631.

Anhalt, G.J., Labib, R.S., Voorhees, J.J., Beals, T.F., and Diaz, L.A. (1982). Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N. Engl. J. Med. 306*, 1189–1196.

Anhalt, G.J., Till, G.O., Diaz, L.A., Labib, R.S., Patel, H.P., and Eaglstein, N.F. (1986). Defining the role of complement in experimental pemphigus vulgaris in mice. *J. Immunol. 137*, 2835–2840.

Anhalt, G.J., Kim, S., Stanley, J.R., Korman, N.J., Jabs, D.A., Kory, M., Izumi, H., Ratrie, H., Mutasim, D., Ariss-Abdo, L., and Labib, R.S. (1990). Paraneoplastic pemphigus. An autoimmune mucocutaneous disease associated with neoplasia. *N. Engl. J. Med. 323*, 1729–1735.

Anhalt, G.J. and Diaz, L.A. (2001). Prospects for autoimmune disease: Research advances in pemphigus. *JAMA. 285*, 652–654.

Anhalt, G.J. (2004). Paraneoplastic pemphigus. *J. Investig. Dermatol. Symp. Proc. 9*, 29–33.

Aoki, V., Millikan, R.C., Rivitti, E.A., Hans-Filho, G., Eaton, D.P., Warren, S.J., Li, N., Hilario-Vargas, J., Hoffmann, R.G., and Diaz, L.A. (2004). Environmental risk factors in endemic pemphigus foliaceus (fogo selvagem). *J. Investig. Dermatol. Symp. Proc. 9*, 34–40.

Bagheri, B. and Hall, R.P. III. (2000). Dermatitis herpetiformis. In R.E. Jordan (ed.), *Atlas of Bullous Disease* (pp. 99–107). New York: Churchill Livingstone.

Balding, S.D., Prost, C., Diaz, L.A., Bernard, P., Bedane, C., Aberdam, D., and Giudice, G.J. (1996). Cicatricial pemphigoid autoantibodies react with multiple sites on the BP180 extracellular domain. *J. Invest. Dermatol. 106*, 141–146.

Beutner, E.H., and Jordon, R.E. (1964). Demonstration of skin antibodies in sera of patients with pemphigus vulgaris by indirect immunofluorescent staining. *Proc. Soc. Exp. Biol. Med. 117*, 505–510.

Bhol, K., Natarajan, K., Nagarwalla, N., Mohimen, A., Aoki, V., and Ahmed, A.R. (1995). Correlation of peptide specificity and IgG subclass with pathogenic and nonpathogenic autoantibodies in pemphigus vulgaris: a model for autoimmunity. *Proc. Natl. Acad. Sci. USA 92*, 5239–5243.

Budinger, L., Borradori, L., Yee, C., Eming, R., Ferencik, R., Grosse-Wilde, H., Merk, H.F., Yancey, K.B., and Hertl, M. (1998). Identification and characterization of autoreactive $CD4^+$ T cell responses to the extracellular domain of bullous pemphigoid antigen 2 in patients with bullous pemphigoid and normals. *J. Clin. Invest. 102*, 2082–2089.

Caldelari, R., de Bruin, A., Baumann, D., Suter, M.M., Bierkamp, C., Balmer, V., and Muller, E. (2001). A central role for the armadillo protein plakoglobin in the autoimmune disease pemphigus vulgaris. *J. Cell. Biol. 153*, 823–834.

Chen, M., Saadat, P., Atha, T., Lipman, K., Ram, R., and Woodley, D.T. (2004). A passive transfer model of epidermolysis bullosa acquisita using antibodies generated against the noncollagenous (NC1) domain of human type VII collagen on human skin grafted onto mice. *J. Invest. Dermatol. 122*, A11.

Christophoridis, S., Budinger, L., Borradori, L., Hunziker, T., Merk, H.F., and Hertl, M. (2000). IgG, IgA and IgE autoantibodies against the ectodomain of BP180 in patients with bullous and cicatricial pemphigoid and linear IgA bullous dermatosis. *Br. J. Dermatol. 143*, 349–355.

Civatte, A. (1943). Diagnostic histopathologique de la dermatite polymorphe doulouresseou maladie de During-Brocq. *Ann. Dermatol. Syph. 3*, 1–30.

Delgado, J.C., Turbay, D., Yunis, E J., Yunis, J.J., Morton, E.D., Bhol, K., Norman, R., Alper, C.A., Good, R.A., and Ahmed, R.A. (1996). Common major histocompatibility complex class II allele HLA-DQB1*0301 is present in clinical variants of pemphigoid. *Proc. Natl. Acad. Sci. USA 93*, 8569–8571.

Delgado, J.C., Hameed, A., Yunis, J.J., Bhol, K., Rojas, A.I., Rehman, S.B., Khan, A.A., Ahmad, M., Alper, C.A., Ahmed, A.R., and Yunis, E.J. (1997). Pemphigus vulgaris autoantibody response is linked to HLA-DQB1*0503 in Pakistani patients. *Hum. Immunol. 57*, 110–119.

Diaz, L.A. and Marcelo, C.L. (1978). Pemphigoid and pemphigus antigens in cultured epidermal cells. *Br. J. Dermatol. 98*, 631–637.

Diaz, L.A., Sampaio, S.A.P., Rivitti, E.A., Martins, C.R., Cunha, P.R., Lombardi, C., Almeida, F.A., Castro, R.M., Macca, M.L., Lavrado, C., Filho, G.H., Borges, P., Chaul, A., Minelli, L., Empinotti, J.C., Friedman, H., Campbell, I., Labib, R.S., and Anhalt, G.J. (1989). Endemic pemphigus foliaceus (Fogo Selvagem): II. Current and historic epidemiologic studies. *J. Invest. Dermatol. 92*, 4–12.

Diaz, L.A., and Giudice, G.J. (2000). End of the century overview: skin blisters. *Arch. Dermatol. 136*, 106–112.

Diaz, L.A., Arteaga, L.A., Hilario-Vargas, J., Valenzuela, J.G., Li, N., Warren, S., Aoki, V., Hans-Filho, G., Eaton, D., dos Santos, V., Nutman, T.B., Antunez, A., Qaqish, B.F., Sampaio, S.A.P., and Rivitti E.A. (2004). Anti-desmoglein-1 antibodies in onchocerciasis, leishmaniasis and Chagas disease suggest a possible etiological link to Fogo Selvagem. *J. Invest. Dermatol*. *123*, 1045–1051.

Dimson, O.G., Giudice, G.J., Fu, C.L., Van den Bergh, F., Warren, S.J., Janson, M.M., and Fairley, J.A. (2003). Identification of a potential effector function for IgE autoantibodies in the organ-specific autoimmune disease bullous pemphigoid. *J. Invest. Dermatol. 120*, 784–788.

Ding, X., Aoki, V., Mascaro, J.M. Jr, Lopez-Swiderski, A., Diaz, L.A., and Fairley, J.A. (1997). Mucosal and mucocutaneous (generalized) pemphigus vulgaris show distinct autoantibody profiles. *J. Invest. Dermatol. 109*, 592–596.

Ding, X., Diaz, L.A., Fairley, J.A., Giudice, G.J., and Liu, Z. (1999). The antidesmoglein-1 autoantibodies in pemphigus vulgaris sera are pathogenic. *J. Invest. Dermatol. 112*, 739–743.

Domloge-Hultsch, N., Anhalt, G.J., Gammon, W.R., Lazarova, Z., Briggaman, R., Welch, M., Jabs, D.A., Huff, C., and Yancey, K.B. (1994). Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. *Arch. Dermatol. 130*, 1521–1529.

Dupuy, A., Viguier, M., Bedane, C., Cordonliani, F., Blaise, S., Aucouturier, F., Bonnetblanc, J.-M., Morel, P., Dubertret, L., and Bachelez, H. (2004). Treatment of refractory pemphigus vulgaris with Rituximab (Anti-CD20 monoclonal antibody). *Arch. Dermatol. 140*, 91–96.

Egan, C.A., Hanif, N., Taylor, T.B., Meyer, L.J., Petersen, M.J., and Zone, J.J. (1999). Characterization of the antibody response in esophageal cicatricial pemphigoid. *Br. J. Dermatol. 140*, 859–864.

Egan, C.A., Lazarova, Z., Darling, T.N., Yee, C., Yancey, K.B. (2003). Antiepiligrin cicatricial pemphigoid: clinical findings, immunopathogenesis, and significant associations. *Medicine (Baltimore) 82*, 177–186.

España, A., Diaz, L.A., Mascaro, J.M. Jr., Giudice, G.J., Fairley, J.A., Till, G.O., and Liu, Z., (1997). Mechanisms of acantholysis in pemphigus foliaceus Clin. *Immunol. Immunopathol. 85*, 83–89.

Fan, J.L., Memar, O., McCormick, D.J., and Prabhakar, B.S. (1999). BALB/c mice produce blister-causing antibodies upon immunization with a recombinant human desmoglein 3. *J. Immunol. 163*, 6228–6235.

Futamura S., Martins C.R., Rivitti, E.A., Labib, R.S., Diaz, L.A., and Anhalt, G.J. (1989). Ultrastructural studies of acantholysis induced *in vivo* by passive transfer of IgG from endemic pemphigus foliaceus (fogo selvagem). *J. Invest. Dermatol. 93*, 480–485.

Gammon, W.R., Heise, E.R., Burke, W.A., Fine, J.D., Woodley, D.T., and Briggaman, R.A. (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.

Getsios, S., Huen, A.C., and Green, K.J. (2004). Working out the strength and flexibility of desmosomes. *Nat. Rev. Mol. Cell. Biol. 5*, 271–281.

Giudice, G.J., Emery, D.J., and Diaz, L.A. (1992). Cloning and primary structural analysis of the bullous pemphigoid autoantigen BP180. *J. Invest. Dermatol. 99*, 243–250.

Giudice, G.J., Emery, D.J., Zelickson, B.D., Anhalt, G.J., Liu, Z., and Diaz, L.A. (1993). Bullous pemphigoid and herpes gestationis autoantibodies recognize a common non-collagenous site on the BP180 ectodomain. *J. Immunol. 151*, 5742–5750.

Haase, C., Budinger, L., Borradori, L., Yee, C., Merk, H. F., Yancey, K., and Hertl, M. (1998). Detection of IgG autoantibodies in the sera of patients with bullous and gestational pemphigoid: ELISA studies utilizing a baculovirus-encoded form of bullous pemphigoid antigen 2. *J. Invest. Dermatol. 110*, 282–286.

Hacker-Foegen, M.K., Janson, M., Amagai, M., Fairley, J.A., and Lin, M.S. (2003). Pathogenicity and epitope characteristics of anti-desmoglein-1 from pemphigus foliaceus patients expressing only IgG1 autoantibodies. *J. Invest. Dermatol. 121*, 1373–1378.

Hashimoto, T. (2001). Immunopathology of IgA pemphigus. *Clin. Dermatol. 19*, 683–689.

Jones, C.C., Hamilton, R.G., and Jordon, R.E. (1988). Subclass distribution of human IgG autoantibodies in pemphigus. *J. Clin. Immunol. 8*, 43–49.

Jordon, R.E., Beutner, E.H., Witebsky, E., Blumental, G., Hale, W.L., and Lever, W.F. (1967). Basement zone antibodies in bullous pemphigoid. *JAMA 200*, 751–756.

Jordon, R.E., Heine, K.G., Tappeiner, G., Bushkell, L.L., and Provost, T.T. (1976). The immunopathology of herpes gestations. Immunofluorescent studies and characterization of the "HG factor". *J. Clin. Invest. 57*, 1426–1433.

Katz, S.I., Hertz, K.C., and Yaoita, H. (1976). Herpes gestations. Immunopathology and characterization of the HG factor. *J. Clin. Invest. 57*, 1434–1441.

Kitajima, Y. (2003). Current and prospective understanding of clinical classification, pathomechanisms and therapy in pemphigus. *Arch. Dermatol. Res. 295*, S17–23.

Koch, P.J., Mahoney, M.G., Ishikawa, H., Pulkkinen, L., Uitto, J., Shultz, L., Murphy, G.F., Whitaker-Menezes, D., and Stanley, J.R. (1997). Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J. Cell. Biol. 137*, 1091–1102.

Korman, N.J., and Cooper, K.D. (2000). Cicatricial pemphigoid. In R.E. Jordan (ed.), *Atlas of Bullous Disease* (pp. 63–73). New York: Churchill Livingstone.

Kricheli, D., David, M., Frusic-Zlotkin, M., Goldsmith, D., Rabinov, M., Sulkes, J., and Milner, Y. (2000). The distribution of pemphigus vulgaris-IgG subclasses and their reactivity with desmoglein 3 and 1 in pemphigus patients and their first-degree relatives. *Br. J. Dermatol. 143*, 337–342.

Labib, R.S., Anhalt, G.J., Patel, H.P., Mutasim, D.F., and Diaz, L.A. (1986). Molecular heterogeneity of bullous pemphigoid antigens as detected by immunoblotting. *J. Immunol. 136*, 1231–1235.

Laffitte, E., Skaria, M., Jaunin, F., Tamm, K., Saurat, J.H., Favre, B., and Borradori, L. (2001). Autoantibodies to the extracellular and intracellular domain of bullous pemphigoid 180, the putative key autoantigen

in bullous pemphigoid, belong predominantly to the IgG1 and IgG4 subclasses. *Br. J. Dermatol. 144*, 760–768.

Lapiere, J.C., Woodley, D.T., Parente, M.G., Iwasaki, T., Wynn, K.C., Christiano, A.M., and Uitto, J. (1993). Epitope mapping of type VII collagen. Identification of discrete peptide sequences recognized by sera from patients with acquired epidermolysis bullosa. *J. Clin. Invest. 92*, 1831–1839.

Lazarova, Z., Yee, C., Darling, T., Briggaman, R.A., and Yancey, K.B. (1996). Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. *J. Clin. Invest. 98*, 1509–1518.

Lever, W.F. (1965). *Pemphigus and Pemphigoid*. Springfield, IL: Charles C. Thomas.

Li, N., Liu, Z., and Diaz, L.A. (2002). Pemphigus foliaceus autoantibodies recognize two dominant pathogenic epitopes located in EC1 and EC2 domains of desmoglein-1. *J. Invest. Dermatol. 119*, A305.

Li, N., Aoki, V., Hans-Fiho, G., Rivitti, E.A., and Diaz, L.A. (2003). The role of intramolecular epitope spreading in the pathogenesis of endemic pemphigus foliaceus (fogo selvagem). *J. Exp. Med. 197*, 1501–1510.

Lin, M.S, Swartz, S.J,. Lopez, A., Ding, X., Fernandez-Vina, M.A., Stastny, P., Fairley, J.A, and Diaz, L.A. (1997). Development and characterization of desmoglein-3 specific T cells from patients with pemphigus vulgaris. *J. Clin. Invest. 99*, 31–40.

Lin, M.S., Gharia, M.A., Swartz, S.J., Diaz, L.A., and Giudice, G.J. (1999). Identification and characterization of epitopes recognized by T lymphocytes and autoantibodies from patients with herpes gestationis. *J. Immunol. 162*, 4991–4997.

Lin, M.S., Fu, C.L., Aoki, V., Hans-Filho, G., Rivitti, E.A., Moraes, J.R., Moraes, M.E., Lazaro, A.M., Giudice, G.J., Stastny, P., and Diaz, L.A. (2000a). Desmoglein-1-specific T lymphocytes from patients with endemic pemphigus foliaceus (fogo selvagem). *J. Clin. Invest. 105*, 207–213.

Lin, M.S., Fu, C.L., Giudice, G.J., Olague-Marchan, M., Lazaro, A.M., Stastny, P., and Diaz, L.A. (2000b). Epitopes targeted by bullous pemphigoid T lymphocytes and autoantibodies map to the same sites on the bullous pemphigoid 180 ectodomain. *J. Invest. Dermatol. 115*, 955–961.

Liu, Z., Diaz, L.A., Troy, J.L., Taylor, A.F., Emery, D.J., Fairley, J.A., and Giudice, G.J. (1993). A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. *J. Clin. Invest. 92*, 2480–2488.

Liu, Z. (2004). Bullous pemphigoid: using animal models to study the immunopathology. *J. Investig. Dermatol. Symp. Proc. 9*, 41–46.

Loiseau, P., Lecleach, L., Prost, C., Lepage, V., Busson, M., Bastuji-Garin, S., Roujeau, J.C., and Charron, D. (2000). HLA class II polymorphism contributes to specify desmoglein derived peptides in pemphigus vulgaris and pemphigus foliaceus. *J. Autoimmun. 15*, 67–73.

Lombardi, M.L., Mercuro, O., Ruocco, V., Lo Schiavo, A., Lombari, V., Guerrera, V., Pirozzi, G., and Manzo, C. (1999). Common human leukocyte antigen alleles in pemphigus vulgaris and pemphigus foliaceus Italian patients. *J. Invest. Dermatol. 113*, 107–110.

Mahoney, M.G., Aho, S., Uitto, J., and Stanley, J.R. (1998). The members of the plakin family of proteins recognized by paraneoplastic pemphigus antibodies include periplakin. *J. Invest. Dermatol. 111*, 308–313.

Mahoney, M.G., Wang, Z., Rothenberger, K., Koch, P.J., Amagai, M., and Stanley, J.R. (1999a). Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J. Clin. Invest. 103*, 461–468.

Mahoney, M.G., Wang, Z.H., Stanley, J.R. (1999b). Pemphigus vulgaris and pemphigus foliaceus antibodies are pathogenic in plasminogen activator knockout mice. *J. Invest. Dermatol. 113*, 22–25.

Mascaro, J.M., Jr., Espana, A., Liu, Z., Ding, X., Swartz, S.J., Fairley, J.A., and Diaz, L.A. (1997). Mechanisms of acantholysis in pemphigus vulgaris: role of IgG valence *Clin. Immunol. Immunopathol. 85*, 90–96.

Miyagawa, S., Amagai, M., Niizeki, H., Yamashina, Y., Kaneshige, T., Nishikawa, T., Shirai, T., and Inoko, H. (1999). HLA-DRB1 polymorphisms and autoimmune responses to desmogleins in Japanese patients with pemphigus. *Tissue Antigens. 54*, 333–340.

Moraes, M.E., Fernandez-Vina, M., Lazaro, A., Diaz, L. A., Filho, G.H., Friedman, H., Rivitti, E., Aoki, V., Stastny, P., and Moraes, J.R. (1997). An epitope in the third hypervariable region of the DRB1 gene is involved in the susceptibility to endemic pemphigus foliaceus (fogo selvagem) in three different Brazilian populations. *Tissue Antigens 49*, 35–40.

Morrison, L.H., Labib, R.S., Zone, J.J., Diaz, L.A., and Anhalt, G.J. (1988). Herpes gestationis autoantibodies recognize a 180-kD human epidermal antigen. *J. Clin. Invest. 81*, 2023–2026.

Mutasim, D.F., Takahashi Y, Labib, R.S., Anhalt, G.J., Patel, H.P. and Diaz, L.A. (1985). A pool of bullous pemphigoid antigen(s) is intracellular and associated with the basal cell cytoskeleton-hemidesmosome complex . *J. Invest. Dermatol. 84*, 47–53.

Nemzer, P.G., Egan, C.A., and Zone, J.J. (2000). Linear IgA bullous dermatosis. In R. E. Jordan (ed.), *Atlas of Bullous Disease* (pp. 109–123). New York: Churchill Livingstone.

Nishifuji, K., Amagai, M., Kuwana, M., Iwasaki, T., and Nishikawa, T. (2000). Detection of antigen-specific B cells in patients with pemphigus vulgaris by enzyme-linked immunospot assay: requirement of T cell collaboration for autoantibody production. *J. Invest. Dermatol. 114*, 88–94.

Okazaki, A., Miyagawa, S., Yamashina, Y., Kitamura, W., and Shirai, T. (2000). Polymorphisms of HLA-DR and -DQ genes in Japanese patients with bullous pemphigoid. *J. Dermatol. 27*, 149–156.

O'Toole, E.A. and Woodley, D.T. (2000). Epidermolysis bullosa acquisita. In R. E. Jordan (ed.) Atlas of Bullous Disease (pp. 125–138). New York: Churchill Livingstone.

Patel, H. P., Diaz, L.A., Anhalt, G.J., Labib, R.S., and Takahashi, Y. (1984). Demonstration of pemphigus antibodies on the cell surface of murine epidermal cell monolayers and their internalization. *J. Invest. Dermatol. 83*, 409–415.

Provost, T.T., and Tomasi, T.B. (1973). Evidence for complement activation via the alternate pathway in skin disease: Herpes gestations, systemic lupus erythematosus and bullous pemphigoid. *J. Clin. Invest. 52*, 1779–1787.

Rock B., Martins, C.R., Theofilopoulos, A.N., Balderas, S., Anhalt, G.J., Labib, R.S., Futamura, S., Rivitti, E.A. and Diaz L.A. (1989). The pathogenic effect of IgG4 autoantibodies in endemic pemphigus foliaceus (fogo selvagem). *N. Engl. J. Med. 320*, 1463–1469.

Roscoe, J.T., Diaz, L.A., Sampaio, S.A.P., Castro, R.M., Labib, R.S., Takahashi, Y., Patel, H.P., and Anhalt, G.J. (1985). Brazilian pemphigus foliaceus autoantibodies are pathogenic to BALB/c mice by passive transfer. *J. Invest. Dermatol. 85*, 538–541.

Rubenstein, D., Berkowitz, P., Hu, P., Liu, Z., and Enghild, J. (2004). Pemphigus vulgaris IgG induces desmosomal transmembrane signaling. *J. Invest. Dermatol. 122*, A34.

Salopek, T.G., Logsetty, S., and Tredget, E.E. (2002). Anti-CD20 chimeric monocloncal antibody (rituximab) for the treatment of recalcitrant, life-threatening pemphigus vulgaris with implications in the pathogenesis of the disorder. *J. Am. Acad. Dermatol. 47*, 785–788.

Sardy, M., Karpati, S., Merkl, B., Paulsson, M., and Smyth, N. (2002). Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. *J. Exp. Med. 195*, 747–757.

Seignate, J., Guillot, B., Guilhou, J.J., and Meynadier, J., (1987). Probable association between HLA-DR5 and bullous pemphigoid. *Tissue Antigens 30*, 190–191.

Sekiguchi, M., Futei, Y., Fujii, Y., Nishikawa, T., and Amagai, M. (2001). Dominant autoimmune epitopes recognized by pemphigus antibodies map to the N-terminal adhesive region of desmogleins. *J. Immunol. 167*, 5439–5448.

Shimizu, A., Ishiko, A., Ota, T., Tsunoda, K., Amagai, M., and Nishikawa, T. (2004). IgG binds to desmoglein 3 in desmosomes and causes a desmosomal split without keratin retraction in a pemphigus mouse model. *J. Invest. Dermatol. 122*, 1145–1153.

Sinha, A.A., Brautbar, C., Szafer, F., Friedmann, A., Tzfoni, E., Todd, J.A., Steinman, L., and McDevitt, H.O. (1988). A newly characterized HLA DQ beta allele associated with pemphigus vulgaris. *Science 239*, 1026–1029.

Sollid, L.M. (2000). Molecular basis of celiac disease. *Annu. Rev. Immunol. 18*, 53–81.

Stanley, J.R., Hawley-Nelson, P., Yuspa, S.H., Shevach, E.M., and Katz, S.I. (1981). Characterization of bullous pemphigoid antigen: A unique basement membrane protein of stratified epithelia. *Cell 24*, 897–903.

Takahashi, Y., Mutasim, D.F., Patel, H.P., Anhalt, G.J., Labib, R.S., and Diaz, L.A. (1985). Experimentally induced pemphigus vulgaris in neonatal BALB/c mice: a time-course study of clinical, immunologic, ultrastructural, and cytochemical changes. *J. Invest. Dermatol. 84*, 41–46.

Tanaka, T., Parry, D.A.D., Klaus-Kovtun, V., Steinert, P.M., and Stanley, J.R. (1991). Comparison of molecularly cloned bullous pemphigoid antigen to desmoplakin I confirms that they define a new family of cell adhesion junction plaque proteins. *J. Biol. Chem. 266*, 12555–12559.

Tyagi , S., Bhol, K., Natarajan, K., Livir-Rallatos, C., Foster, C.S., and Ahmed, A.R. (1996). Ocular cicatrical pemhigoid antigen: partial sequence and biochemical characterization. *Proc. Natl. Acad. Sci. 93*, 14714–14719.

Veldman, C.M., Gebhard, K.L., Uter, W., Wassmuth, R., Grotzinger, J., Schultz, E., and Hertl, M. (2004). T cell recognition of desmoglein 3 peptides in patients with pemphigus vulgaris and healthy individuals. *J. Immunol. 172*, 3883–3892.

Venning, V.A., Taylor, J.C., Ting, A., and Wojnarowska, F. (1989). HLA type in bullous pemphigoid, cicatrical pemphigoid and linear IgA disease. *Clin. Exp. Dermatol. 14*, 283–285.

Wang, L., Bu, D., Yang, Y., Chen, X., and Zhu, X. (2004). Castleman's tumours and production of autoantibody in paraneoplastic pemphigus. *Lancet 363*, 525–531.

Warren, S.J., Lin, M.S., Giudice, G.J., Hoffmann, R.G., Hans-Filho, G., Aoki, V., Rivitti, E.A., Santos, V., and Diaz, L.A. (2000). The prevalence of antibodies against desmoglein 1 in endemic pemphigus foliaceus in Brazil. Cooperative Group on Fogo Selvagem Research. *N. Engl. J. Med. 343*, 23–30.

Warren, S.J., Arteaga, L.A., Rivitti, E.A., Aoki, V., Hans-Filho, G., Qaqish, B.F., Lin, M.S., Giudice, G.J., and Diaz, L.A. (2003). The role of subclass switching in the pathogenesis of endemic pemphigus foliaceus. *J. Invest. Dermatol. 120*, 104–108.

Wintroub, B.U., Mihm, M.C., Jr., Goetzl, E.J., Soter, N.A., and Austen, K.F. (1978). Morphologic and functional evidence for release of mast-cell products in bullous pemphigoid. *N. Engl. J. Med. 298*, 417.

Wucherpfennig, K.W., Yu, B., Bhol, K., Monos, D.S., Argyris, E., Karr, R.W., Ahmed, A.R., and Strominger, J.L. (1995). Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: Charged residues of a single MHC binding pocket confer selective presentation of self-peptides in pemphigus vulgaris. *Proc. Natl. Acad. Sci. USA 92*, 11935–11939.

Venning, V.A., Taylor, J.C., Ting, A., and Wojnarowska, F. (1989). HLA type in bullous pemphigoid, cicatricial pemphigoid and linear IgA disease. *Clin. Exp. Dermatol. 14*. 283–285.

Zillikens, D., Caux, F., Mascaro, J. M., Wesselmann, U., Schmidt, E., Prost, C., Callen, J.P., Brocker, E.B., Diaz, L.A. and Giudice, G.J. (1999). Autoantibodies in lichen planus pemphigoides react with a novel epitope within the C-terminal NC16A domain of BP180. *J. Invest. Dermatol. 113*,117–121.

Zone, J.J., Egan, C.A., Taylor, T.B., and Meyer, L.J. (2004). IgA autoimmune disorders: development of a passive transfer mouse model. *J. Investig. Dermatol. Symp. Proc. 9*, 47–51.

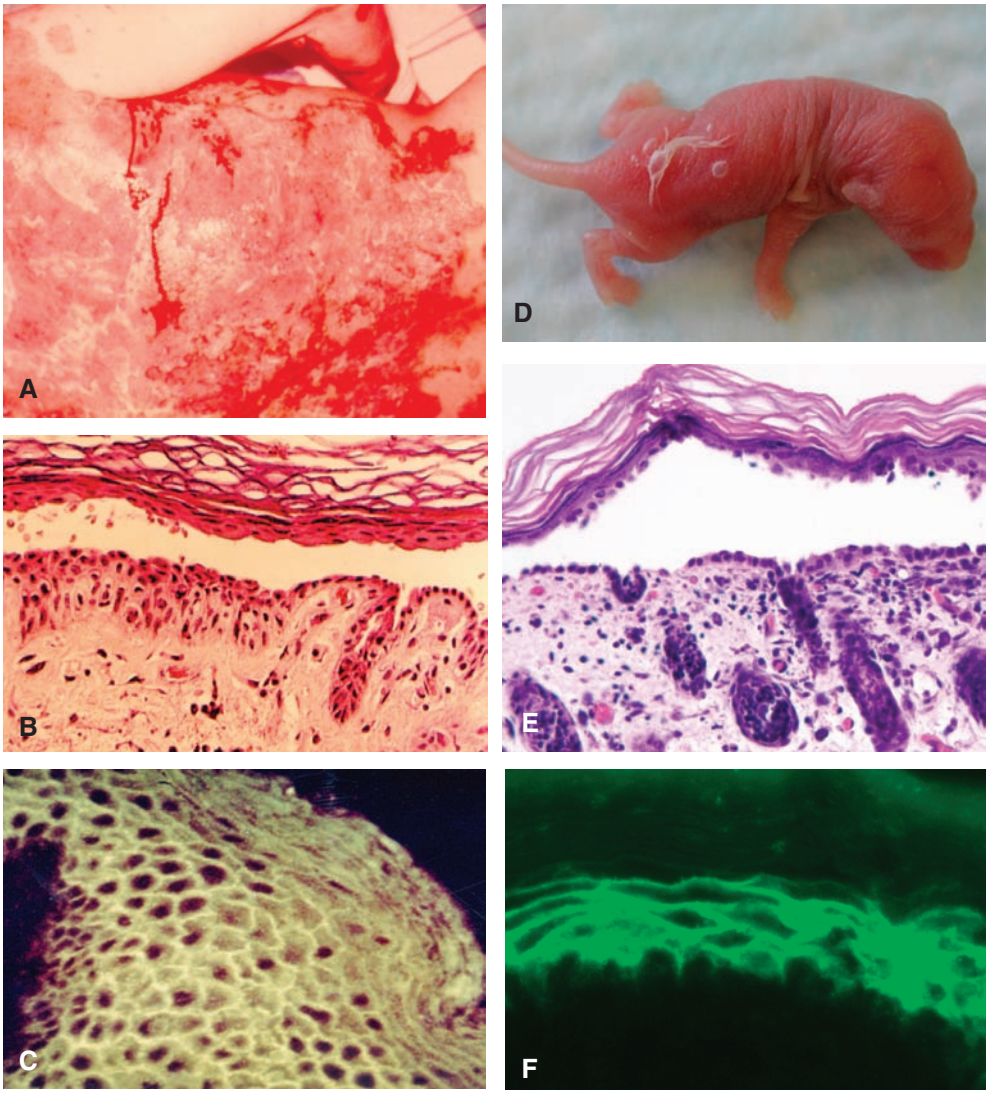


FIGURE 57.2 Left panel, clinical (a), histologic (b), and immunofluorescent (c) features of pemphigus vulgaris (PV). Right panel, mouse model of PV. The key features of the human disease are reproduced in these animals by passive transfer of PV autoantibodies (d–f).

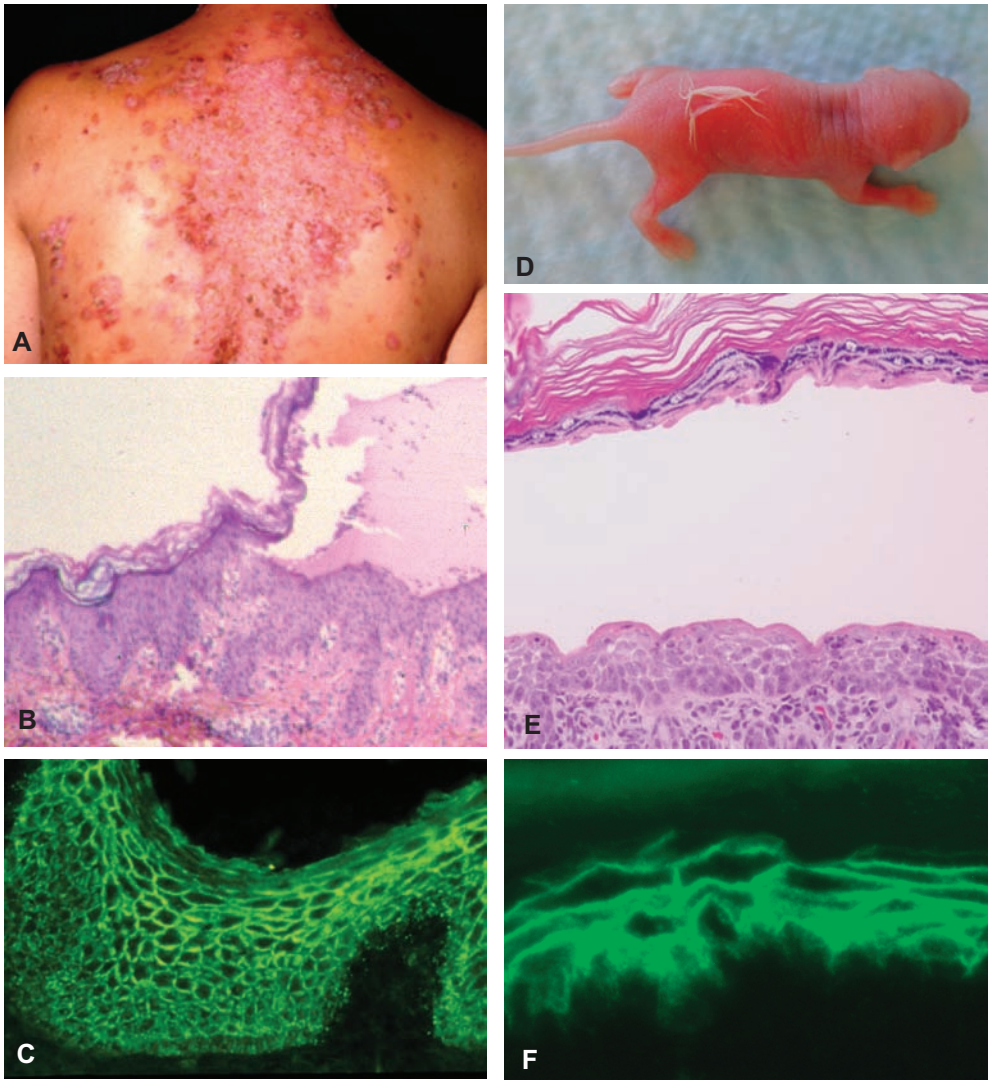


FIGURE 57.4 Left panel, clinical (a), histologic (b), and immunofluorescent (c) features of endemic pemphigus foliaceus (PF) (fogo selvagem). Right panel, mouse model of PF. The animals have been passively transferred with human PF IgG (and IgG4) and develop skin lesions (d), which histologically (e) are similar to the human disease. The human anti-Dsg1 autoantibodies are detected bound to the lesional epidermis (f) and circulating in the mouse serum.

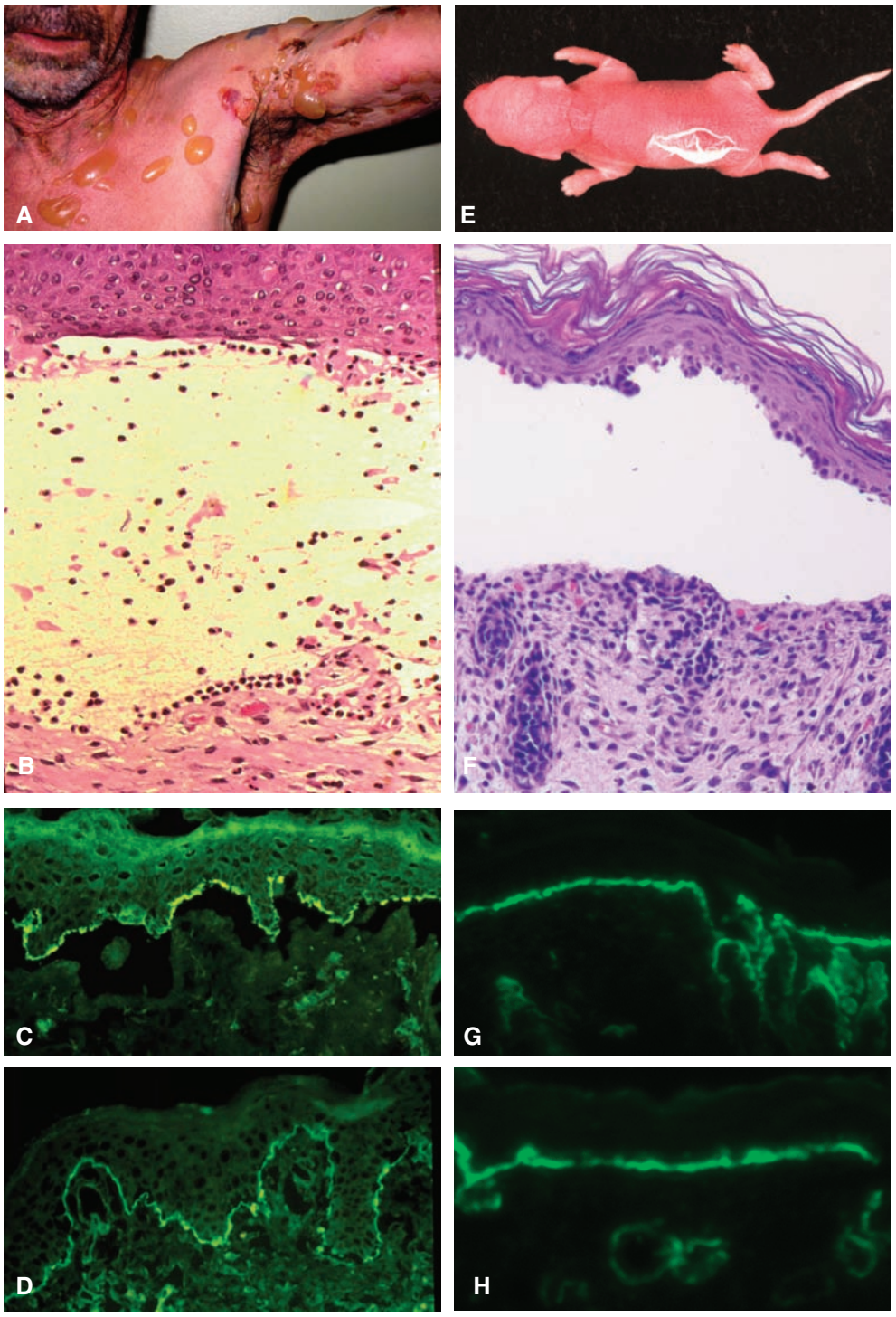


FIGURE 57.6 Bullous pemphigoid (BP). Left panel, key clinical, histologic and immunofluorescent features of BP. Large, tense blisters and erosions are seen in flexural areas (a). The histology reveals subepidermal blisters (b). Direct immunofluorescence (IF) shows in-situ deposition of IgG (c) and C3 (d) at the cutaneous basement membrane zone (BMZ). Right panel, mouse injected with rabbit antimurine BP180 antibodies. The animals develop skin blisters (e). The histology of these lesions shows dermal–epidermal separation with an inflammatory infiltration (f). Direct IF shows in-situ deposition of rabbit IgG (g) and mouse C3 (h) at the BMZ.

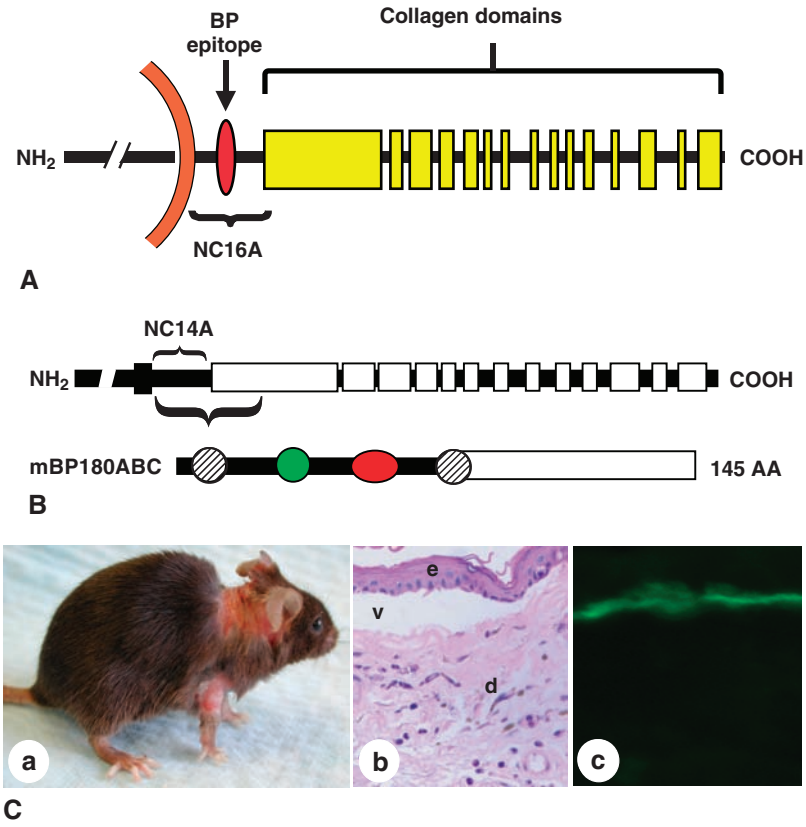


FIGURE 57.8 Active model of bullous pemphigoid (BP). *A*, Schematic diagram of the human BP180 antigen. The C-terminal extracellular region is made up of a series of collagen-like domains (yellow rectangles) interrupted by non-collagen domains (black bars). The major epitope (NC16A) recognized by BP autoantibodies is depicted by the red oval. *B*, Mouse BP180 antigen used to immunize mice. This antigen (referred to as mBP180ABC) contains the largest non-collagen domain (NC14A) and part of the largest collagen-like domain. mBP180ABC harbors four epitopes, one of which (red) is pathogenic. *C*, 8–10-week-old C57BL/6J mice immunized with mBP180ABC. Some of the mice developed BP-like skin lesions, starting the third week after the boosting immunization (a). Histologic examination shows subepidermal blistering (b). Direct immunofluorescence shows in-vivo deposition of autoantibodies at the basement membrane zone (c). d, dermis; e, epidermis; v, vesicle.

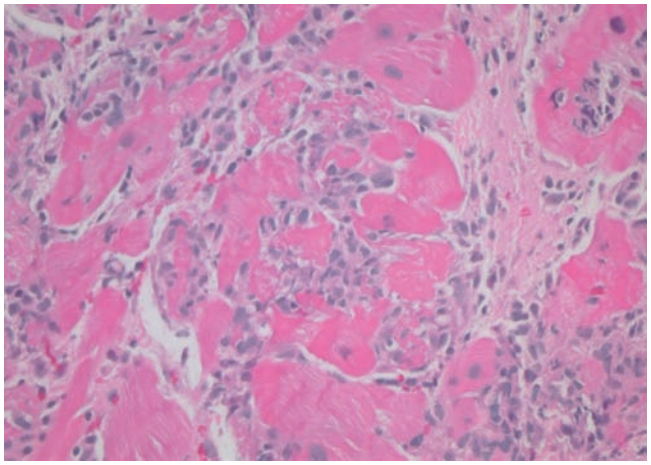


FIGURE 63.1 Lymphocytic myocarditis. There is a heavy infiltrate of large activated lymphocytes throughout the myocardium. Myocyte necrosis is noted in the middle of this image. Fibrosis is present on the right side of the image. (H&E 400×).

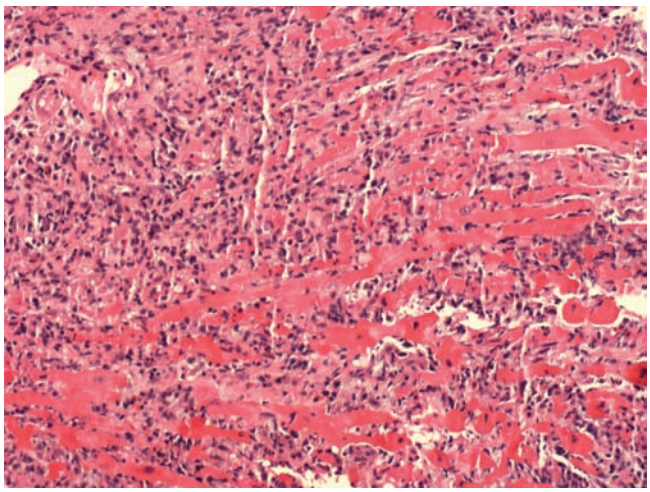


FIGURE 63.3 Fulminant myocarditis. The myocardium is replaced by a marked polymorphous inflammatory infiltrate composed predominantly of lymphocytes and macrophages with rarer eosinophils and neutrophils. Global myocyte injury and loss is noted. No giant cells are present. (H&E 100×).

CHAPTER

58

Non-Bullous Skin Diseases: Alopecia, Vitiligo Psoriasis, and Urticaria

MARGITTA WORM AND WOLFRAM STERRY

Department of Dermatology and Allergy, Charité Campus Mitte, Universitàts Medizin Berlin, Germany

An autoimmune pathomechanism has been discussed or confirmed for several dermatologic diseases within the past decades. The pathogenesis of these diseases is multifactorial and autoimmune mechanisms are often only one component of a complex pathophysiology. Some of these diseases typically affect only the skin and do not involve other organ systems, unlike the systemic autoimmune disorders. However, subsets of patients may be identified who develop either another autoimmune disease, such as Hashimoto's thyroiditis, or in whom another organ system is involved, such as the joints. The autoimmune background of these skin diseases has implications for their treatment, since immune-modulating or -suppressing agents are often effective. The pathophysiologic background of these diseases notably includes an abnormal T-cell response, but also autoantibody production against self-antigens within the skin.

ALOPECIA AREATA

Alopecia areata has a long medical history, first being described in the year 57 AD by Celsus. There is a vast knowledge regarding its pathogenesis and therapy. This disease is quite often associated with certain environmental exposures or particular individual incidents. The high spontaneous remission rate for alopecia areata has led to a wide range of therapies to promote hair growth, including complementary modalities. Strong direct and indirect evidence supports an autoimmune etiology for alopecia areata.

The exact incidence of alopecia areata is not known. A study from the US has revealed a lifetime risk as high as 1.7% (Safavi et al., 1995). The disease occurs most frequently within the second and third decades of life.

Clinical Features

Alopecia areata starts usually with one or several areas of hair loss in the scalp, beard or other hair-bearing sites. The eyebrows or eyelashes are also often affected. The skin area within the area of hair loss appears normal; no signs of atrophy are present (Figure 58.1). Within the areas of active hair shedding, a short fractured hair shaft may be identified, the characteristic "exclamation point" hair. Complete scalp involvement, called alopecia totalis, may occur suddenly or during prolonged progressive disease, and complete or nearly complete loss of the entire body hair, called alopecia universalis, may also occur. In 1–2% of affected

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

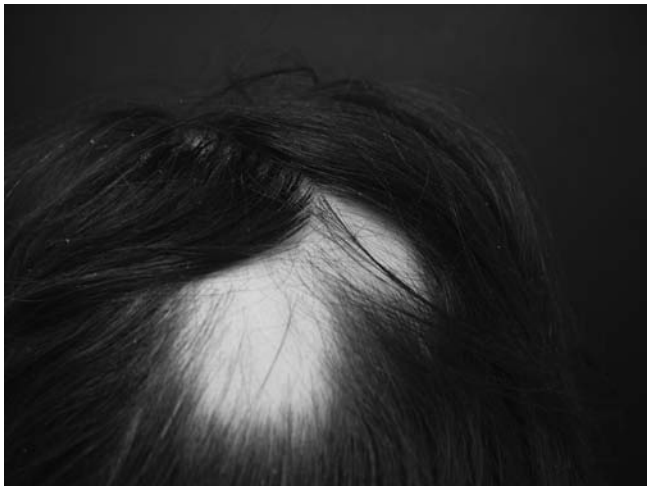


FIGURE 58.1 Typical clinical appearance of alopecia areata.

individuals, alopecia areata is further described as the ophiasis type, in which the hair loss occurs particularly within the retroauricular and occipital area, and alopecia areata diffusa type, which is characterized by a sudden diffuse hair loss without the typical circumscribed areas.

The course of the disease is generally characterized by recurrent hair loss and recovery. However, especially in alopecia areata totalis, universalis, and ophiasis, spontaneous recovery of hair growth often does not occur, resulting in persistent alopecia. Several factors indicative of a poor outcome have been identified, including the early onset of disease, a severe and long-standing course of the disease, and the presence of nail changes or atopic eczema. The nail changes include dimples and transverse furrows. Other organ systems are not affected and the most distressing consequence is the psychosociologic stigmatization of these young patients; social isolation and the development of secondary psychotic diseases are common.

Important differential diagnoses of alopecia areata are trichotillomania, tinea capitis, alopecia syphilitica, cutaneous lupus erythematodes, lichen planopilaris, and idiopathic scarring alopecia.

Pathophysiology

During its cycle the hair turns from an anagen into a catagen and finally into a telogen hair. In the adult scalp the anagen stage (active growth) lasts 3 years, catagen stage (regression) 3 weeks, and telogen stage (resting period) 3 months. At any one time, 84% of the scalp hairs are in the anagen stage, 2% in the catagen stage, and 14% in the telogen stage.

Headington (1991) has suggested that the pathophysiology of alopecia areata can be divided into four distinct stages: 1) acute hair loss; 2) persistent baldness; 3) partial telogen to anagen conversion; and 4) normal recovery. Lymphocytic infiltrates in the peribulbar area of the anagen follicles are observed in all stages. The follicular structures become miniaturized and as a result are identified most superficially in the dermis. In the early active phase of hair loss, diminutive follicles are predominantly observed in the early or late catagen stages and these persist into the chronic active phase of baldness. In long-standing cases, the inflammatory infiltrates in the skin appear to diminish (Messenger et al., 1986). Lymphocytes may invade both the bulbs and the outer root sheaths of early recovering anagen hairs. The lymphocytic infiltrates in the peribulbar area consist of substantial numbers of helper T cells (CD4/CD8 ratio 4 : 1). The ability of the hair follicles to self-recover is retained, and if the hair follicle re-enters the anagen stage, it can be either attacked again by the infiltrating lymphocytes or can undergo spontaneous growth (Messenger et al., 1986). The presence of peri- and intra-follicular $CD4^+$ and $CD8^+$ lymphocytic infiltrates suggests a T-cell-mediated autoimmune pathogenesis of the disease. Recently, a reduction of $CD4^+CD25^+$ regulatory T cells was identified in mice suffering from alopecia areata (Zoller et al., 2002).

Genetic Features

There is a genetic background in 10–25% of alopecia areata patients. Several HLA markers have been associated with alopecia areata, including DR-4, DR-5, DR-6, DR-7, DR-11, and several DQB-1 alleles (McElwee et al., 2001). Studies have identified polymorphisms for proinflammatory genes, including tumor necrosis factor (TNF)-α (Galbraith and Pandey, 1995), interleukin-1 receptor (IL-1R) (Cork et al., 1995), and the IL-1β gene (Galbraith et al., 1999). It is assumed that these polymorphisms within proinflammatory genes are associated with the clinical appearance and severity of alopecia areata.

Animal models have been established to study the pathophysiology of alopecia areata, notably in the C3H/HEJ mouse and the Dundee Experimental Board Rat (DEBR). Initial immunohistochemistry studies from affected patients indicated that the keratinocytes from hair follicles in lesions of alopecia areata are characterized by the expression of major histocompatibility complex (MHC) class I and class II, but also CD54 (Messenger and Bleehen, 1985). Such antigen-presenting molecules are not expressed within the normal hair follicle, because this is an immune-privileged organ that is protected from the immune system (Paus et al., 1994). Several as yet unidentified autoantigens induce a loss of this immune privilege of the hair follicle, resulting in increased expression of MHC- and CD54, and leading to the possibility of antigen presentation by hair follicle cells. The responsible autoantigens in hair follicles have not yet been identified. However, the expression of MHC class I plays a

central role, as $CD8^+$ T cells are located within the center of the peri- and intra-follicular infiltrates close to the MHC class I-expressing keratinocytes. Several studies have demonstrated the central role of $CD8^+$ T cells in the DEBR model by showing hair growth following depletion of $CD8^+$ T cells (McElwee et al., 1996). This was confirmed in a humanized severe combined immune deficiency (SCID) mouse model (Gilhar et al., 1999). $CD8^+$ T cells target the hair follicle keratinocytes and melanocytes by inducing apoptosis through CD95–CD178(CD95L) interaction (Bodemer et al., 2000). It has been shown that animals with a defective CD95–CD178(CD95L) system are resistant to alopecia areata (Freyschmidt-Paul et al., 2003b). The induction of apoptosis within the hair follicle keratinocytes and melanocytes leads to dystrophic changes and the hair shaft is not formed regularly.

Several other cell types may be also involved in alopecia areata, including macrophages, dendritic cells, $CD4^+$ T cells, and B-lymphocytes. The cytokine profile of isolated T cells from mice with alopecia areata reveals both a Th1 and Th2 profile in addition to increased TNF-α and IL-6 production (Zoller et al., 2002). Also, autoantibodies have been described in mouse and man (Tobin et al., 1997), but this is most likely a secondary effect as autoantibodies seem not to be directly involved in the development of hair loss.

Treatment

As mentioned above, the high spontaneous remission rate of alopecia areata means that many treatments have been described. When results of controlled prospective studies are not available, the possibility of spontaneous regrowth of hair needs to be considered.

Corticosteroids are widely used for the treatment of alopecia areata, but only two controlled studies with topical application of steroids have been published (Pascher et al., 1970; Leyden and Kligman, 1972). Another treatment option for alopecia areata is the induction of an allergic contact dermatitis with dinitrochlorobenzol (DNCB) (Happle and Echternacht, 1977). The efficacy of DNCB was confirmed in several controlled studies, therefore excluding the possibility of a spontaneous remission of the disease (Happle and Echternacht, 1977; Freyschmidt-Paul et al., 2003a). However, the success rate ranges from 29 to 78% with a median efficacy rate of 51% (Freyschmidt-Paul et al., 2003a). Future therapeutic approaches could include the application of molecules that are apparently able to penetrate the hair bulbus and inhibit either the CD95–CD178 (CD95L) interaction or MHC class I expression within the hair follicle keratinocytes. Unfortunately, the new topical immune modulators do not reach the hair bulbus in sufficient concentrations to have an effective immunosuppressive effect. Finally, several biologics are being considered, including antibodies to TNF-α/TNF-α receptor antagonists, and molecules, which interfere with costimulatory pathways like CTLA-4, although an efficacy–cost analysis will be necessary for these treatments.

VITILIGO

Clinical Features

Vitiligo is an acquired depigmenting disorder characterized by the loss of melanocytes from the epidermis. It occurs with a frequency of 0.1–2% in various populations. Several types of vitiligo are distinguished according to the distribution of the lesions. Vitiligo that is segmental or focal is called localized vitiligo. Generalized vitiligo, which most commonly affects the face, upper trunk, backs of the hands, genitals, and periocular areas, is characterized by lesions in a symmetrical distribution.

Stable patches of vitiligo often have an irregular border but are sharply demarcated from the surrounding skin (Figure 58.2). In some cases, the surrounding skin is hyperpigmented. A slight rim of erythema at the border may rarely be seen in expanding lesions, together with a thin zone of transitory, partial pigmentation. Hairs and patches of vitiligo usually become wholly white. The scalp and eyelashes are rarely affected.

Pathophysiology

A central process in vitiligo is the destruction of melanocytes at the dermoepidermal junction. The periphery of expanding lesions is hypopigmented rather than completely depigmented, and still shows a few dopa-positive melanocytes and some melanin granules in the basal layer.

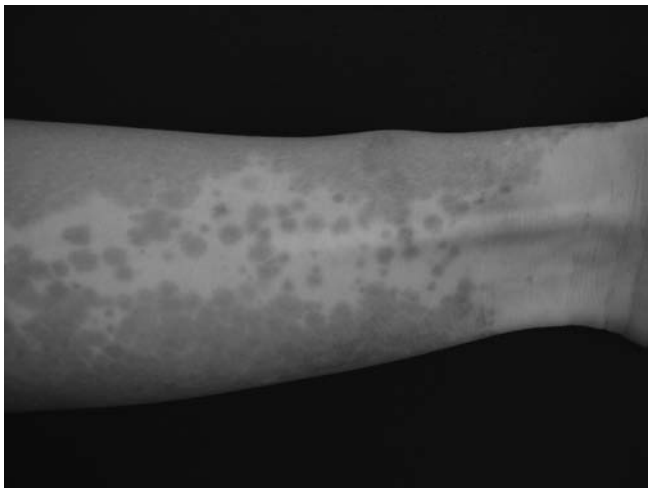


FIGURE 58.2 Vitiligo with typical loss of skin pigment. The border is irregular, but sharply demarcated from the surrounding skin.

In the outer border of patches of vitiligo, melanocytes are often prominent and demonstrate dendritic processes filled with melanin vacuoles. Sometimes, a superficial perivascular mononuclear cell infiltrate is observed at the border of depigmented areas.

Three major hypotheses for the pathogenesis of vitiligo are currently discussed. The first, and longest standing, considers vitiligo to be an autoimmune disease (Ongenae et al., 2003). The second, the neurogenic hypothesis, suggests that an accumulation of neurochemical substances damages the melanocytes, resulting in decreased melanin production (Lazarova et al., 2000). The third, the biochemical hypothesis, implicates cytotoxic metabolites from melanin synthesis as responsible for the destruction of melanocytes (Schallreuter et al., 2004). However, the list of other etiologic factors includes a deficiency of unidentified melanocyte growth factors, genetic defects, and an intrinsic defect in the structure or function of melanocytes (Ongenae et al., 2003).

The association between vitiligo, autoimmune disorders and the presence of organ-specific antibodies, as well as the fact that nonsurgical repigmenting therapies have immune-modulating effects, support the idea of an autoimmune pathogenesis for vitiligo. However, the exact mechanisms remain unclear, including whether autoimmunity is the primary cause or whether an abnormal immune response is simply a secondary effect. Also, different pathogenic pathways may be involved in the various clinical types of vitiligo. The neural hypothesis is thought to be involved in segmental vitiligo, whereas the biochemical or autoimmune hypothesis is thought to relate to generalized or focal non-dermatomal vitiligo.

In addition to the association with other autoimmune disorders and organ-specific antibodies, many other autoimmune features have been described during the course of vitiligo. These include deviations in T-cell and natural killer (NK) cell profiles (al-Fouzan et al., 1995). Immune histochemical studies of the perilesional area in generalized vitiligo reveals $CD4^+$ and $CD8^+$ T cells in the infiltrate (often with an increased CD8:CD4 ratio), which express activation molecules such as IL-2R and MHC class II. The T cells secrete interferon (IFN)-γ, which enhances T-cell trafficking to the skin by increasing CD54 expression (al Badri et al., 1993a; 1993b). An activated phenotype of the T-cell–mediated immune system was confirmed in vitiligo by detecting significantly increased levels of the soluble IL-2R (CD25), especially on T cells in generalized, focal, and non-dermatomal types of vitiligo (Honda et al., 1997). Also, tissue fluids from the margin of hypopigmented macules, especially in active disease, contain higher levels of soluble IL-2R than uninvolved skin of the same patient (Caixia et al., 1999). A substantial number of infiltrating T cells express cutaneous lymphocyte antigen (CLA), which is typical for skin-homing T cells (Le Poole et al., 1996). A recent study localized CLA^+ cytotoxic T cells to disappearing melanocytes in generalized vitiligo. A focal epidermal expression of CD54 and MHC class II at the site of interaction of skin-homing T cells and melanocytes was detected. The primary target of the immune-mediated attack on the keratinocytes–melanocyte unit is unknown. MHC class II-restricted melanocyte killing by T cells has been suggested by some authors (al Badri et al., 1993b). Keratinocytes may contribute to this process by presenting melanocyte antigens in an MHC class II-restricted manner after phagocytosis of melanosomes. However, the changes observed in epidermal keratinocytes with altered expression of antigen-presenting molecules suggest a primary involvement of keratinocytes in vitiligo.

In addition to T-cell infiltrates, macrophages are abundant in the dermis. The role of Langerhans cells, which act as antigen-presenting cells, is unclear. A depletion of epidermal Langerhans cells in active and repigmenting non-segmental vitiligo, and their reappearance in stable nonsegmental vitiligo, have been described (van den Wijngaard et al., 2000). Apoptosis as a primary pathophyiologic event in vitiligo has been suggested recently (Huang et al., 2002). In addition, an imbalance in cytokines in the epidermal microenvironment of lesional vitiligo has been demonstrated (Moretti et al., 2002).

A variety of circulating autoantibodies have been detected in sera from patients with vitiligo (Bystryn, 1989). These autoantibodies are directed to nonpigment cell antigens (common tissue antigens), cytoplasmic pigment cell antigens, and pigment cell surface antigens. Antibodies to surface endocytoplasmatic antigens of melanocytes have been of interest. These autoantibodies belong to the IgG isotypes, and subclasses of IgG1, IgG2, and IgG3 have been described (Harning et al., 1991). The significance of these autoantibodies has so far not been elucidated. It was suggested that antimelanocyte antibodies mediate melanocytotoxicity, whereas coexisting antikeratinocyte antibodies may occur secondary to cellular damage. However, the antibody response may also be secondary to a primary melanocyte destruction mediated by other mechanisms. As yet, only a few autoantigens have been identified. Tyrosinase and tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2) are key enzymes involved in melanin synthesis and have been implicated as autoantigens (Kemp et al., 1998). Passive immunization with TRP-1 monoclonal antibodies induces melanoma regression and vitiligo-like depigmentation in mice (Hara et al., 1995). However, antibody-mediated destruction requires membrane expression of the target antigens. Recently, the sur-face receptor, melanin-concentrating hormone receptor 1 (MCHR1), was detected as an antibody target in 16% of vitiligo sera. Although the antibodies are highly disease-specific, this neuropeptide receptor is

involved in the regulation of melanogenesis but it is not expressed by melanocytes (Kemp et al., 2002). Sox transcription factors, e.g., Sox9, are involved in the differentiation of tissues derived from the neural crest (Cheung and Briscoe, 2003) and were identified as melanocytic autoantigens in vitiligo associated with other autoimmune diseases (Hedstrand et al., 2001).

All-in-all, the pathogenic role of antimelanocyte antibodies remains unclear. Although the incidence and serum level of antibodies to melanocyte antigens was found to correlate with the disease activity, the extent of depigmentation, presence of other autoimmune diseases, and cellular and biochemical effects must be considered (Naughton et al., 1986). Finally, there is evidence that both cellular and humoral immunity cooperate in the destruction of melanocytes in vitiligo patients.

Genetic Features

Familial clustering of cases is not uncommon. A polygenic basis has been suggested. There is an increased frequency of several autoimmune disorders in vitiligo patients and first-degree relatives. These include autoimmune thyroid disease, pernicious anemia, Addison's disease, systemic lupus erythematosus (SLE), and inflammatory bowel disease. Such associations indicate that vitiligo has common etiologic links with other autoimmune diseases (Alkhateeb et al., 2003) Also, coexistence of vitiligo and psoriasis or lichen planus has been reported (Rubisz-Brzezinska et al., 1996; Dhar and Malakar, 1998; Hwang et al., 1998). In vitiligo patients, there is a strong familial association and relation to atopy. In a study from Asia, a significant percentage (60–70%) of affected patients had an associated personal and family history of atopy (Tan et al., 2002). Other studies have suggested vitiligo susceptibility loci on chromosomes 7, 8, and 17 (Spritz et al., 2004). Although these family clusterings and genetic linkage studies suggest that genetic factors predispose to vitiligo, a clear transmission pattern and cosegregation of vitiligo with specific mutations have not been demonstrated.

Treatment

Treatment of vitiligo is based on immune modulatory and immunosuppressive approaches (Valkova et al., 2004). In general, the efficacy of different types of treatment is variable and in some patients only cosmetic covering gives satisfactory results. Topical corticosteroids are most useful on small patches of recent onset. One percent betamethasone valerate may be used on the face and a superpotent agent, such as 0.05% cobetasol propionate, on the body. If there is no response within 3 months, treatment should be stopped; full response, if it occurs, may take many months. Recently, in uncontrolled studies, topical calcipotriol has shown a possible beneficial effect in patchy areas of vitiligo depigmentation, either alone or in combination with ultraviolet (UV) therapy (Cherif et al., 2003; Vasquez-Lopex et al., 2003). Furthermore, UV-light preventing measures using UV-protection emulsions will help by reducing increased pigmentation of the normal skin, as well as protecting the depigmented skin areas.

Local and systemic PUVA-therapy (psoralen + UVA) is the first-line treatment for adults with a reported skin repigmentation of up to 80–90% (Cherif et al., 2003; Valkova et al., 2004). However, as the long treatment duration with PUVA results in significant UVA exposure, new treatments are being sought. These include PAUVA, the systemic application of phenylalanine followed by UVA radiation, and KUVA, in which systemic khellin and UVA are combined (Valkova et al., 2004).

Punch grafting is recommended as a treatment for localized nonprogressive vitiligo resistant to conventional therapies (Agarwal, 2004). Here autologous keratinocytes may act as a source of growth factors like leukotrienes, endothelin, basic epidermal growth factor, and stem cell factor, which stimulate donor melanocytes to proliferate and migrate into the vitiligo patch, or alternatively to release growth factors that diffuse into the surrounding vitiligo patch and stimulate the viable but dormant melanocytes.

The 308-nm excimer laser has been used in the management of stable plaque psoriasis and has been proposed for the treatment of vitiligo (Asawanonda et al., 2000; Baltas et al., 2001; Spencer et al., 2002). Unlike narrow-band UVB phototherapy and PUVA, the 308-nm excimer laser modality can selectively treat single vitiligous patches, sparing nonaffected areas. However, among the physical methods known to be effective for treating vitiligo, the 308-nm XeCL excimer laser may considerably improve vitiligo and is well-tolerated.

PSORIASIS

The term psoriasis is derived from the Greek psora, meaning scurf, itch, rash. The first clear description was by Willan in 1808 (Willan [re-edited] 1950).

Clinical Features

Psoriasis is divided into different subtypes, including psoriasis vulgaris, generalized pustular psoriasis, and localized pustular psoriasis. The most common form is psoriasis vulgaris, which is a common chronic inflammatory skin disorder that affects approximately 2–5% of the white population (Henseler and Christophers, 1985) and 0.1–0.3% of the population in the Far East (Sagoo et al., 2004). It is

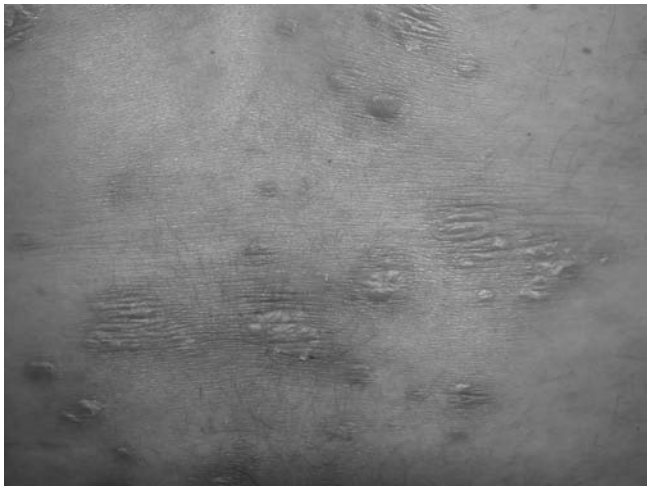


FIGURE 58.3 Sharply demarcated psoriatic plaques.

characterized by pink to red papules and plaques. The lesions are of variable size, sharply demarcated, and covered with scales (Figure 58.3). Both sexes are equally affected and 75% of patients develop the disease before the age of 40 years (Henseler and Christophers, 1985). Two peaks of age onset have been described; one at 20–30 years and a smaller peak at 50–60 years, suggesting that two forms of the disease exist. However, there are exceptions to this since in some families in which there is early onset and severe disease, there appears to be segregation dependent on a highly penetrant autosomal-dominant susceptibility gene distinct from HLA. Psoriasis can also occur with other inflammatory autoimmune diseases, notably psoriatic arthritis (10–30% of cases) (Ruderman and Tambar, 2004).

In the fully developed lesions of psoriasis the histologic picture is characterized by: 1) acanthosis with regular elongation of the rete ridges and thickening in the lower portion; 2) thinning of the suprapapillary epidermis with the occasional presence of small spongioforme pustules; 3) pallor of the upper layers of the epidermis; 4) a diminished to absent granular layer; 5) confluent parakeratosis; 6) the presence of Munro microabscesses (parakeratosis with neutrophils); 7) elongation and edema of the dermal papillae; and 8) tortuous capillaries. Of all these features, Munro microabscesses are characteristic for psoriasis. As a rule Munro microabscesses are found easily in early lesions but are few in number or absent in long-standing lesions. A bleeding point, which may be produced by gentle scraping of the skin (Auspitz sign), corresponds to the tips of the papillae. In ordinary psoriasis, the spongiforme pustule of Kojoi is a very small micropustule that is seen only in early active lesions, whereas it occurs as a macropustule in all variants of generalized pustular psoriasis and represents a characteristic histologic lesion. The epidermis contains an infiltrate of mononuclear cells and neutrophils can often be seen migrating from the capillaries in the papillae into the epidermis.

Pathophyisology

The pathogenesis of psoriasis is due to a combination of a genetic predisposition and particular environmental exposures that include injury, infection, stress or certain medications (Guilhou, 1998). An intriguing characteristic of psoriasis is the Köbner phenomenon, which is the appearance of isomorphic pathologic lesions following skin trauma in patients with preexisting cutaneous disease (Weiss et al., 2002).

Characteristic features of psoriasis suggest an immune-mediated process (Robert and Kupper, 1999). Several lines of direct and indirect evidence suggest that T-cells play a crucial role in pathogenesis (Wrone-Smith and Nickoloff, 1996; Gilhar et al., 1997; Bos and De Rie, 1999). Partially identified antigens, like superantigens but probably also keratinocyte-derived antigens, induce specific $CD4^+$ T cells. The presence of antigen-specific $CD4^+$ T cells secreting type 1 cytokines, IFN-γ, IL-2, and TNF-α was demonstrated in psoriatic skin lesions (Nickoloff, 1991; 1999; Kupper, 2003). In addition, memory T cells present in psoriatic lesions express the CLA molecule on their surface and predominate in the skin, whereas in inflammatory diseases involving tissues other than the skin, predominantly CLA^- T cells are present (Picker et al., 1993). CLA is an adhesion molecule that mediates the initial tethering of T cells to the endothelium in cutaneous postcapillary venules (Picker et al., 1993; Fuhlbrigge et al., 1997). E-selectin is the endothelial ligand for CLA and its expression is upregulated during cutaneous inflammation (Groves et al., 1992). The preferential expression of E-selectin in skin helps select for CLA^+ T cells. Furthermore the activation of T cells through chemokines, other integrins, and cell adhesion molecules is also required (Springer, 1994). The expression of unique chemokine receptors by CLA^+ T cells and the preferential expression of their respective chemokine ligands by skin cells increase the specificity of these T cells for the skin (Campbell et al., 1999). The type 1 cytokine bias has also been observed in circulating blood T cells from psoriatic patients (Lowes et al., 2004). Imbalance between Th1 and Th2 cells in psoriasis was supported by a deficiency of IL-10 in psoriatic lesions. Moreover, with curative antipsoriatic therapy, an increase in IL-10 mRNA expression was observed in peripheral blood mononuclear cells (Asadullah et al., 1998). IL-10 therapy given either intralesionally or subcutaneously resulted in a marked reduction of psoriatic lesions (Asadullah et al., 1998; 1999). These data suggest that psoriasis is an inflammatory $CD4^+$/Th1-mediated autoimmune disorder but the culprit autoantigens have still not been identified.

Further evidence implicating the immune system in psoriasis has come from clinical studies. Drugs that act by suppressing the activity of T cells, such as cyclosporine, FK506, and the recently developed biologics, are effective in treating psoriasis (Krueger, 2002). Further evidence for T-cell-based susceptibility has come from bone marrow transplantations, in which psoriasis of the donor has been transmitted to the recipient (Eedy et al., 1990).

Other evidence has come from animal models. The injection of activated blood-derived T-lymphocytes from a patient with psoriasis into SCID mice bearing autologous human grafted skin resulted in psoriatic plaques and the presence of T-cells with NK cell receptors that accumulate immediately before the onset of acute cutaneous lesions (Nickoloff and Wrone-Smith, 1999). T cells isolated from involved psoriatic skin enhance keratinocyte proliferation. The normal cycle of maturation of keratino-cytes is 28–30 days, whereas in psoriasis this is accelerated to 3–4 days (Guilhou, 1998). During lesion formation, inflammation precedes epidermal hyperproliferation. Also, increased numbers of T cells have been demonstrated in the uninvolved skin of patients with psoriatics (Baadsgaard et al., 1990).

Genetic Features

In 1980 an association of psoriasis with HLA class I alleles was demonstrated, particularly HLA-Cw6 (Tiilikainen et al., 1980). Although not all affected family members are HLA-Cw6$^+$, this locus shows a strong concordance with severity of disease (Gudjonson et al., 2002). As the penetrance of HLA-Cw0602 is only 10%, additional environmental effects or additional genetic susceptibility factors are implicated. Analysis of concurrence of different autoimmune disorders in family members has shown that patients with inflammatory bowel disease and psoriasis share the haplotype HLA-A3, B35, Cw4, and DR-2 (Sels et al., 1997).

The localization of a second psoriasis susceptibility locus to chromosome 17q25 was demonstrated following a genome-wide linkage scan on eight multiply affected families (Tomfohrde et al., 1994). In this study the family contributing the greatest evidence for linkage had 20 affected members and the penetrance of the disease was very high (80–90%). In 1996, linkage of *PSORS3* to chromosome 4q35 was reported in a set of Irish families (Matthews et al., 1996). Additional genome-wide scans have revealed evidence for linkage to chromosome 1q21 (*PSORS4*) in families from the Lazio region of Italy. Others have shown linkage to chromosome 3q21 (*PSORS5*) in Swedish families and 19p13 in German families (Lee et al., 2000). The function of genes at these particular loci has not been determined. However, the identification of multiple susceptibility loci for psoriasis indicates that psoriasis and psoriatic arthritis are genetically heterogeneous (Alarcon-Riquelme, 2003).

Recently, the examination of gene transcripts and peptides with altered expression levels, including a global genome-wide expression study (Zhou et al., 2003), has identified a large number of dysregulated genes and gene clusters, involving particularly epithelial proliferation and the immune system. However, studies have not yet provided sufficient information to identify the molecular defects underlying the disease.

Treatment

Treatment of psoriasis depends on the severity of the disease and the effectiveness of the treatment chosen. It includes local and systemic approaches as well as balneophototherapy. Topical treatment includes the usage of topical vitamin D analogs and retinoids (Takahashi and Iizuka, 2004; Yamauchi, et al., 2004), or combinations of topical treatments (combinations of dithranol, vitamin D3 and retinoids, with a topical corticosteroid can be very effective). The major targets of action of these topical substances are the hyperproliferative keratinocytes. Also, new topical immune modulators like tacrolimus and pimecrolimus are effective in psoriasis (Lebwohl et al., 2004; Wolff, 2004). These substances are approved only for the treatment of atopic dermatitis. Future new developments are most probably the peroxisome proliferation-activating ligands (PPAR-L) (Kuenzli and Saurat, 2004). These have been demonstrated to play an important pathophysiologic role in psoriasis and clinical trials using PPAR-L are underway.

Phototherapy

Phototherapy remains a central treatment option for psoriasis vulgaris. Photochemotherapy and various methods to deliver narrow-band UVB can be used either as monotherapy or in combination with other agents to treat moderate and severe psoriasis. In 1991, a consensus guideline recommended PUVA for patients requiring second-line therapy (Ibbotson et al., 2004). TL-01 UVB has become more widely available and is replacing the less effective broadband sources (Bandow and Koo, 2004). For patients with skin phototypes I–III with chronic plaque psoriasis, TL-01 UVB phototherapy is the treatment of first choice for those requiring photo(chemo)therapy. PUVA should generally be reserved for those patients whose psoriasis does not respond adequately to TL-01 UVB (Dawe et al., 2003).

Current Therapeutic Strategies

Systemic immune therapy includes the suppression of type 1 cytokine-mediated autoimmune reactions and/or immune modulation shifting from a type 1 to type 2 immune response. There is a beneficial effect of the type 2 cytokines

IL-10 and IL-11 (Asadullah et al., 1998). Currently used antipsoriatic drugs include methotrexate, cyclosporine, fumeric acid, azathioprine or mycophenolate mofetil (Geilen et al., 2001; Heydendael et al., 2003; Balasubramaniam et al., 2004; Bigby, 2004). The development of specific immune-modulating or selective suppressive agents includes the large-scale production of humanized, chimeric or purely human antibodies and the development of antisense RNA preparations.

Selective T-cell depletion is achieved with a fusion toxin protein DAB389IL-2 (depletion of activated T-cells expressing IL-2 receptor). It is effective in severe psoriasis, but the side effects are severe and prolonged immunosuppression can occur (Gottlieb et al., 1995; Bagel et al., 1998). Another approach is the blockade of T cells by directly targeting critical surface proteins involved in the process of T-cell activation, or blocking critical type 1 cytokines. Several biologics have already shown considerable clinical efficacy for the treatment of psoriasis in well-designed clinical studies. In a phase II multicenter study on 145 patients, efazilumab (an anti-CD11a antibody that inhibits integrin-binding molecule) induced over 50% improvement in "physicians' global assessment" after 8 weeks of treatment in 48% of patients compared to 15% of patients given placebo (Papp et al., 2001). Anti-TNF-α agents are in clinical trials (Mease et al., 2000; Chaudhari et al., 2001). Etanercept has recently been approved for the treatment of psoriatic arthritis.

Another method of selective depletion of autoreactive lymphocytes is by blocking the T-cell receptor (TCR) assuming that autoreactive T cells in psoriasis are oligoclonal (Chang et al., 1994; Menssen et al., 1995). There is also extensive research on chemokine-blocking drugs. However, the development of selective immune modulatory drugs, which may be considered both anti-inflammatory and immunosuppressive, is complicated by the fact that leukocytes are governed by a complicated network of many and partly redundant chemokines and their receptors (see Chapter 18). Further data are given on various biologics for treatment of psoriasis in Chapter 76.

Psoriasis has a major adverse impact on quality of life and causes much psychological and physical disability. The efficacy of several biologic agents is already proven, and some have been registered for clinical use in psoriasis. Yet, despite these recent advances, the ideal antipsoriatic medication has still to be developed. What is needed is a safe, orally administered compound that provides greater than 80% reduction in disease activity in a significant (>70%) number of patients.

CHRONIC URTICARIA

Hippocrates described urticaria as a distinct entity, but many different subtypes have been recognized during the past century. Approximately 25% of individuals develop an acute urticaria during their lifetime, which is usually a self-limiting disease with the resolution of symptoms in under 6 weeks. If symptoms persist longer, chronic urticaria can be specified, which affects approximately 1% of the population.

Clinical Features

Urticaria is characterized by the rapid appearance of wheals, which may be accompanied by angioedema. Three typical features are characteristics of a wheal: 1) a central swelling of variable size surrounded by a reflex erythema or flare (Figure 58.4); 2) associated itching or sometimes burning; and 3) short duration with normal appearances usually within 24 h. On histologic examination the classical transient wheal shows edema of the epidermis and the upper and mid-dermis, with dilation of postcapillary venules and lymphatics of the upper dermis.

Pathophysiology

Many different factors have been implicated in chronic urticaria, including bacterial infections, adverse reactions to medications or foods, IgE-mediated allergies, and underlying autoimmune diseases, such as SLE.

Depending on the duration of the wheal, a mixed inflammatory perivascular infiltrate of variable intensity consists of neutrophils and/or eosinophils, macrophages, and $CD4^+$ T cells (Haas et al., 1998). In different subtypes of urticaria, alteration of adhesion molecules and cytokine expression is seen in uninvolved skin (Hermes et al., 1999).

Greaves (2002) developed the concept of autoimmune urticaria, postulating that a subgroup of chronic urticaria patients (approximately one-third of patients) has an autoimmune pathophysiology involving activation of cutaneous mast cells. This concept began with the observation that intracutaneous injections of autologous sera caused positive

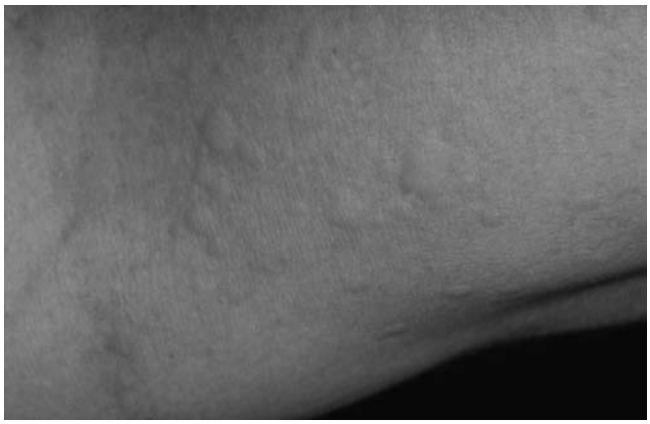


FIGURE 58.4 Urticarial lesions with a central swelling of variable size surrounded by a reflex erythema.

skin reactions (Malmros, 1946). This autologous serum skin test (ASST) procedure has been used to identify autoreactive chronic urticaria patients (Hide et al., 1993), but only skin reactions >1.5 mm should be regarded as positive (Sabroe et al., 1999a). A subgroup of ASST-positive chronic urticaria patients has autoantibodies against FcεRI and/or IgE. Such autologous autoantibodies can degranulate mast cells and basophils and may also induce urticaria-like skin reactions in healthy individuals. These observations were subsequently confirmed by other authors (Fiebiger et al., 1995; 1998; Tong et al., 1997; Sabroe et al., 1999b; Wedi et al., 2000; Zuberbier et al., 2000).

The ASST may be regarded as a useful screening test when used as described by Sabroe et al. (1999b), but its sensitivity and specificity are each only around 80%, and the diagnosis of an autoimmune urticaria cannot be exclusively based on it. Anti-Fcε-RI autoantibodies and the anti-IgE autoantibodies, which may be present in such patients, can be determined either by Western blot or enzyme-linked immunosorbent assay (ELISA). However, these detection systems do not give any explanation of the functional relevance of these antibodies, if present. Only by using cell activation assays can functional data be obtained. This cell activation system is based on enrichment of human basophils, which are stimulated with the patient's sera, with measurements of histamine production. Studies by Kaplan (2002) have suggested that complement also is involved in the activation of mast cells and/or basophils in patients with chronic autoimmune urticaria. The analysis of cell activation assays shows that the presence of anti-FcεRI and IgE autoantibodies does not necessarily lead to mast cell degranulation. This observation has led to the concept that mast cell activating and nonactivating autoantibodies can be distinguished. However, it has also been shown that some patients with a positive ASST have no anti-IgE or anti-IgE receptor antibodies, indicating that there may be other as yet unidentified autoantibodies, e.g., against mast cell membrane components.

Other autoimmune features, particularly antithyroid peroxidase autoantibodies, have been observed in autoimmune urticaria patients (Nettis et al., 2002); thus, among 624 patients with chronic urticaria, there was evidence of thyroid immunity in 14% versus an expected 6% (Nettis et al., 2002).

Treatment

Treatment of chronic urticaria, particularly in patients with an autoimmune background, requires antihistamines and/or leukotrienes in high dosages, and often immune modulatory and/or immunosuppressive treatments are necessary. In some patients even the addition of such treatment is unsatisfactory and they become severely handicapped by the chronic itching course of the disease.

A few reported studies suggest efficacy of removal of autoantibodies by plasmapheresis (Grattan et al., 1992; 2000; Greaves, 2000). Alternatively, immunologic treatment with agents that inhibit antibody formation has been reported, particularly usage of cyclosporine (Fradin et al., 1991; Toubi et al., 1997; Grattan et al., 2000), or high dosages of intravenous immunoglobulin (O'Donnell et al., 1998). However, such treatments are costly and should be reserved for therapy refractory patients.

CONCLUSION

Taken together, several dermatologic diseases with a diverse clinical picture have an autoimmune background. Many other skin diseases, like Behçet's disease, which is characterized by a vasculitis with mucocutaneous, ocular, arthritic, vascular, and other manifestations, have also been discussed as autoimmune diseases. Over the past years more knowledge has been obtained on the mechanisms of many of these diseases. This has had an impact on treatment, which now includes immunosuppressive and immune-modulating agents. As more biologics become available for the treatment of autoimmune diseases, they become an increasing area of interest for dermatology, given the likely application of such substances for the treatment of autoimmune skin diseases. However, in this regard, the severity of the disease has to be considered in relation to the costs of the drug and its potential side effects. As the genetic background plays an important, although as yet undefined, role in autoimmune skin diseases, the possible coexistence of autoimmune diseases of the skin and other tissues must be considered. Every patient presenting with an autoimmune disorder and concomitant skin manifestations should get a dermatologic consultation to unravel associated autoimmune skin disease.

References

Agarwal, U.S. (2004). Satellite repigmentation after punch grafting. *Int. J. Dermatol. 43*, 273–274.

Alacon-Riquelme, M.E. (2003). A RUNX trio with a taste for autoimmunity. *Nat. Genet. 35*, 299–300.

al Badri, A.M., Todd, P.M., Garioch, J.J., Gudgeon, J.E., Stewart, D.G., and Goudie, R.B. (1993a). An immunohistological study of cutaneous lymphocytes in vitiligio. *J. Pathol. 170*, 149–155.

al Badri, A.M., Foulis, A.K., Todd, P.M., Gariouch, J.J., Gudgeon, J.E., Stewart, D.G., Gracie, J.A., and Goudie, R.B. (1993b). Abnormal expression of MHC class II and ICAM-1 by melanocytes in vitiligo. *J. Pathol. 169*, 203–206.

al-Fouzan, A., al-Arbash, M., Fouad, F., Kaaba, S.A., Mousa, M.A., and al-Harbi, S.A. (1995). Study of HLA class I/IL and T lymphocyte subsets in Kuwaiti vitiligo patients. *Eur. J. Immunogenet. 22*, 209–213.

Alkhateeb, A., Fain, P.R., Thody, A., Bennett, D.C., and Spritz, R.A. (2003). Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res. 16*, 208–214.

Asadullah K., Sterry, W., Stephanek, K., Jasulaitis, D., Leupold, M., Audring, H., Volk, H.D., and Docke, W.D. (1998). IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. *J. Clin. Invest. 101*, 783–794.

Asadullah, K., Sabat, R., Wiese, A., Docke, W.D., Volk, H.D., and Sterry, W. (1999). Interleukin-10 in cutaneous disorders: implications for its pathophysiological importance and therapeutic use. *Arch. Dermatol. Res. 291*, 628–636.

Asawanonda, P., Anderson, R.R., Chang, Y., and Taylor, C.R. (2000). 308-nm excimer laser for the treatment of psoriasis: a dose-response study. *Arch. Dermatol. 136*, 619–624.

Baadsgaard, O., Fisher, G., Voorhees, J.J., and Cooper, K.D. (1990). The role of the immune system in the pathogenesis of psoriasis. *J. Invest. Dermatol. 95*, 32S–34S.

Bagel, J., Garland, W.T., Breneman, D., Holick, M., Littlejohn, T.W., Crosby, D., Faust, H., Fivenson, D., and Nichols, J. (1998). Administration of DAB389IL-2 to patients with recalcitrant psoriasis: a double-blind, phase II multicenter trial. *J. Am. Acad. Dermatol. 38*, 938–944.

Balasubramaniam, P., Stevenson, O., and Berth-Jones, J. (2004). Fumaric acid esters in severe psoriasis, including experience of use in combination with other systemic modalities. *Br. J. Dermatol. 150*, 741–746.

Baltas, E., Nagy, P., Bonis, B., Novak, Z., Ignacz, F., Szabo, G., Bor, Z., Dobozy, A., and Kemeny, L. (2001). Repigmentation of localized vitiligo with the xenon chloride laser. *Br. J. Dermatol.* 144, 1266–1267.

Bandow, G.D. and Koo, J.Y. (2004). Narrow-band ultraviolet B radiation: a review of the current literature. *Int. J. Dermatol. 43*, 555–561.

Bigby, M. (2004). A randomized controlled trial of methotrexate and cyclosporine in the treatment of psoriasis. *Arch. Dermatol. 140*, 347–348.

Bodemer, C., Peuchmaur, M., Fraitaig, S., Chatenoud, L., Brousse, N., and De Prost, Y. (2000). Role of cytotoxic T cells in chronic alopecia areata. *J. Invest. Dermatol. 114*, 112–116.

Bos, J.D. and De Rie, M.A. (1999). The pathogenesis of psoriasis: immunological facts and speculations. *Immunol. Today 20*, 40–46.

Bystryn, J.C. (1989). Serum antibodies in vitiligo patients. *Clin. Dermatol. 7*, 136–145.

Caixa, T., Hongwen, F., and Xiran, L. (1999). Levels of soluble interleukin-2 receptor in the sera and skin tissue fluids of patients with vitiligo. *J. Dermatol. Sci.* 21, 59–62.

Campbell, J.J., Haraldsen, G., Pan, J., Rottman, J., Qin, S., Ponath, P., Andrew, D.P., Warnke, R., Ruffing, N., Kassam, N., Wu, L., and Butcher, E.C. (1999). The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature 400*, 776–780.

Chang, J.C., Smith, L.R., Froning, K.J., Schwabe, B.J., Laxer, J.A., Caralli, L.L., Kurland, H.H., Karasek, M.A., Wilkinson, D.I., and Carlo, D.J. (1994). $CD8^+$ T cells in psoriatic lesions preferentially use T-cell receptor V beta 3 and/or V beta 13.1 genes. *Proc. Natl. Acad. Sci. USA 91*, 9282–9286.

Chaudhari, U., Ramona, P., Mulcahy, L.D., Dooley, L.T., Baker, D.G., and Gottlieb, A.B. (2001). Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet 357*, 1842–1847.

Cherif, F., Azaiz, M.I., Ben Hamida, A., Ben, O., and Dhari, A. (2003). Calcipotriol and PUVA as treatment for vitiligo. *Dermatol. Online J. 9*, 4.

Cheung, M. and Briscoe, J. (2003). Neural crest development is regulated by the transcription factor Sox9. *Development 130*, 5681–5693.

Cork, M.J., Tarlow, J.K., Clay, F.E., Crane, A., Blakemore, A.I., McDonagh, A.J., Messenger, A.G., and Duff, G.W. (1995). An allele of the interleukin-1 receptor antagonist as a genetic severity factor in alopecia areata. *J. Invest. Dermatol. 104*, 15S–16S.

Dawe, R.S., Cameron, H., Yule, S., Man, I., Wainwright, N.J., Ibbotson, S.H., and Ferguson, J. (2003). A randomized controlled trial of narrowband ultraviolet B vs bath-psoralen plus ultraviolet A photochemotherapy for psoriasis. *Br. J. Dermatol. 148*, 1194–1204.

Dhar, S. and Malakar, S. (1998). Colocalization of vitiligo and psoriasis in a 9-year-old boy. *Pediatr. Dermatol. 15*, 242–243.

Eedy, D.J., Burrows, D., Bridges, J.M., and Jones, F.G. (1990). Clearance of severe psoriasis after allogenic bone marrow transplantation. *BMJ 300*, 908.

Fiebiger, E., Maurer, D., Holub, H., Reininger, B., Hartmann, G., Woisetschlager, M., Kinet, J.P., and Stingl, G. (1995). Serum Ig autoantibodies directed against the alpha chain of Fc epsilon RI: a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients*?* *J. Clin. Invest. 96*, 2606–2612.

Fiebiger, E., Hammerschmid, F., Stingl, G., and Maurer, D. (1998). Anti-FcepsilonRlalpha autoantibodies in autoimmune-mediated disorders. Identification of a structure-function relationship. *J. Clin. Invest. 101*, 243–251.

Fradin, M.S., Ellis, C.N., Goldfarb, M.T., and Voorhees, J.J. (1991). Oral cyclosporine for severe chronic idiopathic urticaria and angioedema. *J. Am. Acad. Dermatol. 25*, 1065–1067.

Freyschmidt-Paul, P., Happle, R., McElwee, K.J., and Hoffmann, R. (2003a). Alopecia areata: treatment of today and tomorrow. *J. Investig. Dermatol. Symp. Proc. 8*, 12–17.

Freyschmidt-Paul, P., McElwee, K.J., Botchkarev, V., Kissling, S., Wenzel, E., Sundberg, J.P., Happle, R., and Hoffmann, R. (2003b). Fas-deficient C3.MRL-Tnfrsf6(lpr) mice and Fax ligand-deficient C3H/HeJ-Tnfsf6(gld) mice are relatively resistant to the induction of alopecia areata by grafting of alopecia areata-affected skin from C3H/HeJ mice. *J. Investig. Dermatol. Symp. Proc. 8*, 104–108.

Fuhlbrigge, R.C., Kieffer, J.D., Armerding, D., and Kupper, T.S. (1997). Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature 389*, 978–981.

Galbraith, G.M. and Pandey, J.P. (1995). Tumor necrosis factor alpha (TNF-alpha) gene polymorphism in alopecia areata. *Hum. Genet. 96*, 433–436.

Galbraith, G.M., Palesch, Y., Gore, E.A., and Pandey, J.P. (1999). Contribution of interleukin 1beta and KM loci to alopecia areata. *Hum. Hered. 49*, 85–89.

Geilen, C.C., Arnold, M., and Orfanos, C.E. (2001). Mycophenolate mofetil as a systemic antipsoriatic agent: positive experience in 11 patients. *Br. J. Dermatol. 144*, 583–586.

Gilhar, A., David, M., Ullmann, Y., Berkutski, T., and Kalish, R.S. (1997). T-lymphocyte dependence of psoriatic pathology in human psoriatic skin grafted to SCID mice. *J. Invest. Dermatol. 109*, 283–288.

Gilhar, A., Shalaginov, R., Assy, B., Serafimovich, S., and Kalish, R.S. (1999). Alopecia areata is a T-lymphocyte mediated autoimmune disease: lesional human T-lymphocytes transfer alopecia areata to human skin grafts on SCID mice. *J. Investig. Dermatol. Symp. Proc. 4*, 207–210.

Gottlieb, S.L., Gilleaudeau, P., Johnson, R., Estes, L., Woodworth, T.G., Gottlieb, A.B., and Krueger, J.G. (1995). Response of psoriasis to a lymphocyte-selective toxin (DAB3891L-2) suggests a primary immune, but not keratinocyte, pathogenic basis. *Nat. Med. 1*, 442–447.

Grattan, C.E., Francis, D.M., Slater, N.G., Barlow, R.J., and Greaves, M.W. (1992). Plasmapheresis for severe, unremitting, chronic urticaria. *Lancet 339*, 1078–1080.

Grattan, C.E., O'Donnell, B.F., Francis, D.M., Niimi, N., Barlow, R.J., Seed, P.T., Kobza, A., and Greaves, M.W. (2000). Randomized double-blind study of cyclosporin in chronic 'idiopathic' urticaria. *Br. J. Dermatol. 143*, 365–372.

Greaves, M. (2000). Chronic urticaria. *J. Allergy Clin. Immunol. 105*, 664–672.

Greaves, M. (2002). Autoimmune urticaria. *Clin. Rev. Allergy Immunol. 23*, 171–183.

Groves, R.W., Ross, E., Barker, J.N., Ross, J.S., Camp, R.D., and MacDonald, D.M. (1992). Effect of *in vivo* interleukin-1 on adhesion

molecule expression in normal human skin. *J. Invest. Dermatol. 98*, 384–387.

Gudjonsson, J.E., Karason, A., Antonsdottir, A.A., Runarsdottir, E.H., Gulcher, J.R., Stefansson, K., and Valdimarsson, H. (2002). HLA-Cw6-positive and HLA-Cw6-negative patients with Psoriasis vulgaris have distinct clinical features. *J. Invest. Dermatol. 118*, 362–365.

Guilhou, J. (1998). Immunopathogenesis of psoriasis: news in an old concept. *Dermatology 197*, 310–312.

Haas, N., Schadendorf, D., and Henz, B.M. (1998). Differential endothelial adhesion molecule expression in early and late whealing reactions. *Int. Arch. Allergy Immunol. 115*, 210–214.

Happle, R. and Echternacht, K. (1977). Induction of hair growth in alopecia areata with D.N.C.B. *Lancet 2*, 1002–1003.

Hara, I., Takechi, Y., and Houghton, A.N. (1995). Implicating a role for immune recognition of self in tumor rejection: passive immunization against the brown locus protein. *J. Exp. Med. 182*, 1609–1614.

Harning, R., Cui, J., and Bystryn, J.C. (1991). Relation between the incidence and level of pigment cell antibodies and disease activity in vitiligio. *J. Invest. Dermatol. 97*, 1078–1080.

Headington, J.T. (1991). The histopathology of alopecia areata. *J. Invest. Dermatol. 96*, 69S.

Hedstrand, H., Ekwall, O., Olsson, M.J., Landgren, E., Kemp, E.H., Weetman, A.P., Perheentupa, J., Husebye, E., Gustafsson, J., Betterle, C., Kampe, O., and Rorsman, F. (2001). The transcription factors SOX9 and SOX10 are vitiligo autoantigens in autoimmune polyendocrine syndrome type I. *J. Biol. Chem. 276*, 35390–35395.

Henseler, T. and Christophers, E. (1985). Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. *J. Am. Acad. Dermatol. 13*, 450–456.

Hermes, B., Prochazka, A.K., Haas, N., Jurgovsky, K., Sticherline, M., and Henz, B.M. (1999). Upregulation of TNF-alpha and IL-3 expression in lesional and uninvolved skin in different types of urticaria. *J. Allergy Clin. Immunol. 103*, 307–314.

Heyendael, V.M., Spuls, P.I., Opmeer, B.C., de Borgie, C.A., Reitsma, J.B., Goldschmidt, W.F., Bossuty, P.M., Bos, J.D., and de Rie, M.A. (2003). Methotrexate versus cyclosporine in moderate-to-severe chronic plaque psoriasis. *N. Engl. J. Med. 349*, 658–665.

Hide, M., Francis, D.M., Grattan, D.E., Hakimi, J., Kochan, J.P., and Greaves, M.W. (1993). Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N. Engl. J. Med. 328*, 1599–1604.

Honda, Y., Okubo, Y., and Koga, M. (1997). Relationship between levels of soluble interleukin-2 receptors and the types and activity of vitiligo. *J. Dermatol. 24*, 561–563.

Huang, C.L., Nordlund, J.J., and Boissy, R. (2002). Vitiligo a manifestation of apoptosis*? Am. J. Clin. Dermatol. 3*, 301–308.

Hwang, S.M., Ahn, S.K., and Choi, E.H. (1998). Psoriasis occurring in amelanotic lesions. *J. Dermatol. 25*, 66–67.

Ibbotson, S.H., Bilsland, D., Cox, N.H., Dawe, R.S., Diffey, B., Edwards, C., Farr, P.M., Ferguson, J., Hart, G., Hawk, J., Lloyd, J., Martin, C., Moseley, H., McKenna, K., Rhodes, L.E., and Taylor, D.K. (2004). An update and guidance on narrowband ultraviolet B phototherapy: a British Photodermatology Group Workshop Report. *Br. J. Dermatol. 151*, 283–297.

Kaplan, A.P. (2002). Clinical practice. Chronic urticaria and angioedema. *N. Engl. J. Med. 346*, 175–179.

Kemp, E.H., Waterman, E.A., Gawkrodger, D.J., Watson, P.F., and Weetman, A.P. (1998). Autoantibodies to tryosinase-related protein-1 detected in the sera of vitiligo patients using a quantitive radiobinding assay. *Br. J. Dermatol. 139*, 798–805.

Kemp, E.H., Waterman, E.A., Hawes, B.E., O'Neill, K., Gottumukkala, R.V., Gawkrodger, D.J., Weetman, A.P., and Watson, P.F. (2002). The melanin-concentrating hormone receptor 1, a novel target of autoantibody responses in vitiligo. *J. Clin. Invest. 109*, 923–930.

Krueger, J.G. (2002). The immunologic basis for the treatment of psoriasis with new biologic agents. *J. Am. Acad. Dermatol. 46*, 1–23; quiz 23–26.

Kuenzli, S. and Saurat, J.H. (2004). Peroxisome proliferator-activated receptors as new molecular targets in psoriasis. *Curr. Drug Targets Inflamm. Allergy 3*, 205–211.

Kupper, T.S. (2003). Immunologic targets in psoriasis. *N. Engl. J. Med. 349*, 1987–1990.

Lazarova, R., Hristakieva, E., Lazarov, N., and Shani, J. (2000). Vitiligo-related neuropeptides in nerve fibers of the skin. *Arch. Physiol. Biochem. 108*, 262–267.

Le Pooole, I.C., van den Wijngaard, R.M., Westerhof, W., and Das, P.K. (1996). Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *Am J. Pathol. 148*, 1219–1228.

Lebwohl, M., Freeman, A.K., Chapman, M.S., Feldman, S.R., Hartle, J.E., and Henning, A. (2004). Tacrolimus ointment is effective for facial and intertriginous psoriasis. *J. Am. Acad. Dermatol. 51*, 723–730.

Lee, Y.A., Ruschendorf, F., Windemuth, C., Schmitt-Egenolf, M., Stadelmann, A., Nurnberg, G., Stander, M., Wienker, T.F., Reis, A., and Traupe, H. (2000). Genomewide scan in German families reveals evidence for a novel psoriasis-susceptibility locus on chromosome 19p13. *Am. J. Hum. Genet. 67*, 1020–1024.

Leyden, J.L. and Kligman, A.M. (1972). Treatment of alopecia areata with steroid solution. *Arch. Dermatol*. *106*, 924.

Lowes, M.A., Lew, W., and Krueger, J.G. (2004). Current concepts in the immunopathogenesis of psoriasis. *Dermatol. Clin. 22*, 349–369, vii.

Malmros, H. (1946). Autoserumtest. *Nordisk Med.* 29, 150–151.

Matthews, D., Fry, L., Powles, A., Weber, J., McCarthy, M., Fisher, E., Davies, K., and Williamson, R. (1996). Evidence that a locus for familial psoriasis maps to chromosome 4q. *Nat. Genet. 14*, 231–233.

McElwee, K.J., Spiers, E.M., and Oliver, R.F. (1996). *In vivo* depletion of $CD8^+$ T cells restores hair growth in the DEBR model for alopecia areata. *Br. J. Dermatol. 135*, 211–217.

McElwee, K., Freyschmidt-Paul, P., Ziegler, A., Happle, R., and Haffmann, R. (2001). Genetic susceptibility and severity of alopecia areata in human and animal models. *Eur. J. Dermatol. 11*, 11–16.

Mease, P.J., Goffe, B.S., Metz, J., VanderStoep, A., Finck, B., and Burge, D.J. (2000). Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet 356*, 385–390.

Menssen, A., Trommler, P., Vollmer, S., Schendel, D., Albert, E., Gurtler, L., Riethmuller, G., and Prinz, J.C. (1995). Evidence for an antigen-specific cellular immune response in skin lesions of patients with psoriasis vulgaris. *J. Immunol. 155*, 4078–4083.

Messenger, A.G. and Bleehen, S.S. (1985). Expression of HLA-DR by anagen hair follicles in alopecia areata. *J. Invest. Dermatol. 85*, 569–572.

Messenger, A.G., Slater, D.N., and Bleehen, S.S. (1986). Alopecia areata: alterations in the hair growth cycle and correlation with the follicular pathology. *Br. J. Dermatol. 114*, 337–347.

Moretti, S., Spallanzani, A., Amato, L., Hautmann, G., Gallerani, I., Fabiani, M., and Fabbri, P. (2002). New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. *Pigment Cell Res. 15*, 87–92.

Naughton, G.K., Reggiardo, D., and Bystryn, J.C. (1986). Correlation between vitiligo antibodies and extent of depigmentation in vitiligo. *J. Am. Acad. Dermatol. 15*, 978–981.

Nettis, E., Dambra, P., D'Oronzio, L., Cavallo, E., Loria, M. P., Fanelli, M., Ferrannini, A., and Tursi, A. (2002). Reactivity to autologous serum skin test and clinical features in chronic idiopathic urticaria. *Clin. Exp. Dermatol. 27*, 29–31.

Nickoloff, B.J. (1991). The cytokine network in psoriasis. *Arch. Dermatol. 127*, 871–884.

Nickoloff, B.J. (1999). The immunologic and genetic basis of psoriasis. *Arch. Dermatol. 135*, 1104–1110.

Nickoloff, B.J. and Wrone-Smith, T. (1999). Injection of pre-psoriatic skin with $CD4^+$ T cells induces psoriasis. *Am. J. Pathol. 155*, 145–158.

O'Donnell, B.F., Barr, R.M., Black, A.K., Francis, D.M., Kermani, F., Niimi, N., Barlow, R.J., Winkelmann, R.K., and Greaves, M.W. (1998). Intravenous immunoglobulin in autoimmune chronic urticaria. *Br. J. Dermatol. 138*, 101–106.

Ongenae, K., Van Geel, N., and Naeyaert, J.M. (2003). Evidence for an autoimmune pathogenesis of vitiligo. *Pigment Cell Res. 16*, 90–100.

Papp, K., Bissonnette, R., Krueger, J.G., Carey, W., Gratton, D., Gulliver, W.P., Lui, H., Lynde, C.W., Magee, A., Minier, D., Ouellet, J.P., Patel, P., Shapiro, J., Shear, N.H., Kramer, S., Walicke, P., Bauer, R., Dedrick, R.L., Kim, S.S., White, M., and Garavoy, M.R. (2001). The treatment of moderate to severe psoriasis with a new anti-CD11a monoclonal antibody. *J. Am. Acad. Dermatol. 45*, 665–674.

Pascher, F., Kurtin, S., and Andrade, R. (1970). Assay of 0.2 percent fluocinolone acetonide cream for alopecia areata and totalis. Efficacy and side effects including histologic study of the ensuing localized acneform response. *Dermatologica 141*, 193–202.

Paus, R., Eichmuller, S., Hofmann, U., Czarnetzki, B.M., and Robinson, P. (1994). Expression of classical and non-classical MHC class I antigens in murine hair follicles. *Br. J. Dermatol. 131*, 177–183.

Picker, L.J., Kishimoto, T.K., Smith, C.W., Warnock, R.A., and Butcher, E.C. (1991). ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature 349*, 796–799.

Picker, L.J., Treer, J.R., Ferguson-Darnell, B., Collins, P.A., Bergstresser, P.R., and Terstappen, L.W. (1993). Control of lymphocyte recirculation in man. II. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. *J. Immunol. 150*, 1122–1136.

Robert, C. and Kupper, T.S. (1999). Inflammatory skin diseases, T cells, and immune surveillance. *N. Engl. J. Med. 341*, 1817–1828.

Rubisz-Brzezinska, J., Buchner, S.A., and Itin, P. (1996). Vitiligo associated with lichen planus. Is there a pathogenetic relationship? *Dermatology 192*, 176–178.

Ruderman, E.M. and Tambar, S. (2004). Psoriatic arthritis: prevalence, diagnosis, and review of therapy for the dermatologist. *Dermatol. Clin. 22*, 477–486, x.

Sabroe, R.A., Gratten, C.E., Francis, C.M., Barr, R.M., Kobza Black, A., and Greaves, M.W. (1999a). The autologous serum skin test: a screening test for autoantibodies in chronic idiopathic urticaria. *Br. J. Dermatol. 140*, 446–452.

Sabroe, R.A., Seed, P.T., Francis, D.M., Barr, R.M., Black, A.K., and Greaves, M.W. (1999b). Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti-FcepsilonRI and anti-IgE autoantibodies. *J. Am. Acad. Dermatol. 40*, 443–450.

Safavi, K.H., Muller, S.A., Suman, V.J., Moshell, A.N., and Melton, L.J., 3rd (1995). Incidence of alopecia areata in Olmstead County, Minnesota, 1975 through 1989. *Mayo Clin. Proc. 70*, 628–633.

Sagoo, G.S., Cork, M.J., Patel, R., and Tazi-Ahnini, R. (2004). Genome-wide studies of psoriasis susceptibility loci: a review. *J. Dermatol. Sci. 35*, 171–179.

Schallreuter, K.U., Elwary, S.M., Gibbons, N.C., Rokos, H., and Wood, J.M. (2004). Activation/deactivation of acetylcholinesterase by H_2O_2: more evidence for oxidative stress in vitiligo. *Biochem. Biophys. Res. Commun. 315*, 502–508.

Sels, F., Westhovens, R., Emonds, M.P., Vandermeulen, E., and Dequeker, J. (1997). HLA typing in a large family with multiple cases of different autoimmune diseases. *J. Rheumatol. 24*, 856–859.

Spencer, J.M., Nossa, R., and Ajmeri, J. (2002). Treatment of vitiligo with the 308-nm excimer laser: a pilot study. *J. Am. Acad. Dermatol. 46*, 727–731.

Springer, T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell 76*, 301–314.

Spritz, R.A., Gowan, K., Bennett, D.C., and Fain, P.R. (2004). Novel vitiligo susceptibility loci on chromosomes 7 (AIS2) and 8 (AIS3), confirmation of SLEV1 on chromosome 17, and their roles in an autoimmune diathesis. *Am. J. Hum. Genet. 74*, 188–191.

Takahashi, H. and Iizuka, H. (2004). [Treatment of keratinizing skin disorders, psoriasis, by topical vitamin D3]. *Clin. Calcium 14*, 129–132.

Tan, E., Tay, Y.K., Goh, C.L., and Chin Giam, Y. (2002). The pattern and profile of alopecia areata in Singapore—a study of 219 Asians. *Int. J. Dermatol. 41*, 748–753.

Tiilikainen, A., Lassus, A., Karvonen, J., Vartiainen, P., and Julin, M. (1980). Psoriasis and HLA-Cw6. *Br. J. Dermatol. 102*, 179–184.

Tobin, D.J., Sundberg, J.P., King, L.E., Jr., Boggess, D., and Bystryn, J.C. (1997). Autoantibodies to hair follicles in C3H/HeJ mice with alopecia areata-like hair loss. *J. Invest. Dermatol. 109*, 329–333.

Tomfohrde, J., Silverman, A., Barnes, R., Fernandez-Vina, M.A., Young, M., Lory, D., Morris, L., Wuepper, K.D., Stastny, P., and Menter, A. (1994). Gene for familial psoriasis susceptibility mapped to the distal end of human chromosome 17q. *Science 264*, 1141–1145.

Tong, L.J., Balakrishnan, G., Kochan, J.P., Kinet, J.P., and Kaplan, A.P. (1997). Assessment of autoimmunity in patients with chronic urticaria. *J. Allergy Clin. Immunol. 99*, 461–465.

Toubi, E., Blant, A., Kessel, A., and Golan, T.D. (1997). Low-dose cyclosporin A in the treatment of severe chronic idiopathic urticaria. *Allergy 52*, 312–316.

Valkova, S., Trashlieva, M., and Christova, P. (2004). Treatment of vitiligo with local khelin and UVA: comparison with systemic PUVA. *Clin. Exp. Dermatol. 29*, 180–184.

van den Wijngaard, R., Wankowicz-Kalinska, A., Le Poole, C., Tigges, B., Westerhof, W., and Das, P. (2000). Local immune response in skin in generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA^+ T cells at the perilesional site. *Lab. Invest. 80*, 1299–1309.

Vasquez-Lopez, F., Lopez-Escobar, M., and Perez-Oliva, N. (2003). Calcipotriene and vitiligo. *Arch. Dermatol. 139*, 1656–1657.

Wedi, B., Novacovic, V., Koerner, M., and Kapp, A. (2000). Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression—inhibitory effects of anti-inflammatory drugs. *J. Allergy Clin. Immunol. 105*, 552–560.

Weiss, G., Shemer, A., and Trau, H. (2002). The Koebner phenomenon: review of the literature. *J. Eur. Acad. Dermatol. Venereol. 16*, 241–248.

Willan, R. (1950). *Description and Treatment of Cutaneous Diseases*. London: Johnson.

Wolff, K. (2004). Current concepts and review of pimecrolimus in the treatment of psoriasis. *Dermatol. Clin. 22*, 461–465, ix–x.

Wrone-Smith, T. and Nickoloff, B.J. (1996). Dermal injection of immunocytes induces psoriasis. *J. Clin. Invest. 98*, 1878–1887.

Yamauchi, P.S., Rizk, D., and Lowe, N.J. (2004). Retinoid therapy for psoriasis. *Dermatol. Clin. 22*, 467–476, x.

Zhou, X., Krueger, J.G., Kao, M.C., Lee, E., Du, F., Menter, A., Wong, W.H., and Bowcock, A.M. (2003). Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol. Genom. 13*, 69–78.

Zoller, M., McElwee, K.J., Engel, P., and Hoffmann, R. (2002). Transient CD44 variant isoform expression and reduction in CD4(+)/CD25(+) regulatory T cells in C3H/HeJ mice with alopecia areata. *J. Invest. Dermatol. 118*, 983–992.

Zuberbier, T., Henz, B.M., Fiebiger, E., Maurer, D., and Stingl, G. (2000). Anti-FcepsilonRIalpha serum autoantibodies in different subtypes of urticaria. *Allergy 55*, 951–954.

CHAPTER

59

Kidney Disease: Goodpasture's Disease, Lupus Nephritis, ANCA-Associated Glomerulonephritis

BARRAK M. PRESSLER,*† RONALD J. FALK,† AND GLORIA A. PRESTON†

**College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, and †Division of Nephrology and Hypertension, Department of Medicine, University of North Carolina at Chapel Hill. Chapel Hill, North Carolina, USA*

Kidney diseases that are caused by autoimmune processes may result from: 1) direct antibody-mediated attack against kidney antigens (anti-glomerular basement membrane disease); 2) immune complex deposition [systemic lupus erythematosus (SLE)]; or 3) antibody-induced leukocyte activation, endothelial cell injury, and glomerulonephritis [antineutrophil cytoplasmic antibody (ANCA) disease]. Although many other kidney diseases are at least partially immune-mediated in nature, we will concentrate on these three diseases as the best examples of autoimmunity in the kidney.

ANTI-GLOMERULAR BASEMENT MEMBRANE DISEASE

Anti-glomerular basement membrane (anti-GBM) disease is the only kidney disease in which it is definitely known that antibodies develop against a native renal antigen—the α3 helix of collagen IV. Ernest Goodpasture first described two patients with renal failure and pulmonary hemorrhage in the early 20th century, although the eponym "Goodpasture's disease" was not suggested until 1958 (Stanton and Tange, 1958). In the 1960s it was discovered that a subset of patients with these clinical signs had circulating antirenal antigen antibodies with deposition of immunoglobulin in the kidney (Salama et al., 2001a). Currently, the pulmonary–renal syndrome can be caused by more than just anti-GBM disease; cryoglobulinemia, SLE, and systemic vasculitides, such as ANCA disease, are also potential culprits. However "Goodpasture's disease" and "Goodpasture syndrome" are terms reserved for those pulmonary–renal syndrome patients with antibodies against the GBM epitope.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Clinical, Pathologic, and Epidemiologic Features

Collagen IV is a normal constituent of basement membranes and thus is distributed widely throughout the body. Six homologous proteins, numbered α1 through α6, assemble into triple-helical protomers that interlock to form the final collagen IV network (Sundaramoorthy et al., 2002). Most collagen IV is composed entirely of α1.α1.α2 protomers, which are basement membrane proteins produced and assembled during fetal development. During *in-utero* maturation these are gradually replaced by α3.α4.α5 protomers in the basement membranes of the glomerulus, renal tubules, lung, cochlea, and eye (Cashman et al., 1988). α5.α5.α6 protomers replace some of the original protomers in Bowman's capsule, skin, and smooth muscle, but these are not affected in anti-GBM disease (Hudson et al., 2003).

All collagen IV protein helices consist of a middle collagenous region bounded by two noncollagenous domains. The proteins initially associate noncovalently via C-terminal noncollagenous domain interactions, which induce winding of the central collagenous regions into a triple helix (Sundaramoorthy et al., 2002). The final collagen IV network is formed by the globular head–globular head interaction of the C-terminal domains of two adjacent protomers and the association of four N-terminal domain regions into a large lattice. The glomerular capillary endothelial cells and podocyte foot processes attach directly to the collagen IV matrix via integrins and dystroglycans (Mundel and Shankland, 2002).

The overwhelming majority (>99%) of patients with anti-GBM disease develop antibodies specific for a single basement membrane epitope in the collagen IV molecule. The "Goodpasture epitope" is made up of nine discontinuous amino acids in the C-terminal noncollagenous domain of the α3 helix [α3(IV)NC1] (Hellmark et al., 1999; Gunnarsson et al., 2000) (Figure 59.1). Although there are sporadic reports of patients with anti-GBM disease who do not have antibodies against this particular epitope or collagen IV (despite the presence of linear immunoglobulin staining on immunohistochemical examination of renal tissue), these cases are rare.

Anti-GBM disease is rare—responsible for less than 5% of all glomerular disease. In white persons, approximately one new case of anti-GBM disease is diagnosed per million people each year, with the incidence in other ethnic populations even lower (Bolton, 1996; Pusey, 2003). Anti-GBM disease has been reported in individuals of all ages and both genders. A bimodal distribution exists, with disease increasing in frequency in young-to-middle aged men and elderly women; however, the first peak may be due to increased exposure to exacerbating environmental factors such as cigarette smoke (Donaghy and Rees, 1983; Hudson et al., 2003; Pusey, 2003).

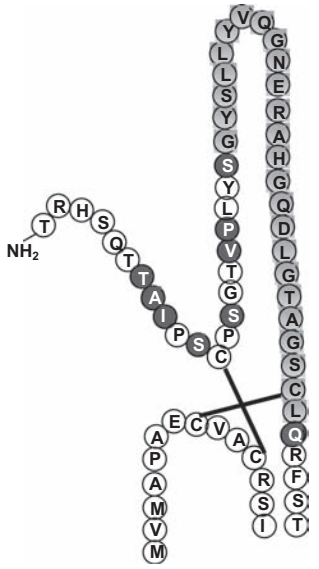


FIGURE 59.1 Schematic of the region of α3(IV)NC1 that contains the anti-glomerular basement membrane antibody epitope. The critical amino acids which make up the discontinuous epitope are colored in blue. Grey amino acids represent a nonantigenic loop that separates a distant antigenic glutamine residue from the other eight antigenic amino acid residues. The thick black lines are disulfide bonds between cysteine residues. Modified from Gunnarsson et al., 2000. See color plate section.

Rapidly progressive glomerulonephritis and pulmonary hemorrhage are the typical clinical manifestations of anti-GBM disease. Patients with pulmonary hemorrhage may be asymptomatic with bleeding only noted upon thoracic radiography or airway endoscopy, or may present with a range of clinical signs from fatigue to cough to fulminant hemoptysis. Many anti-GBM patients with lung involvement have a history of cigarette smoking or other environmental exposure that may exacerbate pulmonary signs (Donaghy and Rees, 1983). Anti-GBM patients with lung involvement usually do not have advanced kidney disease at first diagnosis because pulmonary-related symptoms are more easily observed by the patient, and thus diagnosis may occur earlier (Hudson et al., 2003).

Thirty to 40% of anti-GBM patients have isolated kidney involvement (Pusey, 2003). As with pulmonary disease, glomerulonephritis due to anti-GBM disease may cause a spectrum of clinical signs and clinicopathologic findings at initial presentation. Rarely, patients may present with incidental proteinuria; however, most have clinical signs referable to more severe kidney dysfunction. Patients typically have an active urine sediment, proteinuria, and hematuria. Reduction in renal function may be minimal at the time of diagnosis, although most patients are azotemic and many require dialysis. Younger patients commonly have rapidly progressive pulmonary and kidney disease, whereas older patients have a higher incidence of slowly progressive

kidney disease without precipitous declines in renal and pulmonary function (Hudson et al., 2003). Hypertension is uncommon until endstage kidney disease is imminent.

The most common finding by light microscopy in renal biopsies from patients with anti-GBM disease is diffuse necrotizing crescentic glomerulonephritis. More than 50% of glomeruli are typically affected. GBMs may be disrupted and the mesangium and Bowman's space filled with infiltrating lymphocytes, macrophages, and neutrophils. Chronically affected kidneys may exhibit tubulo-interstitial nephritis and fibrosis of glomeruli. Although the light microscopic appearance of renal biopsies from patients with anti-GBM disease usually reflects the rapidly progressive nature of the disease, examination of kidneys from patients with mild or early disease may reveal normal to mildly affected glomeruli. Similarly, patients with chronic smoldering disease without severe clinicopathologic abnormalities may have global sclerosis and fibrosis without obvious glomerulonephritis and necrosis. ANCA disease or membranous nephropathy is occasionally found in patients with anti-GBM disease; histologic changes typical of these diseases may thus occur as well. Electron microscopy of kidneys from patients with anti-GBM disease reveals nonspecific findings of inflammation, including leukocyte infiltration. As expected the GBM is periodically disrupted, but electron-dense deposits are typically absent.

Regardless of appearance on light or electron microscopy, immunofluorescence microscopic findings are diagnostic for anti-GBM disease. Anti-IgG stains show intense diffuse linear staining of the GBM in all patients (Figure 59.2). Occasional patients have staining of tubular membranes as well, depending on the specificity of the anti-GBM antibodies. Antibodies are predominantly IgG1 and IgG4, although IgM and IgA may also occur. C3 deposition is also typical although not universal in anti-GBM disease; C1q deposition is rare. Diabetic nephropathy and fibrillary glomerulonephritis also are associated with linear staining by immunofluorescence microscopy, but the site of immunoglobulin deposition is not the GBM and the pattern of staining is not identical.

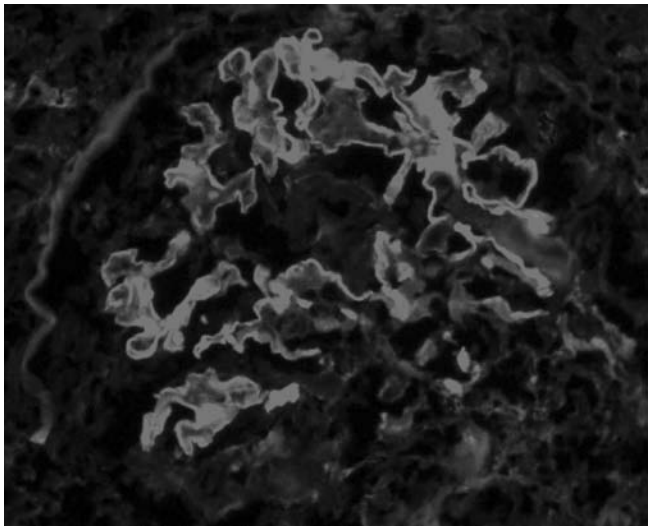


FIGURE 59.2 Glomerulus from a patient with anti-glomerular basement membrane (GBM) disease showing linear staining of the GBM for IgG. There is negative staining in the right lower quadrant of the glomerulus at a site of necrosis in which the GBM has been destroyed. See color plate section.

Courtesy of J. Charles Jennette.

Autoimmune Features

The role of autoantibodies in disease pathogenesis has been clearly demonstrated. Anti-GBM antibodies bind to the renal and alveolar basement membranes, inducing complement fixation and leukocyte recruitment and activation. Passive transfer of serum from human patients or animal models to naïve animals results in glomerulonephritis. Disease remission and amelioration of renal damage in humans is directly associated with removal of antibodies via plasmapheresis and decrease in anti-GBM antibody titer.

Although anti-α3(IV)NC1 antibodies are the diagnostic hallmark of anti-GBM disease, disease induction and progression is likely dependent on cell-mediated mechanisms as well. The precise role of autoreactive T cells is not understood, although differences in the peripheral blood T-cell repertoire of affected patients have been reported (Cairns et al., 2003). The α3(IV)NC1 Goodpasture epitope is expressed in normal human thymic tissue, and thus autoreactive T lymphocytes should be eliminated during negative selection; however, peripheral blood T cells with anti-α3(IV)NC1 specificity and a type 1 helper T (Th1) cytokine profile are found in patients with acute anti-GBM disease (Derry et al., 1995; Merkel et al., 1996; Salama et al., 2001a; Cairns et al., 2003). One study has provided evidence that patients may have decreased numbers of α3(IV)NC1-specific $CD4^+CD25^+$ regulatory T cells (Tregs), thus allowing autoreactive T lymphocytes to proliferate and induce disease (Salama et al., 2003).

Genetic Features

Susceptibility of humans and mice to anti-GBM disease is highly associated with particular major histocompatibility complex (MHC) II alleles. Approximately 80% of patients with anti-GBM disease carry the HLA-DRB1*1501 or HLA-DRB1*04 alleles, as compared to only 20% of individuals in the general population (Phelps and Rees, 1999). Mouse strains with the MHC $H\text{-}2^s$, $H\text{-}2^b$, or $H\text{-}2^d$ alleles develop experimental anti-GBM disease following immunization with α3(IV)NC1, whereas those with $H\text{-}2^k$ are resistant; similarly, rat strains with MHC Rt1-l alleles have

differing susceptibility to disease as compared to those with Rt1-k alleles (Kalluri et al., 1997; Reynolds et al., 1998). This may reflect an increased ability to present antigenic α3(IV)NC1-derived peptides for recognition by self-reactive T cells in the periphery, or on the contrary an inability to present α3(IV)NC1-derived peptides to T lymphocytes in the thymus during cell maturation, which would normally compromise deletion of autoreactive cells. Regardless, development of disease is polygenic, as two different WKY rat strains with identical MHC haplotypes are not equally susceptible to experimental anti-GBM disease (Reynolds et al., 2002a).

Environmental Influences

Epidemiologic studies and case reports have suggested an association between anti-GBM disease and several environmental agents. The first description of "Goodpasture syndrome" was in two patients with influenza-like clinical signs, and sporadic reports of an association between these two diseases can still be found (Salama et al., 2001b). The facts that clusters of anti-GBM disease do occur, and that 20–60% of patients report a prodromal upper respiratory tract infection, increase the suspicion of involvement of infection or toxin-triggered events (Perez et al., 1974). Hydrocarbon and cigarette smoke exposures have been associated with anti-GBM disease, particularly in patients with significant pulmonary hemorrhage (Stevenson et al., 1995). Similarly, lithotripsy, antimyeloperoxidase ANCAs, and other causes of "renal trauma" have occasionally been reported to be associated with the development of anti-GBM disease (Xenocostas et al., 1999).

Historically it was hypothesized that damage to the pulmonary or renal basement membranes by environmental agents exposed a previously cryptic α3(IV)NC1 antigen, thus allowing anti-GBM disease to develop. However, purified antibodies cause disease in animal models without concurrent tissue damage, and thymic medullary cells in normal children express the anti-GBM epitope, implying that the immune system at one point is tolerant of this region of the protein (Wong et al., 2001). It is more likely that the above-mentioned conditions exacerbate pre-existing disease, and asymptomatic patients thus develop overt clinical signs through increased accessibility of antigen to preexisting antibodies. Patients with asymptomatic or clinically mild anti-GBM disease may thus have faster progression of their disease, and a false temporal association is assumed when fulminant clinical signs develop.

Animal Models

Experimental anti-GBM disease can be induced in chickens and rodents by immunization with sonicated GBM collagen, purified collagen IV NC1 trimers, or recombinant α3(IV)NC1. Less commonly, a prime-boost strategy using allotypic antibodies is used to induce experimental anti-GBM disease in mice and rats (Wakayama et al., 2000). In this technique, initial immunization with polyclonal immunoglobulins from a nonhost species is given so that the recipient animal is sensitized to the Fc antibody fragment of the donor species. A second immunization with purified anti-GBM antibodies from the same foreign species leads to a faster inflammatory response and glomerulonephritis. However, because the resultant glomerulonephritis is likely due to the anti-allotypic antibody response, rather than a true anti-GBM immune response, this model is not favored by most investigators.

Animals with experimental anti-GBM disease develop variable degrees of proteinuria, hematuria, azotemia, and pulmonary hemorrhage. Although analogous to anti-GBM disease, rodents with experimental anti-GBM disease have linear antibody staining of the tubular basement membranes and non-α3(IV)NC1 regions of the GBM in addition to the Goodpasture epitope; this occurs because antibodies develop against multiple components of the collagen immunogen. In rodents, the frequency of disease induction, rate of onset of clinical signs, and severity of experimental anti-GBM disease is highly strain dependent. For example, WKY/CR rats and SJL or DBA mice develop rapidly progressive glomerulonephritis with large numbers of glomeruli affected, whereas WKY/Olac rats and several mouse strains (including NOD and AKR mice) develop less severe to negligible disease (Kalluri et al., 1997; Reynolds et al., 1998; Hopfer et al., 2003). Mice deficient in the FcγIIb antibody receptor are particularly susceptible to experimental anti-GBM disease (Nakamura et al., 2000). When immunized with collagen, greater than 90% of FcγIIb receptor-deficient mice develop anti-GBM disease within 8 weeks; in other mouse models disease typically develops in 25–60% of mice, and may take up to 3 months to manifest (Nakamura et al., 2000; Hopfer et al., 2003).

Induction of experimental anti-GBM disease has been accomplished by transfer or stimulation of T cells specific for anti-GBM epitopes. Disease has been produced in chickens by transfer of mononuclear cells from birds previously immunized with bovine collagen, and in rats by activation or transfer of α3(IV)NC1-specific T cells or immunization with a peptide fragment of α3(IV)NC1 (Wu et al., 2003; 2004). This disease-inducing peptide is presumptively a T-cell epitope that activates α3(IV)NC1-specific T cells (Wu et al., 2003; 2004). The peptide used in these studies significantly overlaps with the known α3(IV)NC1 Goodpasture antigen. However, antibodies induced in this peptide model are of a different specificity from naturally-occurring antibodies from human patients, and glomerulonephritis develops prior to the production of anti-GBM antibodies.

Pathogenic Mechanisms

As discussed above, the initiation and progression of anti-GBM disease likely requires both antibody- and cell-mediated mechanisms. Production of α3(IV)NC1-specific antibodies requires presentation of antigen-derived peptides by cognate T cells. Binding of antibodies to the GBM results in leukocyte recruitment through Fc receptor binding, and complement fixation and activation via the classic pathway.

Studies of animal models have provided additional data on the likely immunogenesis of anti-GBM disease. Glomerulonephritis can be induced following immunization with components of collagen IV both in B-cell-competent (i.e., antibody producing) and -deficient mice and chickens (Dean et al., 2005). Additionally, disease is induced in $RAG^{-/-}$ mice (i.e., B- and T-cell deficient) by transferring serum from affected B-cell-competent mice. Infiltrating T cells in WKY rats with experimental disease are of restricted clonality, suggesting a specific T-lymphocyte response rather than a generalized inflammatory recruitment of all effector T cells (Walters et al., 2004). Depletion of $CD8^+$ T cells, blockade of the CD40 costimulatory pathway, or blockade of the CD28-CD80(B7) "second signal" pathway all prevent induction of experimental anti-GBM disease in rats, providing further evidence of a critical role of T lymphocytes (Reynolds et al., 2000; 2002b; 2004). Blockade of various cytokines in rodent models likewise modulates the severity of experimental anti-GBM disease. Studies on the role of macrophages in animal models demonstrate that antibodies are required for cell recruitment, and that these cells contribute to glomerular damage (Kaneko et al., 2003; Kuroda et al., 2004).

Immunologic Markers in Diagnosis

Demonstration of anti-GBM antibodies within serum or by immunofluorescence antibody staining of renal biopsy specimens is diagnostic for disease. The rare patient will have anti-GBM antibodies that are not specific for the α3(IV)NC1 antigen, although the clinical course and renal biopsy findings are identical to those with classic anti-GBM disease (Salama et al., 2002; Levy et al., 2004). Magnitude of serum antibody titer does not predict patient survival or need for dialysis at diagnosis; however, anti-GBM antibody titer is directly associated with eventual maintenance of renal function in those patients who are not dialysis dependent at initial presentation (Segelmark et al., 2003). Anti-GBM antibody affinity does not correlate with outcome (Segelmark, et al., 2003).

Approximately 30% of patients with anti-GBM disease have ANCAs; 5% of ANCA vasculitis patients have anti-GBM antibodies (Levy et al., 2004). ANCAs are specific for myeloperoxidase in the vast majority of these patients, although antibodies against proteinase-3 and other neutrophil cytoplasmic antigens may occur (Levy, et al., 2004). Differentiation of these two diseases in the presence of both antibodies is based on clinical features, relative magnitude of titers, and renal biopsy findings. The relative concentration and specificity of the anti-GBM antibodies in patients with or without ANCAs are similar (Hellmark et al., 1997; Levy et al., 2004). Patients with both anti-GBM antibodies and ANCAs tend to be significantly older, are more likely to need dialysis at the time of presentation, and have systemic inflammatory disease that is more characteristic of ANCA-associated vasculitis (Levy et al., 2004). Despite these differences patients with both antibodies do not have significantly different mortality or frequency of pulmonary hemorrhage from patients with anti-GBM antibodies only. Because of the relapsing nature of ANCA-associated vasculitis and the potential for systemic clinical signs, patients with anti-GBM disease should have ANCA titers measured routinely.

Treatment and Outcome

The mainstays of treatment of anti-GBM disease are immunosuppression and reduction in circulating anti-GBM antibodies by plasmapheresis. The high association between the presence of anti-GBM antibodies and ongoing glomerulonephritis in affected patients makes decrease in antibody titer a priority. Plasmapheresis effectively removes unbound anti-α3(IV)NC1 antibodies, while cytotoxic agents inhibit continued production of new antibodies by inhibition of B-cell function. Glucocorticoids decrease antibody production and also reduce inflammation within the kidney via inhibition of leukocyte recruitment and function.

A standardized treatment regimen has been proposed based on successful treatment of a large cohort of patients (Levy et al., 2001). Initial treatment is with high-dose oral prednisolone and cyclophosphamide; prednisolone is tapered over several months, while cyclophosphamide is continued for 2–3 months. Plasma exchange is performed daily until the anti-GBM titer returns to normal concentrations or for 1 week; post-exchange transfusion of fresh frozen plasma is recommended if pulmonary hemorrhage is present (Levy et al., 2001). Supportive treatment for renal failure and pulmonary compromise is given as needed. Given this protocol, patients with initial serum creatinine concentration less than 5.7 mg/dl (435 μmol/L) have significantly greater long-term survival; patients who require dialysis at presentation or have global crescentic glomerulonephritis on renal biopsy are more likely to remain dependent on dialysis long-term (Levy et al., 2001). All the patients with initial serum creatinine less than 5.7 mg/dl survived, with 95% renal survival. At the time of last follow-up, 84% of this cohort was still alive, with 74% renal survival (median follow-up time was 10 years) (Levy et al., 2001).

Following resolution of clinical signs and reduction of anti-GBM antibodies to normal levels, immunosuppressive therapy can be tapered and discontinued in most patients. Continued monitoring of urine for increases in protein or blood, and occasional measurement of anti-GBM serum antibody concentration, are recommended during dose reductions. Long-term prognosis for patients who survive the immediate post-diagnosis period is good. The 1-year survival rate is typically greater than 75% (Pusey, 2003). Patients with anti-GBM disease often remain free of clinical signs for life after successful treatment, although relapse occurs rarely (Pusey, 2003).

A rarer type of anti-GBM disease occurs in patients with Alport syndrome following renal transplantation. Alport syndrome is due to a mutation in the α5 chain of collagen IV; patients typically have progressive proteinuria and glomerulopathy, and eventual renal failure (Hudson et al., 2003). Following renal transplantation a subset of Alport syndrome patients develop antibodies against the transplanted normal collagen IV molecule, to which the transplant recipient would not expect to be tolerant due to lack of thymic expression during maturation. However, the immunosuppressive regimen given to renal transplant recipients is usually sufficient to prevent development of anti-GBM disease.

SYSTEMIC LUPUS ERYTHEMATOSUS

SLE is a multiorgan disease with diverse manifestations. For a thorough discussion of this disease and its nonrenal clinical and autoimmune features the reader is referred to Chapters 27 and 28. The focus here will be the effects of SLE on the kidney, referred to as lupus nephritis.

The term "lupus erythematosus" was first used in the mid-to-late 1800s to describe patients with cutaneous lesions such as malar or discoid rashes (Lahita, 2004). This disease was recognized as a systemic illness in 1872, when the first list of criteria for diagnosis was published (Lahita, 2004). Lupus nephritis was first reported in the early 20th century in two patients with SLE-associated facial rashes; a second report described the typical pathologic glomerular lesions in lupus nephritis (Osler, 1904; Lahita, 2004). Cases of renal-limited SLE were recognized soon thereafter (Lahita, 2004). Concepts on the nature of SLE were revolutionized by the description by Hargraves (1969) of the lupus erythematosus (LE) cell, which prompted studies in the 1950s on the detection of antinuclear autoantibodies in SLE (see Chapters 28 and 72).

Clinical, Pathologic, and Epidemiologic Features

Four of eleven diagnostic criteria outlined by a 1982 modified American Rheumatism Association standardized protocol are required for diagnosis of SLE (Tan et al., 1982). One of these 11 diagnostic criteria is renal involvement, referred to as lupus nephritis. Although renal biopsy is required for definitive diagnosis of lupus nephritis, persistent proteinuria (>0.5 g/day or greater than 3+ albumin on urine dipstick) or the presence of cellular casts are acceptable for a presumptive diagnosis of nephritis, particularly if a biopsy is contraindicated (Tan et al., 1982). Presence of additional criteria, such as clinicopathologic findings more specific for SLE (i.e., anti-dsDNA antibodies or hypocomplementemia) may also increase the likelihood of correct diagnosis of lupus nephritis in the absence of histopathology (Dooley et al., 2004).

The incidence of lupus nephritis in SLE at the time of first diagnosis is high. Early studies reported clinicopathologic evidence of kidney disease in greater than 50% of patients, and in some reports approached 80% (Dubois and Tuffanelli, 1964; Estes and Christian, 1971). However, likely due to earlier diagnosis of SLE, the incidence of nephritis at diagnosis has decreased to approximately 25–50% depending on the diagnostic criteria used (Alarcon et al., 2002; Cooper et al., 2002).

Lupus nephritis is much more common and severe in black persons at the time of diagnosis, as compared to white persons (Ballou et al., 1982; Cervera et al., 1993; Appel and Valeri, 1994; Dooley et al., 1997; Cooper et al., 2002). In the USA, development of kidney disease in patients without overt lupus nephritis at diagnosis is more likely in Hispanic individuals (Alarcon et al., 2002). Although SLE is significantly more common in females, males may be more likely to have renal involvement, regardless of ethnicity (Cooper et al., 2002). Likewise, although SLE is primarily a disease of adulthood, children are much more likely to have severe kidney disease (Cervera et al., 1993; McCurdy et al., 1992).

The clinical signs and histologic changes seen in lupus nephritis are highly variable. Clinical manifestations range from asymptomatic to acute nephritic disease, nephrotic syndrome, or endstage kidney disease. The severity of disease often correlates with the type of histologic change. Thus, biopsy and characterization of the renal lesions in patients with lupus nephritis are recommended to refine prognosis and to guide therapy. Additionally, serial biopsies have been associated with an improved long-term prognosis (Bajaj et al., 2000; Yoo et al., 2000).

The standardized classification system for lupus nephritis was revised in 2004 under the auspices of the International Society of Nephrology (ISN) and the Renal Pathology Society (RSP) (Table 59.1) (Weening et al., 2004). This updated classification scheme is a revision of the previously used World Health Organization (WHO) system. In this system lupus nephritis is subdivided based on the type and severity of glomerular pathology; however, tubular, interstitial, and vascular lesions often occur in SLE patients as well, and may independently influence clinical presentation and prognosis. For a thorough discussion of the updated classi-

TABLE 59.1 Abbreviated classification of lupus nephritis, revised by the International Society of Nephrology (ISN) and the Renal Pathology Society (RSP), 2004

Class I	Minimal mesangial lupus nephritis	Normal light microscopy; mesangial immune deposits visible only by immunofluorescence microscopy. Formerly known as class IIa
Class II	Mesangioproliferative lupus nephritis	Mesangial proliferation visible by light microscopy. Presence of immune deposits, glomerulonephritis, or fibrosis exclude classification as class II. Formerly known as class IIb
Class III	Focal lupus nephritis	Glomerulonephritis with lesions found in <50% of glomeruli. Lesions are subclassified based on activity (active versus chronic). Lesions are usually segmental
Class IV	Diffuse lupus nephritis	Glomerulonephritis with lesions found in 50% or more of glomeruli. Lesions are subclassified based on activity (active versus chronic) and distribution of glomerular lesions (segmental versus global)
Class V	Membranous lupus nephropathy	May occur in conjunction with class III or class IV lesions
Class VI	Advanced sclerosing lupus nephritis	Most glomeruli (minimum of 90%) are globally sclerosed and "endstage"

fication, interpretation, and differences from the previous system the reader is referred to Weening et al. (2004).

Autoimmune Features

SLE results from hyperactivation of B cells, production of autoantibodies against multiple self-antigens, and activation of cell-mediated immunity secondary to deposition of immune complexes (see Chapters 16 and 28).

Although immune deposits in the kidneys of patients with SLE typically contain IgG, IgM, IgA, and complement, IgG clearly predominates. Antibodies result in lupus nephritis through complement fixation and leukocyte recruitment via Fc receptor ligation (Couser, 1990). Sites of antibody deposition within the kidney may vary, and localization to the subepithelial or subendothelial spaces versus basement membrane, antibody affinity and isotype, and antigen-specificity of the various autoantibodies all may influence the development and severity of lupus nephritis (Vlahakos et al., 1992).

Autoreactive T cells are present in SLE. Lymphocyte infiltration of affected organs is common, particularly the kidneys in lupus nephritis. Persistent or aberrant T-cell activation may be the initiating defect in SLE; abnormal 154(CD40L) expression on lymphocytes and decreased $CD4^+CD25^+$ Treg activity have both been implicated (Desai-Mehta et al., 1996; Powrie and Maloy, 2003).

Genetic Features

Of the genes that have been associated with the development of SLE, Fc receptor polymorphisms have the strongest association with development of nephritis (see Chapter 16). Allelic or single nucleotide polymorphisms of the FcγRIIa, IIb and IIIa genes have been identified with increased risk for development of SLE (Salmon et al., 1996; Wu et al., 1997; Seligman et al., 2001; Edberg et al., 2002; Su et al., 2004). These altered Fc receptors may increase susceptibility to lupus nephritis or affect its rate of progression by altering or inhibiting clearance of immune complexes, or by inappropriately activating leukocytes, which bind deposited complexes (Salmon and Roman, 2001; Tsao, 2004).

Environmental Influences

Studies have not identified any environmental factors that predispose specifically to lupus nephritis. The association of certain autoantibody subsets with kidney disease may argue that unidentified environmental factors dictate the final repertoire of the SLE autoantibodies, and thus expressions of lupus nephritis. Unlike spontaneous SLE, drug-induced SLE syndromes are less likely to cause lupus nephritis (Shapiro et al., 1984; Speirs et al., 1989; Short and Lockwood, 1995).

Animal Models

SLE naturally occurs in dogs, cats, and horses. Although disease in these species is rare and poorly characterized, reports indicate that most cases would satisfy the standardized diagnostic criteria used in humans. Approximately 50% of dogs with SLE develop lupus nephritis based on the presence of proteinuria; progression to renal failure is a common cause of morbidity and mortality (Chabanne et al., 1999). Histopathologic findings in renal biopsies have not been well characterized in this species, but immune complex deposition is a common finding, with variable amounts of proliferative or membranous changes (Center et al., 1987; Chabanne et al., 1999). SLE is much rarer in cats and horses, but nephritis is still a consistent finding (Werner and Gorman, 1984; Pedersen and Barlough, 1991).

Both induced and spontaneously-occurring mouse models of SLE exist (see Chapter 26). Most of these models develop nephritis to varying degrees. The best-characterized spontaneous models of lupus nephritis are the MRL/lpr and NZB/NZW F1 mice (Peutz-Kootstra et al., 2001). MRL/lpr mice are homozygously defective for the Fas^{lpr}

gene, which is essential for normal lymphocyte apoptosis (Theofilopoulos et al., 1985a). These mice invariably develop immune complex glomerulonephritis (Theofilopoulos and Dixon, 1985). NZB/NZW F1 mice spontaneously develop autoantibodies with similar antigenic targets as humans with SLE; lupus nephritis develops due to immune complex deposition (Theofilopoulos et al., 1985b). Although the precise gene or genes responsible for NZB/NZW F1 mouse autoimmunity is unclear, polymorphisms in class II MHC molecules, tumor necrosis factor (TNF)-α, and the Sle gene loci have been implicated (Mocsai et al., 1999; Morel et al., 1999; Rozzo et al., 1999). The finding that these and various other mouse strains can all develop lupus nephritis despite a wide variety of genetic defects supports the hypothesis that naturally-occurring SLE in humans is a syndrome with multiple etiologies.

Pathogenic Mechanisms

Lupus nephritis is predominantly an immune complex disease. Antibody–antigen complexes may form in the peripheral circulation and then be deposited within the kidney, or unbound antigens may first be planted within the renal parenchyma with later *in situ* antibody binding. There is also evidence that lupus nephritis may be due in part to autoantibodies against native renal antigens, particularly components of the GBM and extracellular matrix, such as α-actinin and laminin (Ben-Yehuda et al., 1995; Mostoslavsky et al., 2001).

Regardless of the site of complex formation, not all autoantibodies are equally likely to result in kidney disease in SLE patients. Although serum autoantibody titers do correlate with presence and severity of nephritis, some patients with high titers do not develop kidney disease, whereas others with low titers may have severe nephritis (Chien et al., 2001; Horvath et al., 2001; Reveille, 2004; Linnik et al., 2005). Eluted antibodies from the kidneys of patients with lupus nephritis are not consistently of the same antigen specificity as circulating autoantibodies (Mannik et al., 2003). Autoantigen or immune complex charge and isoelectric point may determine affinity of binding to the GBM or mesangium (Vogt et al., 1990; Suenaga and Abdou, 1993; Woitas and Morioka, 1996). Anti-DNA and antihistone antibodies, in particular, may be more nephritogenic than other autoantibodies due to their overall charge (Xie et al., 2003; Cortes-Hernandez et al., 2004). Anti-dsDNA antibodies may also cross-react with native GBM antigens, and linear staining of renal tissue may be visible by immunofluorescence (Vlahakos et al., 1992; Foster et al., 1993; Lefkowith et al., 1996; Mostoslavsky et al., 2001). Autoantibodies that are frequently found deposited within kidneys of lupus patients include anti-C1q-CLR, anti-SS-A, anti-Sm, anti-SS-B, antihistone, and antichromatin antibodies (Mannik et al., 2003).

The severity of interstitial T-cell infiltration in renal biopsies in lupus nephritis correlates with renal function (Alexopoulos et al., 1990). Infiltrating T cells in the kidney are often oligoclonal and of different specificity from peripheral blood T cells, indicating expansion of a specific subpopulation of cells rather than nonspecific recruitment during inflammation (Massengill et al., 1998; Murata et al., 2002). Recruitment of inflammatory cells is not solely dependent upon binding of immune complex autoantibodies with renal Fc receptors, as experimental Fcγ receptor knockout mice still have localization of immune complexes to the kidney, although nephritis is significantly reduced (Clynes et al., 1998). This finding implies that inflammation is more dependent upon binding of nonrenal Fc receptors, likely those found on circulating leukocytes.

Immunologic Markers in Diagnosis

The sole method for definitive diagnosis of lupus nephritis is renal biopsy; however, if biopsy is contraindicated then the simultaneous presence of proteinuria, anti-dsDNA antibodies, hypocomplementemia, and active urine sediment are accepted by the American College of Rheumatology to be substitutable secondary criteria (Dooley et al., 2004).

Lupus nephritis can occur in any SLE patient with any autoantibody profile; however, specific antibodies or antibody repertoires are associated with a higher incidence of kidney disease (Lefkowith et al., 1996). The strongest association identified thus far is with anti-RNA–protein conjugate antibodies. Anti-Ro/SSA or anti-Sm antibodies, with or without concurrent anti-U_1RNP, predict development of nephritis in approximately 50% of SLE patients. In contrast, anti-U_1RNP alone or concurrent anti-Ro/SSA and anti-La/SSB are protective, with less than 10% of patients developing nephritis (Lefkowith et al., 1996). These associations between specific autoantibodies and lupus nephritis may be modified by other heritable factors, as the predictive value of these repertoires varies with race and gender (McCarty et al., 1993). Other antibodies associated with nephritis include anti-C1q-CLR, antiheparan sulfate, and anti-endothelial cell antibodies (D'Cruz et al., 1991; Coremans et al., 1995; Ravirajan et al., 2001).

SLE and other diseases caused by immune complexes cause hypocomplementemia. Measurement of serum complement concentrations, particularly the C4 and C5 components and total hemolytic complement (CH_{50}), may aid in prediction of lupus nephritis (Appel et al., 1978; Jarrett et al., 1981; Milis et al., 1992; Wada et al., 2004).

Treatment and Outcome

Immunosuppressive therapy is the mainstay of treatment of lupus nephritis. Unfortunately, the optimal drug or drugs and the appropriate treatment regimens are still unclear. It

does appear that treatment should be tailored to the class of lupus nephritis present on renal biopsy. However, study results are often conflicting and the recent modification of the lupus nephritis classification system makes interpretation of historic studies even more difficult. Regardless, it appears that mild forms of lupus nephritis (class I and II) carry a generally good prognosis, and for these, aggressiveness of treatment should instead be based on the severity of any extrarenal disease manifestations. The poorer long-term prognosis of class III and IV lupus nephritis justifies aggressive immunosuppressive therapy. Cyclophosphamide in particular is used for treatment of cases with proliferative lupus nephritis and membranous lupus nephropathy who do not respond to corticosteroid monotherapy. Several studies have shown no benefit from plasmapheresis despite the positive association between decreased antinuclear antibody titers and improved outcome (Lewis, 1992; Wei et al., 1993; Wallace et al., 1998).

Of newer drugs mycophenolate mofetil (MMF) is an attractive alternative in patients with cyclophosphamide toxicity or for rescue therapy in cyclophosphamide-resistant lupus nephritis (Glicklich and Acharya, 1998; Dooley et al., 1999). Patients with newly diagnosed diffuse proliferative lupus nephritis are equally likely to remit with oral MMF and prednisone, oral cyclophosphamide and prednisone, or azathioprine and prednisone; additionally, the incidence of serious infection is reduced with MMF (Chan and Hooi, 2000; Chan et al., 2000). Induction with intravenous (rather than oral) cyclophosphamide at high- or low-dose regimens (either of which is followed by azathioprine) appears to be equally effective at inducing and maintaining remission of proliferative lupus nephritis (Houssiau and Jadoul, 2002). Long-term maintenance with oral azathioprine or MMF appears safer than continued intravenous cyclophosphamide (Contreras et al., 2004). An emerging SLE-specific therapy is LJP 394, a synthetic construct of short dsDNA sequences known to be epitopes for SLE autoantibodies (Alarcon-Segovia et al., 2003).

Several independent factors have been identified as predictors of long-term renal function and patient survival. A prospective cohort study of patients with lupus nephritis has shown that severe renal failure (serum creatinine greater than 2.4 mg/dl), black race, hematocrit less than 20%, and chronic or active histologic lesions, particularly if crescents or interstitial fibrosis are present, are associated with a worse outcome (Austin et al., 1994). Other studies have identified black race, hypertension, and length and number of times out of remission as risk factors for disease progression (Esdaile et al., 1989; Ward and Studenski, 1992; Dooley et al., 1997; Alarcon et al., 2002). Clinicopathologic findings of azotemia, nephritic urine sediment, hypocomplementemia, thrombocytopenia, high anti-DNA antibody titer, and proliferative glomerulonephritis with or without simultaneous membranous nephropathy also predict a worse outcome (Banfi et al., 1985; Esdaile et al., 1989; Nossent et al., 1990; Reveille et al., 1990; Derksen and Meilof, 1992). Histopathologic lesions associated with aggressive, ongoing inflammation (i.e., diffuse proliferative and progressive focal proliferative nephritis) are more serious than mesangial or membranous nephropathy (Baldwin et al., 1970; Gladman et al., 1989).

ANCA GLOMERULONEPHRITIS

Here we focus on the pathogenesis, autoimmune features, and treatment of ANCA-associated glomerulonephritis and renal failure. Nonrenal manifestations of ANCA-associated small vessel vasculitis are discussed in Chapter 65.

Glomerulonephritis and renal failure were associated with small vessel vasculitis prior to the initial description of ANCAs (see Chapter 65). The similar histologic appearance of glomerular lesions noted in Wegener granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and renal-limited small vessel vasculitis were only explained once the unifying presence of ANCAs was discovered.

Clinical, Pathologic, and Epidemiologic Features

The clinical presentation of ANCA disease can vary widely, depending on the disease phenotype (i.e., Wegener' granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, or renal-limited small vessel vasculitis), organ system(s) affected, and chronicity and severity of disease. Regardless of these variables, all ANCA-associated small vessel vasculitis patients have arteriolar and capillary wall inflammation. Fibrinoid necrosis of the vessel walls with karyorrhexis and leukocyte infiltration are classic lesions.

Rapidly progressive glomerulonephritis secondary to vasculitis is one of the most common clinical manifestations of ANCA disease. Renal involvement is found in approximately 90% of patients with microscopic polyangiitis, 80% of patients with Wegener granulomatosis, 45% of patients with the Churg-Strauss syndrome, and by definition 100% of patients with renal-limited small vessel ANCA vasculitis (Jennette and Falk, 1997a; 1997b). The severity of kidney disease at presentation is variable, although proteinuria, hematuria, and renal failure are regularly seen. Kidney disease in patients with Wegener granulomatosis may be more aggressive than in those with microscopic polyangiitis or Churg-Strauss syndrome (Franssen et al., 2000).

The epidemiology of ANCA disease is presented in Chapter 65.

Renal biopsy in patients with ANCA disease glomerulonephritis reveals vascular lesions and necrotizing glomerulonephritis with crescents (filling of Bowman's space with inflammatory cells and cellular debris secondary

to rupture of the visceral epithelium). These lesions are severe, with 50% or more of glomeruli often involved; glomerulonephritis is global, diffuse, and crescents are frequently circumferential. These glomerular lesions are indistinguishable from the rapidly progressive glomerulonephritis seen on light microscopy in patients with anti-GBM disease. Affected glomeruli sclerose over time; a single kidney biopsy specimen may contain glomeruli with both acute and endstage lesions. However, unlike anti-GBM disease, ANCA disease is pauci-immune; immunofluorescent microscopy fails to reveal deposition of significant amounts of immunoglobulin. Electron microscopy reveals generalized inflammation, breaks in the GBM, and a lack of electron-dense immune deposits.

Autoimmune Features

ANCAs occur in greater than 80% of patients with pauci-immune small vessel vasculitis. These antibodies are specific for neutrophil and monocyte granule proteins, with a given patient typically having antibody reactivity to a single antigen. The major neutrophil granule protein targets of ANCA are proteinase-3 (PR3, a serine protease) and myeloperoxidase (MPO, a lysosomal enzyme that produces hypochlorous acid). Indirect immunofluorescence staining of neutrophils using serum from ANCA patients results in cytoplasmic fluorescence. PR3-ANCAs produce a cytoplasmic staining pattern (a C-ANCA pattern), whereas MPO-ANCAs produce a perinuclear cytoplasmic staining pattern (P-ANCA). The majority of Wegener granulomatosis patients have PR3-specific ANCAs, whereas Churg-Strauss syndrome and renal-limited vasculitis patients usually have MPO-ANCAs; microscopic polyangiitis patients are equally likely to have PR3- or MPO-ANCAs. A minority of patients in all disease phenotypes have ANCAs that are specific for neither PR3 nor MPO. ANCAs are IgG, mostly of the IgG1 subclass, with lesser amounts of IgG3 and IgG4. There is no single epitope on either PR3 or MPO that is uniformly recognized by ANCAs from different patients; additionally a single ANCA patient can have antibodies against multiple epitopes of a single protein target.

The role of T cells in the pathogenesis of ANCA disease is unclear. Autoreactive T cells specific for PR3 or MPO are inconsistently present, and may be found in some normal patients (King et al., 1998; van der Geld et al., 2000; Popa et al., 2002; Winek et al., 2004). However, there is evidence that the T-cell repertoire in general in ANCA patients is not normally regulated, as cells may be generally activated above normal levels, or have exaggerated skewing of cytokine profiles in response to antigenic stimulation (Christensson et al., 2000; Masutani et al., 2003). $CD4^+$ T cells from ANCA patients, which proliferate in response to ANCA antigens or nonspecific stimulation, may be either Th1 or Th2; cytokine polarization may be linked to ANCA disease subtype, organ distribution, or whether disease is localized or systemic (Tomer et al., 1999; Masutani et al., 2003; Sanders et al., 2003).

Genetic Features

There have been no genetic associations made as yet between any specific allele or gene and development of glomerulonephritis (as opposed to other organ involvement) in patients with ANCA disease. Genetic features of ANCA disease in general are discussed in Chapter 65.

Environmental Influences

Although statistical associations have been made between environmental exposure to some chemicals and development of ANCA disease (see Chapter 65), no specific links have been made to kidney disease.

Animal Models

The best model of ANCA disease thus far has been induced in $MPO^{-/-}$ mice (see Chapter 65), and there are no naturally-occurring animal models. However, anti-MPO ANCAs and systemic autoimmune disease have been reported in cats treated with propylthiouracil (Aucoin et al., 1985; Waldhauser and Uetrecht, 1996). The frequency of occurrence of these side effects has precluded use of propylthiouracil in treatment of naturally-occurring hyperthyroidism in this species, and unfortunately extensive studies have not been done characterizing the resultant disease. Naturally-occurring ANCAs have recently been described in dogs with inflammatory bowel disease (Allenspach et al., 2004). The presence of ANCA correlates with disease, but the antigen specificity of canine ANCAs is unknown.

Pathogenic Mechanisms

Binding of ANCAs to their specific antigens in combination with Fc region binding to an Fc receptor results in neutrophil and monocyte activation with induction of the respiratory burst, degranulation, and release of cellular inflammatory products into the microenvironment (Falk et al., 1990b; Lai and Lockwood, 1991; Keogan et al., 1992; Savage et al., 1992; Mulder et al., 1994; Porges et al., 1994; Johnson et al., 1997b; Kettritz et al., 1997). Interaction of these leukocytes and their products with the endothelium results in inflammatory damage and vasculitis (Falk et al., 1990b; Dolman et al., 1993; Gross et al., 1993; De'Oliviera et al., 1995).

The extent to which the $F(ab')_2$ region contributes to ANCA-mediated effects is debated. However antibody–antigen interactions are clearly fundamental for the neu-

trophilic perturbations required for vascular injury (Lai and Lockwood, 1991; Keogan et al., 1992; Kettritz et al., 1997; Alcorta et al., 2002; Yang et al., 2002). ANCA-mediated effects on neutrophils and monocytes depend on both the $F(ab')_2$ region binding to its antigen and the engagement of the Fc region with the Fc receptor (Porges et al., 1994; Mulder et al., 1995; Reumaux et al., 1995; Johnson et al., 1997b; Kocher et al., 1998; Kobold et al., 1999; Locke et al., 1999; Yang et al., 2002).

Binding of ANCA is known to affect gene expression in leukocytes, and thus presumptively alter intra- and intercellular signaling. *In vitro* treatment of leukocytes from healthy donors with ANCA IgG or $F(ab')_2$ results in activation of a number of genes, including differentiation-dependent gene 2 (DIF-2), interleukin-8 (IL-8), cyclooxygenase-2 (COX-2), IL-1β, and p21(ras) (Brooks et al., 1996; Yang et al., 2002; Williams and Savage, 2005). It is unknown how exactly ANCAs mediate their effects on signaling pathways. ANCAs may cross-link surface antigens, resulting in clusters of associated molecules in lipid raft-like structures, particularly if the antigens are bound to other cell-surface proteins such as Mac-1 (CD11b/CD18) or other β_2 integrins (Kurosawa et al., 2000; Calderwood et al., 2005).

Alterations in intracellular signaling pathways have been shown to occur following ANCA binding. ANCA induce significant GTPase activity through activation of G_i proteins and diminish FMLP-induced PKC activation and IP_3 generation, thus possibly modulating calcium-dependent pathways (Lai and Lockwood, 1991; Williams et al., 2003; Williams and Savage, 2005). ANCA-induced signals are synergistic with TNF-α effects, indicating that autoantibodies could affect Src-family kinase pathways (Reumaux et al., 1995; Kettritz et al., 1997). Similarly ANCA-activated transcription of the IL-1β gene may imply activation of p38 MAPK (Brooks et al., 1996; Hashimoto et al., 1999). Importantly, gene expression profiling of patients with ANCA disease should become a noninvasive approach to determining disease activity, as demonstrated for IgA nephropathy (Preston et al., 2004).

The lack of ANCAs or ANCA–antigen deposits within the renal vasculature has made it particularly challenging to demonstrate a causal role for these autoantibodies in glomerulonephritis. The endothelium was originally considered a direct target of ANCA-mediated effects because of the hallmark histologic pattern of necrotizing vascular injury, but how ANCAs interact with endothelial cells is unclear as PR3 and MPO are restricted to myeloid lineage cells (Chen et al., 1995; Sturrock, 1996). It was speculated that cytokines induced PR3 and MPO expression on the endothelial cell surface, but PR3 or MPO mRNA transcripts could not be demonstrated in endothelial cells using TaqMan PCR (Mayet et al., 1993a; 1993b; 1997; Johnson et al., 1997a; King et al., 1995; Sibelius et al., 1998; Pendergraft et al., 2000).

A mechanistic link between ANCA binding to neutrophils and glomerular lesions was deduced from studies of neutrophil PR3. Extracellular PR3 can traverse the endothelial plasma membrane and induce apoptosis (Yang et al., 2001). Cell death is linked with sustained activation of c-Jun N-terminal kinase 1 (JNK 1), a primary apoptosis signaling pathway in endothelial cells (Preston et al., 2002). PR3 also functions as a caspase-like protease, cleaving proteins that are normal caspase substrates during the apoptotic program (Jiang et al., 1994; Levkau et al., 1998; Kang et al., 2001; Preston et al., 2002; Witko-Sarsat et al., 2002; Pendergraft et al., 2004b). Cleavage of NF-κB, $p21^{Waf1/Cip1/Sdi1}$, and IL-1β (caspase-1-like activity) and the Sp1 transcription factor (caspase-2- and -3-like activity) has been reported (Coeshott et al., 1999; Pendergraft et al., 2004b; Piedrafita and Pfahl, 1997). Inflamed bowel tissue shows detectible levels of PR3 protein coincident with proteolytically-cleaved $p21^{Waf1/Cip1/Sdi1}$ (Pendergraft et al., 2004b). Thus, PR3 is not merely a protease that degrades matrix proteins but also specifically processes angiotensinogen, TGF-β1, and TNF-α (Csernok et al., 1996; Coeshott et al., 1999; Bank and Ansorge, 2001; Ramaha and Patston, 2002). Less is known about MPO-induced endothelial cell injury. Endothelial cells also internalize MPO by an energy-dependent process (Baldus et al., 2001; Yang et al., 2001). However, unlike PR3, MPO does not cause apoptosis but rather increases intracellular oxidant radical concentration.

The epitope targets of PR3 and MPO ANCA are unknown. The data point to a limited number of epitopes, all of which require an intact tertiary structure (van der Geld et al., 2004). Because both MPO and PR3 have distinct functional domains, ANCA-binding sites could be responsible for the differences in disease presentation.

Dogma is that neutrophil granule protein genes are irreversibly silenced before the cells leave the bone marrow. However, mature leukocytes from the majority of ANCA patients with active disease have high levels of PR3 and MPO mRNA (Yang et al., 2004). Increased transcripts correlate with disease activity and absolute neutrophil values, but not with "left shift" (i.e., neutrophil immaturity), drug treatment regimen, serum inflammatory cytokine concentration, hematuria, proteinuria, ANCA titer, serum creatinine, sex, or age (Yang et al., 2004). This anomaly appears to be ANCA disease specific, as upregulation of these genes was not observed in patients with endstage kidney disease, rheumatoid arthritis or SLE, or in healthy volunteers. Thus, increased transcription of PR3 and MPO may lead to increased protein on the cell surface, conferring risk for the development of ANCA disease (Halbwachs-Mecarelli et al., 1995; van Rossum et al., 2004). In addition to the presence of anti-MPO or -PR3 autoantibodies, a second critical component in the etiology of this disease may be the reactivation of once-silenced genes, leading to increased antigen availability.

Immunogenesis of ANCA: Theory of Autoantigen Complementarity

A recent report offers a new theory of how PR3-ANCA disease, and perhaps other autoimmune diseases, could develop (Pendergraft et al., 2004a). This theory proposes that the initiator of the autoimmune response is not an autoantigen or an exogenous mimic, but rather a peptide or protein that is "antisense" or "complementary" to the autoantigen. The first immune response is production of an antibody specific for this complementary protein. This is followed by an antiantibody (i.e., anti-idiotypic antibody) response, whereby a second immunoglobulin is produced that reacts not only with the first antibody, but also with the corresponding "sense" or self-protein because of surface contour complementarity.

The initiating event in autoantigen complementarity is an immune response against a protein complementary in structure to the autoantigen (Figure 59.3A and B). Complementary proteins could be of exogenous microbial origin; for example, complementary PR3 has homologies with proteins from many different microbes and viruses. Among these microbes are Ross River virus, *Staphylococcus aureus*, and *Entamoeba histolytica*, all of which have been previously linked with ANCA disease (Davies, 1982; Pudifin et al., 1994; Stegeman et al., 1994). Pertinent to this finding, the onset of ANCA disease is commonly associated with a flu-like illness (Falk et al., 1990a). Alternatively, patients may be producing complementary PR3 protein themselves. Recent reports of naturally-occurring antisense transcripts in humans indicate that individuals do in fact transcribe antisense RNA (Lehner et al., 2002). However, PR3 antisense RNA has not been reported in the antisense transcriptome database, indicating that antisense PR3 has not yet been identified in healthy individuals. Leukocytes from PR3-ANCA subjects reveal the presence of PR3 antisense RNA in approximately 50% of cases (Pendergraft et al., 2004a). Whether these antisense transcripts are or can be translated into a protein product to produce the complementary protein is unknown, but translation of an antisense transcript has been reported (Van Den Eynde et al., 1999). It may be that aberrantly expressed antisense transcripts are a component of many human diseases (Lavorgna et al., 2004).

Among patients with PR3-ANCA disease, 21% have antibodies specific for a recombinant protein complementary to PR3, whereas only 1% of normal patients or patients with non-PR3 glomerular diseases are seroreactive to this protein (Pendergraft et al., 2004a). These antibodies may themselves induce an immune response, which is the second proposed event in autoantigen complementarity. Antibodies are known occasionally to act as antigens themselves, and elicit antiantibody antibodies (known as anti-idiotypes), according to the idiotypic network theory (Jerne, 1974). The antigen-binding region (paratope) of an antibody elicits an immune response that results in an anti-idiotype directed against the antigen-binding region. Production of anti-idiotypic antibodies following immunization with antigens or antibodies has been extensively reported (Erlanger et al., 1986; Hill and Erlanger, 1988; Erlanger, 1989; Shoenfeld, 1994; 2004; Shoenfeld et al., 2002). Therefore, anticomplementary PR3 antibodies produced following exposure to complementary PR3 could themselves induce an immune response, resulting in the generation of anti-anticomplementary PR3 antibodies. In fact, affinity-purified anti-PR3-ANCAs bind specifically to anticomplementary-PR3 antibodies, proving that these antibodies are an idiotypic pair (Pendergraft et al., 2004a).

Antigen–antibody complementarity states that the variable region of an antibody molecule is structurally and chemically complementary to the epitope region of the bound antigen (Smith et al., 1987; Blalock and Bost, 1988; Bost and Blalock, 1989; Blalock, 1990; Root-Bernstein and Rallo, 2004). Therefore, because anticomplementary PR3 antibodies bind both complementary PR3 and their anti-idiotypic antibody, both these molecules would be expected to be structurally identical at the epitope regions. It follows then that just as complementary PR3 is expected to bind PR3, the anti-idiotypic antibodies would bind PR3 as well; these anti-idiotypic antibodies are PR3-ANCA and represent the third and final event proposed by autoantigen complementarity (Figure 59.3C).

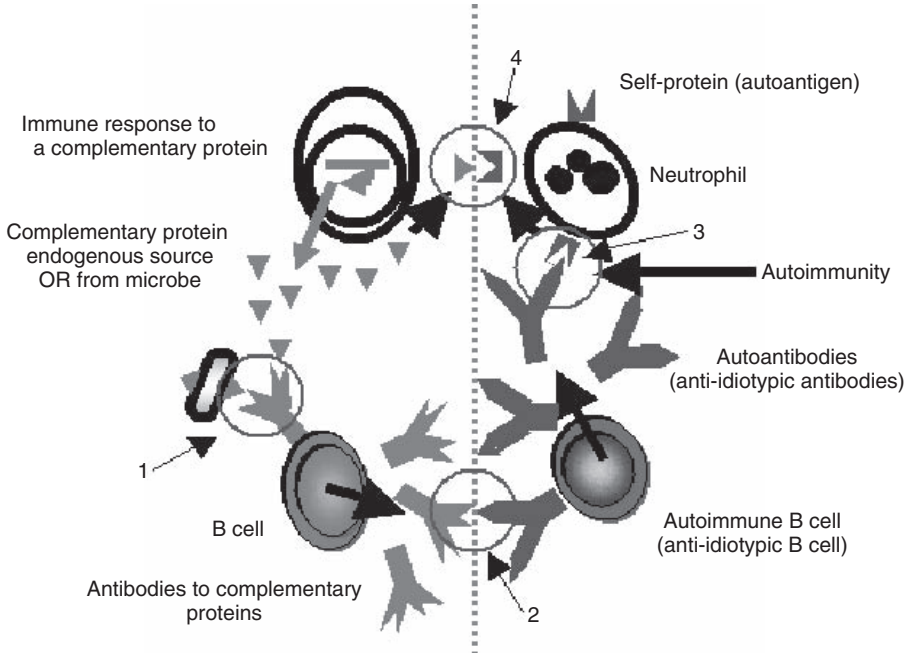
To test the feasibility of these three events, mice were immunized with recombinant complementary human PR3 protein and then evaluated for production of antihuman PR3 antibodies. As predicted, the mice produced not only anticomplementary PR3 antibodies, but antihuman PR3 antibodies as well; PR3-reactive antibodies produced a cytoplasmic (C-ANCA) pattern when examined by immunofluorescence (Pendergraft et al., 2004a).

Immunologic Markers in Diagnosis

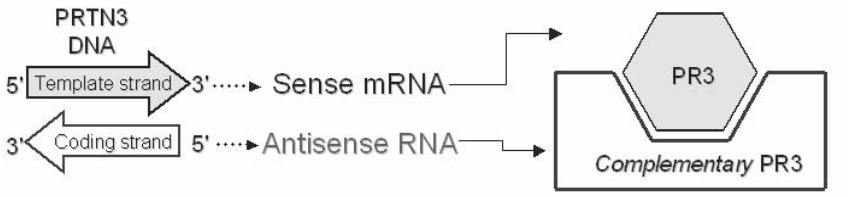
Concurrent presence of kidney disease and ANCA has a relatively high sensitivity and specificity for diagnosis of ANCA glomerulonephritis. Available serum markers, including determination of the precise ANCA target antigen, have little use in predicting presence or later development of kidney disease if clinicopathologic evidence of renal insufficiency or failure is absent. Use of immunologic markers for diagnosis of ANCA disease in general is reviewed in Chapter 65.

Treatment and Outcome

Immunosuppressive treatment of patients with ANCA disease is described in Chapter 65. The short-term renal outcome of patients with ANCA-associated glomerulonephritis is relatively good, with an average patient and renal

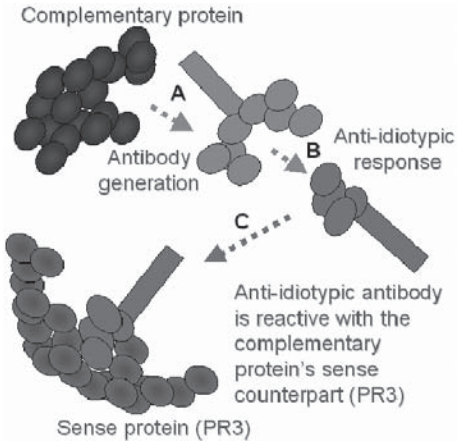


A



- "Antisense RNA" is a general term used to describe a sequence of RNA that is complementary to messenger RNA (mRNA). Antisense RNA is transcribed from the opposite strand of DNA than the one normally used for the RNA transciption.
- A "complementary protein" is one that is translated from antisense RNA (Blalock, 1990). The hydropathy of the complementary protein is exactly opposite to that of the mRNA-coded protein from that same gene. Thus the individual folding patterns result in mirror image structures that can interact in a lock and key manner.

B



C

FIGURE 59.3 *A*, Schematic of the theory of autoantigen complementarity. The theory proposes that the immunogen that begins the sequence of events leading to the production of autoantibodies is not the autoantigen or its mimic, but rather its complementary peptide or its mimic. Step 1: The complementary proteins may be introduced by invading microbes or they may be produced by the individual through translation of antisense RNA. An antibody is produced in response to the complementary protein. Step 2: A second antibody is elicited against the first antibody, referred to as an anti-idiotypic response. Step 3: The resultant anti-idiotypic antibodies react with the autoantigen, whose amino acid sequence is complementary to the sequence of the initiating antigen. Step 4: Complementary proteins have a natural affinity because the hydropathy of one is the opposite of the other. *B*, Definition of terms used in the theory of autoantigen complementarity. *C*, Schematic of anti-PR3 antibody (ANCA) production as a result of an anti-idiotypic response.

survival at 1-year of approximately 75%. Outcome is worse in older patients, patients with endstage kidney disease, or those with severe azotemia at first diagnosis (Booth et al., 2003). Higher creatinine or lower glomerular filtration rate at the time of presentation, persistent proteinuria, and chronic renal lesions, including interstitial fibrosis, tubular atrophy, and glomerulosclerosis, are negative prognostic indicators (Hauer et al., 2002; Neumann et al., 2005). Long-term remission without disease relapse occurs in 60–75% of patients.

CONCLUDING REMARKS AND FUTURE PROSPECTS

Anti-GBM disease, SLE, and ANCA-associated glomerulonephritis are among several kidney diseases that are immune mediated in nature. Other diseases may be consequences of infectious agents, resulting in immune complex deposition, notably membranoproliferative glomerulonephritis or cryoglobulinemic glomerulonephritis, or due to breaks in mucosal immunity, such as IgA nephropathy, or may be secondary to *in situ* formation of immune complexes, such as membranous nephropathy (Table 59.2). There is also an array of tubulo-interstitial nephritides that may be immune mediated in origin, particularly those associated with drug hypersensitivity or autoantibodies to the tubular basement membrane. Each of these entities presents the opportunity for substantial review, although each shares common effector pathways of inflammation with the three diseases described in detail. Membranous glomerulopathy and IgA glomerulonephritis bear names derived from their immunohistologic patterns of kidney injury without regard to their primary pathogenesis, which remains unclear—most target antigens involved in the genesis or maintenance of aberrant immune responses affecting the kidneys remain elusive. Studies using complementary proteins, as described herein, may afford clues as to these, as yet, unknown agents. Certainly, if these inciting antigens and precise epitopes were to be established, more specific and less toxic drugs could be used to supplement or even replace the nonspecific immunomodulating drugs that have hardly changed over the past 30 years.

TABLE 59.2 Infectious agents commonly associated with glomerulonephritides

Bacteria	*Streptococcus* spp.; *Staphylococcus* spp.; chronic bacterial infections of multiple species (atrioventricular shunt infections, endocarditis, osteomyelitis, deep abscesses); *Treponema pallidum* (syphilis); *Salmonella typhi* (typhoid fever)
Viruses	Hepatitis A, B, and C; human immunodeficiency virus; Epstein–Barr virus
Protozoa	*Plasmodium* spp. (malaria)
Parasites	*Schistosoma* spp.

References

Alarcon, G.S., McGwin, G., Jr., Petri, M., Reveille, J.D., Ramsey-Goldman, R., and Kimberly, R.P. (2002). Baseline characteristics of a multiethnic lupus cohort: PROFILE. *Lupus 11*, 95–101.

Alarcon-Segovia, D., Tumlin, J.A., Furie, R.A., McKay, J.D., Cardiel, M.H., Strand, V., Bagin, R.G., Linnik, M.D., and Hepburn, B. (2003). LJP 394 for the prevention of renal flare in patients with systemic lupus erythematosus: results from a randomized, double-blind, placebo-controlled study. *Arthritis Rheum. 48*, 442–454.

Alcorta, D., Preston, G., Munger, W., Sullivan, P., Yang, J.J., Waga, I., Jennette, J.C., and Falk, R. (2002). Microarray studies of gene expression in circulating leukocytes in kidney diseases. *Exp. Nephrol. 10*, 139–149.

Alexopoulos, E., Seron, D., Hartley, R.B., and Cameron, J.S. (1990). Lupus nephritis: correlation of interstitial cells with glomerular function. *Kidney Int. 37*, 100–109.

Allenspach, K., Luckschander, N., Styner, M., Seibold, F., Doherr, M., Aeschbach, D., and Gaschen, F. (2004). Evaluation of assays for perinuclear antineutrophilic cytoplasmic antibodies and antibodies to *Saccharomyces cerevisiae* in dogs with inflammatory bowel disease. *Am. J. Vet. Res. 65*, 1279–1283.

Appel, A.E., Sablay, L.B., Golden, R.A., Barland, P., Grayzel, A.I., and Bank, N. (1978). The effect of normalization of serum complement and anti-DNA antibody on the course of lupus nephritis: a two year prospective study. *Am. J. Med. 64*, 274–283.

Appel, G.B. and Valeri, A. (1994). The course and treatment of lupus nephritis. *Annu. Rev. Med. 45*, 525–537.

Aucoin, D., Peterson, M.E., Hurvitz, A.I., Drayer, D.E., Lahita, R.G., Quimby, F.W., and Reidenberg, M.M. (1985). Propylthiouracil-induced immune-mediated disease in the cat. *J. Pharmacol. Exp. Ther. 234*, 13–18.

Austin, H.A., 3rd, 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.

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*, 2822–2826.

Baldus, S., Eiserich, J.P., Mani, A., Castro, L., Figueroa, M., Chumley, P., Ma, W., Tousson, A., White, C.R., Bullard, D.C., Brennan, M.L., Lusis, A.J., Moore, K.P., and Freeman, B.A. (2001). Endothelial transcytosis of myeloperoxidase confers specificity to vascular ECM proteins as targets of tyrosine nitration. *J. Clin. Invest. 108*, 1759–1770.

Baldwin, D.S., Lowenstein, J., Rothfield, N.F., Gallo, G., and McCluskey, R.T. (1970). The clinical course of the proliferative and membranous forms of lupus nephritis. *Ann. Intern. Med. 73*, 929–942.

Ballou, S.P., Khan, M.A., and Kushner, I. (1982). Clinical features of systemic lupus erythematosus: differences related to race and age of onset. *Arthritis Rheum. 25*, 55–60.

Banfi, G., Mazzucco, G., Barbiano di Belgiojoso, G., Bestetti Bosisio, M., Stratta, P., Confalonieri, R., Ferrario, F., Imbasciati, E., and Monga, G. (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.

Bank, U. and Ansorge, S. (2001). More than destructive: neutrophil-derived serine proteases in cytokine bioactivity control. *J. Leukoc. Biol. 69*, 197–206.

Ben-Yehuda, A., Rasooly, L., Bar-Tana, R., Breuer, G., Tadmor, B., Ulmansky, R., and Naparstek, Y. (1995). The urine of SLE patients

contains antibodies that bind to the laminin component of the extracellular matrix. *J. Autoimmun. 8*, 279–291.

Blalock, J.E. (1990). Complementarity of peptides specified by 'sense' and 'antisense' strands of DNA. *Trends Biotechnol. 8*, 140–144.

Blalock, J.E., and Bost, K.L. (1988). Ligand receptor characteristics of peptides encoded by complementary nucleic acids: implications for a molecular recognition code. *Recent Prog. Horm. Res. 44*, 199–222.

Bolton, W.K. (1996). Goodpasture's syndrome. *Kidney Int. 50*, 1753–1766.

Booth, A.D., Almond, M.K., Burns, A., Ellis, P., Gaskin, G., Neild, G.H., Plaisance, M., Pusey, C.D., and Jayne, D.R. (2003). Outcome of ANCA-associated renal vasculitis: a 5-year retrospective study. *Am. J. Kidney Dis. 41*, 776–784.

Bost, K.L. and Blalock, J.E. (1989). Production of anti-idiotypic antibodies by immunization with a pair of complementary peptides. *J. Mol. Recog. 1*, 179–183.

Brooks, C.J., King, W.J., Radford, D.J., Adu, D., McGrath, M., and Savage, C.O. (1996). IL-1 beta production by human polymorphonuclear leucocytes stimulated by anti-neutrophil cytoplasmic autoantibodies: relevance to systemic vasculitis. *Clin. Exp. Immunol. 106*, 273–279.

Cairns, L.S., Phelps, R.G., Bowie, L., Hall, A.M., Saweirs, W.W., Rees, A.J., and Barker, R.N. (2003). The fine specificity and cytokine profile of T-helper cells responsive to the alpha3 chain of type IV collagen in Goodpasture's disease. *J. Am. Soc. Nephrol. 14*, 2801–2812.

Calderwood, J.W., Williams, J.M., Morgan, M.D., Nash, G.B., and Savage, C.O. (2005). ANCA induces β2 integrin and CXC chemokine-dependent neutrophil-endothelial cell interactions that mimic those of highly cytokine-activated endothelium. *J. Leukoc. Biol. 33*, 33–43.

Cashman, S.J., Pusey, C.D., and Evans, D.J. (1988). Extraglomerular distribution of immunoreactive Goodpasture antigen. *J. Pathol. 155*, 61–70.

Center, S.A., Smith, C.A., Wilkinson, E., Erb, H.N., and Lewis, R.M. (1987). Clinicopathologic, renal immunofluorescent, and light microscopic features of glomerulonephritis in the dog: 41 cases (1975–1985). *J. Am. Vet. Med. Assoc. 190*, 81–90.

Cervera, R., Khamashta, M.A., Font, J., Sebastiani, G.D., Gil, A., Lavilla, P., Domenech, I., Aydintug, A.O., Jedryka-Goral, A., and de Ramon, E. (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 (Baltimore) 72*, 113–124.

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.

Chan, A.Y. and Hooi, L.S. (2000). Outcome of 85 lupus nephritis patients treated with intravenous cyclophosphamide: a single centre 10 year experience. *Med. J. Malaysia. 55*, 14–20.

Chan, T.M., Li, F.K., Tang, C.S., Wong, R.W., Fang, G.X., Ji, Y.L., Lau, C.S., Wong, A.K., Tong, M.K., Chan, K.W., and Lai, K.N. (2000). Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. *N. Engl. J. Med. 343*, 1156–1162.

Chen, H.M., Zhang, P., Voso, M.T., Hohaus, S., Gonzalez, D.A., Glass, C.k., Zhang, D.E., and Tenen, D.G. (1995). Neutrophils and monocytes express high levels of PU-1 (Spi-1) but not Spi-B. *Blood 85*, 2918–2928.

Chien, J.W., Lin, C.Y., and Yang, L.Y. (2001). Correlation between anti-Ro/La titers and clinical findings of patients with systemic lupus erythematosus. *Zhonghua Yi Xue Za Zhi (Taipei) 64*, 283–291.

Christensson, M., Pettersson, E., Sundqvist, K.G., and Christensson, B. (2000). T cell activation in patients with ANCA-associated vasculitis: inefficient immune suppression by therapy. *Clin. Nephrol. 54*, 435–442.

Clynes, R., Dumitru, C., and Ravetch, J.V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science 279*, 1052–1054.

Coeshott, C., Ohnemus, C., Pilyavskaya, A., Ross, S., Wieczorek, M., Kroona, H., Leimer, A.H., and Cheronis, J. (1999). Converting enzyme-independent release of tumor necrosis factor alpha and IL-1beta from a stimulated human monocytic cell line in the presence of activated neutrophils or purified proteinase 3. *Proc. Natl. Acad. Sci. USA 96*, 6261–6266.

Contreras, G., Pardo, V., Leclercq, B., Lenz, O., Tozman, E., O'Nan, P., and Roth, D. (2004). Sequential therapies for proliferative lupus nephritis. *N. Engl. J. Med. 350*, 971–980.

Cooper, G.S., Parks, C.G., Treadwell, E.L., St Clair, E.W., Gilkeson, G.S., Cohen, P.L., Roubey, R.A., and Dooley, M.A. (2002). Differences by race, sex and age in the clinical and immunologic features of recently diagnosed systemic lupus erythematosus patients in the southeastern United States. *Lupus 11*, 161–167.

Coremans, I.E., Spronk, P.E., Bootsma, H., Daha, M.R., van der Voort, E.A., Kater, L., Breedveld, F.C., and Kallenberg, C.G. (1995). Changes in antibodies to C1q predict renal relapses in systemic lupus erythematosus. *Am. J. Kidney Dis. 26*, 595–601.

Cortes-Hernandez, J., Ordi-Ros, J., Labrador, M., Bujan, S., Balada, E., Segarra, A., and Vilardell-Tarres, M. (2004). Antihistone and anti-double-stranded deoxyribonucleic acid antibodies are associated with renal disease in systemic lupus erythematosus. *Am J Med. 116*, 165–173.

Couser, W.G. (1990). Mediation of immune glomerular injury. *J. Am. Soc. Nephrol. 1*, 13–29.

Csernok, E., Szymkowiak, C.H., Mistry, N., Daha, M.R., Gross, W.L., and Kekow, J. (1996). Transforming growth factor-beta (TGF-beta) expression and interaction with proteinase 3 (PR3) in anti-neutrophil cytoplasmic antibody (ANCA)- associated vasculitis. *Clin. Exp. Immunol. 105*, 104–111.

Davies, D.J., Moran, J.E., Niall, J.F., and Ryan, G.B. (1982). Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br. Med. J. 285*, 606–610.

D'Cruz, D.P., Houssiau, F.A., Ramirez, G., Baguley, E., McCutcheon, J., Vianna, J., Haga, H.J., Swana, G.T., Khamashta, M.A., and Taylor, J.C. (1991). Antibodies to endothelial cells in systemic lupus erythematosus: a potential marker for nephritis and vasculitis. *Clin. Exp. Immunol. 85*, 254–261.

Dean, E.G., Wilson, G.R., Li, M., Edgtton, K.L., O'Sullivan K.M., Hudson, B.G., Holdsworth, S.R., and Kitching, A.R. (2005). Experimental autoimmune Goodpasture's disease: A pathogenetic role for both effector cells and antibody in injury. *Kidney Int. 67*, 566–575.

De'Oliviera, J., Gaskin, G., Dash, A., Rees, A.J., and Pusey, C.D. (1995). Relationship between disease activity and anti-neutrophil cytoplasmic antibody concentration in long-term management of systemic vasculitis. *Am. J. Kidney Dis. 25*, 380–389.

Derksen, R.H. and Meilof, J.F. (1992). Anti-Ro/SS-A and anti-La/SS-B 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.

Derry, C.J., Ross, C.N., Lombardi, G., Mason, P.D., Rees, A.J., Lechler, R.I., and Pusey, C.D. (1995). Analysis of T-cell responses to the auto-antigen in Goodpasture's disease. *Clin. Exp. Immunol*. *100*, 262–268.

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.

Dolman, K.M., Gans, R.O., Vervaat, T.J., Zevenbergen, G., Maingay, D., Nikkels, R.E., Donker, A.J., von dem Borne, A.E., and Goldschmeding, R. (1993). Vasculitis and antineutrophil cytoplasmic autoantibodies associated with propylthiouracil therapy. *Lancet 342*, 651–652.

Donaghy, M. and Rees, A.J. (1983). Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. *Lancet 2*, 1390–1393.

Dooley, M.A., Hogan, S., Jennette, C., and Falk, R. (1997). Cyclophosphamide therapy for lupus nephritis: poor renal survival in black Americans. Glomerular Disease Collaborative Network. *Kidney Int. 51*, 1188–1195.

Dooley, M.A., Cosio, F.G., Nachman, P.H., Falkenhain, M.E., Hogan, S.L., Falk, R.J., and Hebert, L.A. (1999). Mycophenolate mofetil therapy in lupus nephritis: clinical observations. *J. Am. Soc. Nephrol. 10*, 833–839.

Dooley, M.A., Aranow, C., and Ginzler, E.M. (2004). Review of ACR renal criteria in systemic lupus erythematosus. *Lupus 13*, 857–860.

Dubois, E.L. and Tuffanelli, D.L. (1964). Clinical manifestations of systemic lupus erythematosus. Computer analysis of 520 cases. *JAMA 190*, 104–111.

Edberg, J.C., Langefeld, C.D., Wu, J., Moser, K.L., Kaufman, K.M., Kelly, J., Bansal, V., Brown, W.M., Salmon, J., Rich, S.S., Harley, J.B., and Kimberly, R.P. (2002). Genetic linkage and association of Fc gamma receptor IIIA (CD16A) on chromosome 1q23 with human systemic lupus erythematosus. *Arthritis Rheum. 46*, 2132–2140.

Erlanger, B.F. (1989). Auto-anti-idiotypy, autoimmunity and some thoughts on the structure of internal images. *Int. Rev. Immunol. 5*, 131–137.

Erlanger, B.F., Cleveland, W.L., Wassermann, N.H., Ku, H.H., Hill, B.L., Sarangarajan, R., Rajagopalan, R., Cayanis, E., Edelman, I.S., and Penn, A.S. (1986). Auto-anti-idiotype: a basis for autoimmunity and a strategy for anti-receptor antibodies. *Immunol. Rev. 94*, 23–37.

Esdaile, J.M., Levinton, C., Federgreen, W., Hayslett, J.P., and Kashgarian, M. (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.

Estes, D. and Christian, C.L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine (Baltimore) 50*, 85–95.

Falk, R.J. and Jennette, J.C. (1988). Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N. Engl. J. Med. 318*, 1651–1657.

Falk, R.J., Hogan, S., Carey, T.S., and Jennette, J.C. (1990a). Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network [see comments]. *Ann. Intern. Med. 113*, 656–663.

Falk, R.J., Terrell, R.S., Charles, L.A., and Jennette, J.C. (1990b). Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. *Proc. Natl. Acad. Sci. USA 87*, 4115–4119.

Foster, M.H., Cizman, B., and Madaio, M.P. (1993). Nephritogenic autoantibodies in systemic lupus erythematosus: immunochemical properties, mechanisms of immune deposition, and genetic origins. *Lab. Invest. 69*, 494–507.

Franssen, C.F., Stegeman, C.A., Kallenberg, C.G., Gans, R.O., De Jong, P.E., Hoorntje, S.J., and Tervaert, J.W. (2000). Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int. 57*, 2195–2206.

Gladman, D.D., Urowitz, M.B., Cole, E., Ritchie, S., Chang, C.H., and Churg, J. (1989). Kidney biopsy in SLE. I. A clinical-morphologic evaluation. *Q. J. Med. 73*, 1125–1133.

Glicklich, D. and Acharya, A. (1998). Mycophenolate mofetil therapy for lupus nephritis refractory to intravenous cyclophosphamide. *Am. J. Kidney Dis. 32*, 318–322.

Gross, W.L., Schmitt, W.H., and Csernok, E. (1993). ANCA and associated diseases: immunodiagnostic and pathogenetic aspects. *Clin. Exp. Immunol. 91*, 1–12.

Gunnarsson, A., Hellmark, T., and Wieslander, J. (2000). Molecular properties of the Goodpasture epitope. *J. Biol. Chem. 275*, 30844–30848.

Halbwachs-Mecarelli, L., Bessou, G., Lesavre, P., Lopez, S., and Witko-Sarsat, V. (1995). Bimodal distribution of proteinase 3 (PR3) surface expression reflects a constitutive heterogeneity in the polymorphonuclear neutrophil pool. *FEBS Lett. 374*, 29–33.

Hargraves, M.M. (1969). Discovery of the LE cell and its morphology. *Mayo Clin. Proc. 44*, 579–599.

Hashimoto, S., Matsumoto, K., Gon, Y., Maruoka, S., Takeshita, I., Hayashi, S., Koura, T., Kujime, K., and Horie, T. (1999). p38 Mitogen-activated protein kinase regulates IL-8 expression in human pulmonary vascular endothelial cells. *Eur. Respir. J. 13*, 1357–1364.

Hauer, H.A., Bajema, I.M., Van Houwelingen, H.C., Ferrario, F., Noel, L.H., Waldherr, R., Jayne, D.R., Rasmussen, N., Bruijn, J.A., and Hagen, E.C. (2002). Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int. 62*, 1732–1742.

Hellmark, T., Niles, J.L., Collins, A.B., McCluskey, R.T., and Brunmark, C. (1997). Comparison of anti-GBM antibodies in sera with or without ANCA. *J. Am. Soc. Nephrol. 8*, 376–385.

Hellmark, T., Burkhardt, H., and Wieslander, J. (1999). Goodpasture disease. Characterization of a single conformational epitope as the target of pathogenic autoantibodies. *J. Biol. Chem. 274*, 25862–25868.

Hill, B.L. and Erlanger, B.F. (1988). Monoclonal antibodies to the thyrotropin receptor raised by an autoantiidiotypic protocol and their relationship to monoclonal autoantibodies from Graves' patients. *Endocrinology 122*, 2840–2850.

Hopfer, H., Maron, R., Butzmann, U., Helmchen, U., Weiner, H.L., and Kalluri, R. (2003). The importance of cell-mediated immunity in the course and severity of autoimmune anti-glomerular basement membrane disease in mice. *FASEB J. 17*, 860–868.

Horvath, L., Czirjak, L., Fekete, B., Jakab, L., Pozsonyi, T., Kalabay, L., Romics, L., Miklos, K., Varga, L., Prohaszka, Z., Szakacs, A., Nagy, E., Daha, M.R., and Fust, G. (2001). High levels of antibodies against Clq are associated with disease activity and nephritis but not with other organ manifestations in SLE patients. *Clin. Exp. Rheumatol. 19*, 667–672.

Houssiau, F.A. and Jadoul, M. (2002). Cytotoxic therapy of lupus nephritis: recent developments. *Nephrol. Dial. Transplant. 17*, 955–957.

Hudson, B.G., Tryggvason, K., Sundaramoorthy, M., and Neilson, E.G. (2003). Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N. Engl. J. Med. 348*, 2543–2556.

Jarrett, M.P., Sablay, L.B., Walter, L., Barland, P., and Grayzel, A.I. (1981). The effect of continuous normalization of serum hemolytic complement on the course of lupus nephritis: a five year prospective study. *Am. J. Med. 70*, 1067–1072.

Jennette, J.C. and Falk, R.J. (1997a). Diagnosis and management of glomerular diseases. *Med. Clin. North Am. 81*, 653–677.

Jennette, J.C. and Falk, R.J. (1997b). Small-vessel vasculitis. *N. Engl. J. Med. 337*, 1512–1523.

Jerne, N.K. (1974). Towards a network theory of the immune system. *Ann. Immunol. (Paris) 125C*, 373–389.

Jiang, H., Lin, J., Su, Z.Z., Collart, F.R., Huberman, E., and Fisher, P.B. (1994). Induction of differentiation in human promyelocytic HL-60 leukemia cells activates p21, WAF1/CIP1, expression in the absence of p53. *Oncogene 9*, 3397–3406.

Johnson, P.A., Alexander, H.D., McMillan, S.A., and Maxwell, A.P. (1997a). Up-regulation of the endothelial cell adhesion molecule intercellular adhesion molecule-1 (ICAM-1) by autoantibodies in autoimmune vasculitis. *Clin. Exp. Immunol. 108*, 234–242.

Johnson, P.A., Alexander, H.D., McMillan, S.A., and Maxwell, A.P. (1997b). Up-regulation of the granulocyte adhesion molecule Mac-1 by autoantibodies in autoimmune vasculitis. *Clin. Exp. Immunol. 107*, 513–519.

Kalluri, R., Danoff, T.M., Okada, H., and Neilson, E.G. (1997). Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. *J. Clin. Invest. 100*, 2263–2275.

Kaneko, Y., Sakatsume, M., Xie, Y., Kuroda, T., Igashima, M., Narita, I., and Gejyo, F. (2003). Macrophage metalloelastase as a major factor for glomerular injury in anti-glomerular basement membrane nephritis. *J. Immunol. 170*, 3377–3385.

Kang, K.H., Lee, K.H., Kim, M.Y., and Choi, K.H. (2001). Caspase-3-mediated cleavage of the NF-κB subunit p65 at the NH2-terminus potentiates naphtoquinone analog-induced apoptosis. *J. Biol. Chem. 24*, 24.

Keogan, M.T., Esnault, V.L., Green, A.J., Lockwood, C.M., and Brown, D.L. (1992). Activation of normal neutrophils by anti-neutrophil cytoplasm antibodies. *Clin. Exp. Immunol. 90*, 228–234.

Kettritz, R., Jennette, J.C., and Falk, R.J. (1997). Crosslinking of ANCA-antigens stimulates superoxide release by human neutrophils. *J. Am. Soc. Nephrol. 8*, 386–394.

King, W.J., Adu, D., Daha, M.R., Brooks, C.J., Radford, A.A. and Savage, C.O.S. (1995). Endothelial cells and renal epithelial cells do not express the Wegener's autoantigen, proteinase 3. *Clin. Exp. Immunol. 102*, 98–105.

King, W.J., Brooks, C.J., Holder, R., Hughes, P., Adu, D., and Savage, C.O. (1998). T lymphocyte responses to anti-neutrophil cytoplasmic autoantibody (ANCA) antigens are present in patients with ANCA-associated systemic vasculitis and persist during disease remission. *Clin. Exp. Immunol. 112*, 539–546.

Kobold, A.C., Kallenberg, C.G., and Tervaert, J.W. (1999). Monocyte activation in patients with Wegener's granulomatosis. *Ann. Rheum. Dis. 58*, 237–245.

Kocher, M., Edberg, J.C., Fleit, H.B., and Kimberly, R.P. (1998). Antineutrophil cytoplasmic antibodies preferentially engage Fc gamma RIIIb on human neutrophils. *J. Immunol. 161*, 6909–6914.

Kuroda, T., Yoshida, Y., Kamiie, J., Kovalenko, P., Nameta, M., Fujinaka, H., Yaoita, E., Endo, T., Ishizuka, S., Nakabayashi, K., Yamada, A., Nagasawa, T., and Yamamoto, T. (2004). Expression of MMP-9 in mesangial cells and its changes in anti-GBM glomerulonephritis in WKY rats. *Clin. Exp. Nephrol. 8*, 206–215.

Kurosawa, S., Esmon, C.T., and Stearns-Kurosawa, D.J. (2000). The soluble endothelial protein C receptor binds to activated neutrophils: involvement of proteinase-3 and CD11b/CD18. *J. Immunol. 165*, 4697–4703.

Lahita, R. (2004). *Introduction*. In Lahita, R. (ed.) Systemic Lupus Erythematosus 4th edn. San Diego: Elsevier.

Lai, K.N. and Lockwood, C.M. (1991). The effect of anti-neutrophil cytoplasm autoantibodies on the signal transduction in human neutrophils. *Clin. Exp. Immunol. 85*, 396–401.

Lavorgna, G., Dahary, D., Lehner, B., Sorek, R., Sanderson, C.M., and Casari, G. (2004). In search of antisense. *Trends Biochem. Sci. 29*, 88–94.

Lefkowith, J.B., Kiehl, M., Rubenstein, J., DiValerio, 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.

Lehner, B., Williams, G., Campbell, R.D., and Sanderson, C.M. (2002). Antisense transcripts in the human genome. *Trends Genet. 18*, 63–65.

Levkau, B., Koyama, H., Raines, E.W., Clurman, B.E., Herren, B., Orth, K., Roberts, J.M., and Ross, R. (1998). Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. *Mol, Cell. 1*, 553–563.

Levy, J.B., Turner, A.N., Rees, A.J., and Pusey, C.D. (2001). Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann, Intern, Med. 134*, 1033–1042.

Levy, J.B., Hammad, T., Coulthart, A., Dougan, T., and Pusey, C.D. (2004). Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int. 66*, 1535–1540.

Lewis, E.J. (1992). Plasmapheresis therapy is ineffective in SLE. Lupus Nephritis Collaborative Study Group. *J. Clin. Apheresis. 7*, 153.

Linnik, M.D., Hu, J.Z., Heilbrunn, K.R., Strand, V., Hurley, F.L., and Joh, T. (2005). Relationship between anti-double-stranded DNA antibodies and exacerbation of renal disease in patients with systemic lupus erythematosus. *Arthritis Rheum. 52*, 1129–1137.

Locke, I.C., Leaker, B., and Cambridge, G. (1999). A comparison of the characteristics of circulating anti-myeloperoxidase autoantibodies in vasculitis with those in non-vasculitic conditions. *Clin. Exp. Immunol. 115*, 369–376.

Mannik, M., Merrill, C.E., Stamps, L.D., and Wener, M.H. (2003). Multiple autoantibodies form the glomerular immune deposits in patients with systemic lupus erythematosus. *J. Rheumatol. 30*, 1495–1504.

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.

Masutani, K., Tokumoto, M., Nakashima, H., Tsuruya, K., Kashiwagi, M., Kudoh, Y., Fukuda, K., Kanai, H., Akahoshi, M., Otsuka, T., Hirakata, H., and Iida, M. (2003). Strong polarization toward Th1 immune response in ANCA-associated glomerulonephritis. *Clin. Nephrol. 59*, 395–405.

Mayet, W.J., Csernok, E., Szymkowiak, C., Gross, W.L., and Meyer zum Buschenfelde, K.H. (1993a). Human endothelial cells express proteinase-3, the target antigen of anticytoplasmic antibodies in Wegener's granulomatosis. *Blood 82*, 1221–1229.

Mayet, W.J., Hermann, E.M., Csernok, E., Gross, W.L., and Meyer zum Buschenfelde, K.H. (1993b). *In vitro* interactions of c-ANCA (antibodies to proteinase 3) with human endothelial cells. *Adv. Exp. Med. Biol. 336*, 109–113.

Mayet, W.J., Schwarting, A., Orth, T., Sibelius, U., Hattar, K. and Meyer zum Buschenfelde, K.H. (1997). Signal transduction pathways of membrane expression of proteinase 3 (PR-3) in human endothelial cells. *Eur. J. Clin. Invest. 27*, 893–899.

McCarty, G.A., Harley, J.B., and Reichlin, M. (1993). A distinctive autoantibody profile in black female patients with lupus nephritis. *Arthritis Rheum. 36*, 1560–1565.

McCurdy, D.K., Lehman, T.J., Bernstein, B., Hanson, V., King, K.K., Nadorra, R., and Landing, B.H. (1992). Lupus nephritis: prognostic factors in children. *Pediatrics 89*, 240–246.

Merkel, F., Kalluri, R., Marx, M., Enders, U., Sevanovic, S., Giegerich, G., Neilson, E.G., Rammensee, H.G., Hudson, B.G., and Weber, M. (1996). Autoreactive T-cells in Goodpasture's syndrome recognize the N-terminal NC1 domain on alpha 3 type IV collagen. *Kidney Int. 14*, 1127–1133.

Milis, L., Timmermans, V., Morris, C.A., Pussell, B.A., and Charlesworth, J.A. (1992). The value of complement measurements in the assessment of lupus activity. *Australas. N. Z. J. Med. 22*, 338–344.

Mocsai, A., Ligeti, E., Lowell, C.A., and Berton, G. (1999). Adhesion-dependent degranulation of neutrophils requires the Src family kinases Fgr and Hck. *J. Immunol. 162*, 1120–1126.

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*, 176–181.

Mostoslavsky, G., Fischel, R., Yachimovich, N., Yarkoni, Y., Rosenmann, E., Monestier, M., Baniyash, M., and Eilat, D. (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.

Mulder, A.H., Heeringa, P., Brouwer, E., Limburg, P.C., and Kallenberg, C.G. (1994). Activation of granulocytes by anti-neutrophil cytoplasmic

antibodies (ANCA): a Fc gamma RII-dependent process. *Clin. Exp. Immunol. 98*, 270–278.

Mulder, A.H., Stegeman, C.A., and Kallenberg, C.G. (1995). Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis: a predominant role for the IgG3 subclass of ANCA. *Clin. Exp. Immunol. 101*, 227–232.

Mundel, P. and Shankland, S.J. (2002). Podocyte biology and response to injury. *J. Am. Soc. Nephrol. 13*, 3005–3015.

Murata, H., Matsumura, R., Koyama, A., Sugiyama, T., Sueishi, M., Shibuya, K., Tsutsumi, A., and Sumida, T. (2002). T cell receptor repertoire of T cells in the kidneys of patients with lupus nephritis. *Arthritis Rheum. 46*, 2141–2147.

Nakamura, A., Yuasa, T., Ujike, A., Ono, M., Nukiwa, T., Ravetch, J.V., and Takai, T. (2000). Fcgamma 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*, 899–906.

Neumann, I., Kain, R., Regele, H., Soleiman, A., Kandutsch, S., and Meisl, F.T. (2005). Histological and clinical predictors of early and late renal outcome in ANCA-associated vasculitis. *Nephrol. Dial. Transplant. 20*, 96–104.

Nossent, H.C., Henzen-Logmans, S.C., Vroom, T.M., Berden, J.H., and Swaak, T.J. (1990). Contribution of renal biopsy data in predicting outcome in lupus nephritis. Analysis of 116 patients. *Arthritis Rheum. 33*, 970–977.

Osler, W. (1904). On the visceral manifestations of the erythema group of skin diseases. *Am. J. Med. Sci. 127*, 1.

Pedersen, N. and Barlough, J.E. (1991). Systemic lupus erythematosus in the cat. *Feline Pract. 19*, 5.

Pendergraft, W.F., Alcorta, D.A., Segelmark, M., Yang, J.J., Tuttle, R., Jennette, J.C., Falk, R.J., and Preston, G.A. (2000). ANCA antigens, proteinase 3 and myeloperoxidase, are not expressed in endothelial cells. *Kidney Int. 57*, 1981–1990.

Pendergraft, W.F., 3rd, Preston, G.A., Shah, R.R., Tropsha, A., Carter, C.W., Jr., Jennette, J.C., and Falk, R.J. (2004a). Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat. Med. 10*, 72–79.

Pendergraft, W.F., 3rd, Rudolph, E.H., Falk, R.J., Jahn, J.E., Grimmler, M., Hengst, L., Jennette, J.C., and Preston, G.A. (2004b). Proteinase 3 sidesteps caspases and cleaves p21(Waf1/Cip1/Sdi1) to induce endothelial cell apoptosis. *Kidney Int. 65*, 75–84.

Perez, G.O., Bjornsson, S., Ross, A.H., Aamato, J., and Rothfield, N. (1974). A mini-epidemic of Goodpasture's syndrome clinical and immunological studies. *Nephron 13*, 161–173.

Peutz-Kootstra, C.J., de Heer, E., Hoedemaeker, P.J., Abrass, C.K., and Bruijn, J.A. (2001). Lupus nephritis: lessons from experimental animal models. *J. Lab. Clin. Med. 137*, 244–260.

Phelps, R.G. and Rees, A.J. (1999). The HLA complex in Goodpasture's disease: a model for analyzing susceptibility to autoimmunity. *Kidney Int. 56*, 1638–1653.

Piedrafita, F.J. and Pfahl, M. (1997). Retinoid-induced apoptosis and Sp1 cleavage occur independently of transcription and require caspase activation. *Mol. Cell. Biol. 17*, 6348–6358.

Popa, E.R., Franssen, C.F., Limburg, P.C., Huitema, M.G., Kallenberg, C.G., and Tervaert, J.W. (2002). *In vitro* cytokine production and proliferation of T cells from patients with anti-proteinase 3- and antimyeloperoxidase-associated vasculitis, in response to proteinase 3 and myeloperoxidase. *Arthritis Rheum. 46*, 1894–1904.

Porges, A.J., Redecha, P.B., Kimberly, W.T., Csernok, E., Gross, W.L., and Kimberly, R.P. (1994). Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. *J. Immunol. 153*, 1271–1280.

Powrie, F. and Maloy, K.J. (2003). Immunology. Regulating the regulators. *Science 299*, 1030–1031.

Preston, G.A., Zarella, C.S., Pendergraft, W.F., 3rd, Rudolph, E.H., Yang, J.J., Sekura, S.B., Jennette, J.C., and Falk, R.J. (2002). Novel effects of neutrophil-derived proteinase 3 and elastase on the vascular endothelium involve *in vivo* cleavage of NF-kappaB and proapoptotic changes in JNK, ERK, and p38 MAPK signaling pathways. *J. Am. Soc. Nephrol. 13*, 2840–2849.

Preston, G.A., Waga, I., Alcorta, D.A., Sasai, H., Munger, W.E., Sullivan, P., Phillips, B., Jennette, J.C., and Falk, R.J. (2004). Gene expression profiles of circulating leukocytes correlate with renal disease activity in IgA nephropathy. *Kidney Int. 65*, 420–430.

Pudifin, D.J., Duursma, J., Gathiram, V., and Jackson, T.F. (1994). Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. *Clin. Exp. Immunol. 97*, 48–51.

Pusey, C.D. (2003). Anti-glomerular basement membrane disease. *Kidney Int. 64*, 1535–1550.

Ramaha, A. and Patston, P.A. (2002). Release and degradation of angiotensin I and angiotensin II from angiotensinogen by neutrophil serine proteinases. *Arch. Biochem. Biophys. 397*, 77–83.

Ravirajan, C.T., Rowse, L., MacGowan, J.R., and Isenberg, D.A. (2001). An analysis of clinical disease activity and nephritis-associated serum autoantibody profiles in patients with systemic lupus erythematosus: a cross-sectional study. *Rheumatology (Oxford) 40*, 1405–1412.

Reumaux, D., Vossebeld, P.J., Roos, D., and Verhoeven, A.J. (1995). Effect of tumor necrosis factor-induced integrin activation on Fc gamma receptor II-mediated signal transduction: relevance for activation of neutrophils by anti-proteinase 3 or anti-myeloperoxidase antibodies. *Blood 86*, 3189–3195.

Reveille, J.D. (2004). Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus 13*, 290–297.

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.

Reynolds, J., Mavromatidis, K., Cashman, S.J., Evans, D.J., and Pusey, C.D. (1998). Experimental autoimmune glomerulonephritis (EAG) induced by homologous and heterologous glomerular basement membrane in two substrains of Wistar-Kyoto rat. *Nephrol. Dial. Transplant. 13*, 44–52.

Reynolds, J., Tam, F.W., Chandraker, A., Smith, J., Karkar, A.M., Cross, J., Peach, R., Sayegh, M.H., and Pusey, C.D. (2000). CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis. *J. Clin. Invest. 105*, 643–651.

Reynolds, J., Cook, P.R., Ryan, J.J., Norsworthy, P.J., Glazier, A.M., Duda, M.A., Evans, D.J., Aitman, T.J., and Pusey, C.D. (2002a). Segregation of experimental autoimmune glomerulonephritis as a complex genetic trait and exclusion of Col4a3 as a candidate gene. *Exp. Nephrol. 10*, 402–407.

Reynolds, J., Norgan, V.A., Bhambra, U., Smith, J., Cook, H.T., and Pusey, C.D. (2002b). Anti-CD8 monoclonal antibody therapy is effective in the prevention and treatment of experimental autoimmune glomerulonephritis. *J. Am. Soc. Nephrol. 13*, 359–369.

Reynolds, J., Khan, S.B., Allen, A.R., Benjamin, C.D., and Pusey, C.D. (2004). Blockade of the CD154-CD40 costimulatory pathway prevents the development of experimental autoimmune glomerulonephritis. *Kidney Int. 66*, 1444–1452.

Root-Bernstein, R. and Rallo, A. (2004). Antigenic complementarity resulting in idiotype-antiidiotype immune complexes: possible contributor to AIDS pathogenesis and autoimmunity. *Autoimmunity 37*, 203–210.

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*, 2623–2630.

Salama, A.D., Chaudhry, A.N., Ryan, J.J., Eren, E., Levy, J.B., and Pusey, C.D. (2001a). In Goodpasture's disease, CD4(+) T cells escape thymic

deletion and are reactive with the auto-antigen alpha3(IV)NC1. *J. Am. Soc. Nephrol. 12*, 1908–1915.

Salama, A.D., Levy, J.B., Lightstone, L., and Pusey, C.D. (2001b). Goodpasture's disease. *Lancet 358*, 917–920.

Salama, A.D., Dougan, T., Levy, J.B., Cook, H.T., Morgan, S.H., Naudeer, S., Maidment, G., George, A.J., Evans, D., Lightstone, L., and Pusey, C.D. (2002). Goodpasture's disease in the absence of circulating anti-glomerular basement membrane antibodies as detected by standard techniques. *Am. J. Kidney Dis. 39*, 1162–1167.

Salama, A.D., Chaudhry, A.N., Holthaus, K.A., Mosley, K., Kalluri, R., Sayegh, M.H., Lechler, R.I., Pusey, C.D., and Lightstone, L. (2003). Regulation by CD25+ lymphocytes of autoantigen-specific T-cell responses in Goodpasture's (anti-GBM) disease. *Kidney Int. 64*, 1685–1694.

Salmon, J.E., and Roman, M.J. (2001). Accelerated atherosclerosis in systemic lupus erythematosus: implications for patient management. *Curr. Opin. Rheumatol. 13*, 341–344.

Salmon, J.E., Millard, S., Schachter, L.A., Arnett, F.C., Ginzler, E.M., Gourley, M.F., Ramsey-Goldman, R., Peterson, M.G., 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.

Sanders, J.S., Stegeman, C.A., and Kallenberg, C.G. (2003). The Th1 and Th2 paradigm in ANCA-associated vasculitis. *Kidney Blood Press. Res. 26*, 215–220.

Savage, C.O., Pottinger, B.E., Gaskin, G., Pusey, C.D., and Pearson, J.D. (1992). Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am. J. Pathol. 141*, 335–342.

Segelmark, M., Hellmark, T., and Wieslander, J. (2003). The prognostic significance in Goodpasture's disease of specificity, titre and affinity of anti-glomerular-basement-membrane antibodies. *Nephron Clin. Pract. 94*, c59–68.

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.

Shapiro, K.S., Pinn, V.W., Harrington, J.T., and Levey, A.S. (1984). Immune complex glomerulonephritis in hydralazine-induced SLE. *Am. J. Kidney Dis. 3*, 270–274.

Shoenfeld, Y. (1994). Idiotypic induction of autoimmunity: a new aspect of the idiotypic network. *FASEB J. 8*, 1296–1301.

Shoenfeld, Y. (2004). The idiotypic network in autoimmunity: antibodies that bind antibodies that bind antibodies. *Nat Med. 10*, 17–18.

Shoenfeld, Y., Rauova, L., Gilburd, B., Kvapil, F., Goldberg, I., Kopolovic, J., Rovensky, J., and Blank, M. (2002). Efficacy of IVIG affinity-purified anti-double-stranded DNA anti-idiotypic antibodies in the treatment of an experimental murine model of systemic lupus erythematosus. *Int. Immunol. 14*, 1303–1311.

Short, A.K., and Lockwood, C.M. (1995). Antigen specificity in hydralazine associated ANCA positive systemic vasculitis. *Q. J. Med. 88*, 775–783.

Sibelius, U., Hattar, K., Schenkel, A., Noll, T., Csernok, E., Gross, W.L., Mayet, W.J., Piper, H.M., Seeger, W., and Grimminger, F. (1998). Wegener's granulomatosis: anti-proteinase 3 antibodies are potent inductors of human endothelial cell signaling and leakage response. *J. Exp. Med. 187*, 497–503.

Smith, L.R., Bost, K.L., and Blalock, J.E. (1987). Generation of idiotypic and anti-idiotypic antibodies by immunization with peptides encoded by complementary RNA: a possible molecular basis for the network theory. *J. Immunol. 138*, 7–9.

Speirs, C., Fielder, A.H., Chapel, H., Davey, N.J., and Batchelor, J.R. (1989). Complement system protein C4 and susceptibility to hydralazine-induced systemic lupus erythematosus. *Lancet 1*, 922–924.

Stanton, M.C. and Tange, J.D. (1958). Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). *Australas. Ann. Med. 7*, 132–144.

Stegeman, C.A., Tervaert, J.W., Sluiter, W.J., Manson, W.L., de Jong, P.E., and Kallenberg, C.G. (1994). Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. *Ann. Intern. Med. 120*, 12–17.

Stevenson, A., Yaqoob, M., Mason, H., Pai, P., and Bell, G.M. (1995). Biochemical markers of basement membrane disturbances and occupational exposure to hydrocarbons and mixed solvents. *Q. J. Med. 88,* 23–28.

Sturrock, A., Franklin, K.F. and Hoidal, J.R. (1996). Human Proteinase-3 expression is regulated by PU.1 in conjunction with a cytidine-rich element. *J. Biol. Chem. 271*, 32392–32402.

Su, K., Wu, J., Edberg, J.C., Li, X., Ferguson, P., Cooper, G.S., Langefeld, C.D., and Kimberly, R.P. (2004). A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcgammaRIIb alters receptor expression and associates with autoimmunity. I. Regulatory FCGR2B polymorphisms and their association with systemic lupus erythematosus. *J. Immunol. 172*, 7186–7191.

Suenaga, R. and Abdou, N.I. (1993). Cationic and high affinity serum IgG anti-dsDNA antibodies in active lupus nephritis. *Clin. Exp. Immunol. 94*, 418–422.

Sundaramoorthy, M., Meiyappan, M., Todd, P., and Hudson, B.G. (2002). Crystal structure of NC1 domains. Structural basis for type IV collagen assembly in basement membranes. *J. Biol. Chem. 277*, 31142–31153.

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.

Theofilopoulos, A.N. and Dixon, F.J. (1985). Murine models of systemic lupus erythematosus. *Adv. Immunol. 37*, 269–390.

Theofilopoulos, A.N., Balderas, R.S., Gozes, Y., Aguado, M.T., Hang, L.M., Morrow, P.R., and Dixon, F.J. (1985a). Association of lpr gene with graft-vs.-host disease-like syndrome. *J Exp Med. 162*, 1–18.

Theofilopoulos, A.N., Prud'Homme, G.J., and Dixon, F.J. (1985b). Autoimmune aspects of systemic lupus erythematosus. *Concepts Immunopathol.* 1, 190–218.

Tomer, Y., Barak, V., Gilburd, B., and Shoenfeld, Y. (1999). Cytokines in experimental autoimmune vasculitis: evidence for a Th2 type response. *Clin. Exp. Rheumatol. 17*, 521–526.

Tsao, B.P. (2004). Update on human systemic lupus erythematosus genetics. *Curr. Opin Rheumatol. 16*, 513–521.

Van Den Eynde, B.J., Gaugler, B., Probst-Kepper, M., Michaux, L., Devuyst, O., Lorge, F., Weynants, P., and Boon, T. (1999). A new antigen recognized by cytolytic T lymphocytes on a human kidney tumor results from reverse strand transcription. *J. Exp. Med. 190*, 1793–1800.

van der Geld, Y.M., Huitema, M.G., Franssen, C.F., van der Zee, R., Limburg, P.C., and Kallenberg, C.G. (2000). *In vitro* T lymphocyte responses to proteinase 3 (PR3) and linear peptides of PR3 in patients with Wegener's granulomatosis (WG). *Clin. Exp. Immunol. 122*, 504–513.

van der Geld, Y.M., Stegeman, C.A., and Kallenberg, C.G. (2004). B cell epitope specificity in ANCA-associated vasculitis: does it matter? *Clin. Exp. Immunol. 137*, 451–459.

van Rossum, A.P., Rarok, A.A., Huitema, M.G., Fassina, G., Limburg, P.C., and Kallenberg, C.G. (2004). Constitutive membrane expression of proteinase 3 (PR3) and neutrophil activation by anti-PR3 antibodies. *J. Leukoc. Biol. 76*, 1162–1170.

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.

Vogt, A., Schmiedeke, T., Stockl, F., Sugisaki, Y., Mertz, A., and Batsford, S. (1990). The role of cationic proteins in the pathogenesis of immune complex glomerulonephritis. *Nephrol. Dial. Transplant. 5*(Suppl 1), 6–9.

Wada, Y., Ito, S., Ueno, M., Nakano, M., Arakawa, M., and Gejyo, F. (2004). Renal outcome and predictors of clinical renal involvement in patients with silent lupus nephritis. *Nephron Clin. Pract. 98*, c105–111.

Wakayama, H., Hasegawa, Y., Kawabe, T., Hara, T., Matsuo, S., Mizuno, M., Takai, T., Kikutani, H., and Shimokata, K. (2000). Abolition of anti-glomerular basement membrane antibody-mediated glomerulonephritis in FcRgamma-deficient mice. *Eur. J. Immunol. 30*, 1182–1190.

Waldhauser, L. and Uetrecht, J. (1996). Antibodies to myeloperoxidase in propylthiouracil-induced autoimmune disease in the cat. *Toxicology 114*, 155–162.

Wallace, D.J., Goldfinger, D., Pepkowitz, S.H., Fichman, M., Metzger, A.L., Schroeder, J.O., and Euler, H.H. (1998). Randomized controlled trial of pulse/synchronization cyclophosphamide/apheresis for proliferative lupus nephritis. *J. Clin. Apheresis 13*, 163–166.

Walters, G., Habib, A.M., Reynolds, J., Wu, H., Knight, J.F., and Pusey, C.D. (2004). Glomerular T cells are of restricted clonality and express multiple CDR3 motifs across different Vbeta T-cell receptor families in experimental autoimmune glomerulonephritis. *Nephron Exp. Nephrol. 98*, e71–81.

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.

Weening, J.J., D'Agati, V.D., Schwartz, M.M., Seshan, S.V., Alpers, C.E., Appel, G.B., Balow, J.E., Bruijn, J.A., Cook, T., Ferrario, F., Fogo, A. B., Ginzler, E.M., Hebert, L., Hill, G., Hill, P., Jennette, J.C., Kong, N.C., Lesavre, P., Lockshin, M., Looi, L.M., Makino, H., Moura, L.A., and Nagata, M. (2004). The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int. 65*, 521–530.

Wei, C.H., Ho, Y.S., Chou, B.L., Sheu, L.F., Shieh, S.D., Chen, A., and Tu, Y.C. (1993). Lupus nephritis, emphasizing tubulointerstitial lesions: a clinico-pathological analysis. *Zhonghua Yi Xue Za Zhi (Taipei) 51*, 27–32.

Werner, L.L., and Gorman, N.T. (1984). Immune-mediated disorders of cats. *Vet. Clin. North Am. Small Anim. Pract. 14*, 1039–1064.

Williams, J.M. and Savage, C.O. (2005). Characterization of the regulation and functional consequences of p21ras activation in neutrophils by antineutrophil cytoplasm antibodies. *J. Am. Soc. Nephrol. 16*, 90–96.

Williams, J.M., Ben-Smith, A., Hewins, P., Dove, S.K., Hughes, P., McEwan, R., Wakelam, M.J., and Savage, C.O. (2003). Activation of the G(i) heterotrimeric G protein by ANCA IgG F(ab′)2 fragments is necessary but not sufficient to stimulate the recruitment of those downstream mediators used by intact ANCA IgG. *J. Am. Soc. Nephrol. 14*, 661–669.

Winek, J., Mueller, A., Csernok, E., Gross, W.L., and Lamprecht, P. (2004). Frequency of proteinase 3 (PR3)-specific autoreactive T cells determined by cytokine flow cytometry in Wegener's granulomatosis. *J. Autoimmun. 22*, 79–85.

Witko-Sarsat, V., Canteloup, S., Durant, S., Desdouets, C., Chabernaud, R., Lemarchand, P., and Descamps-Latscha, B. (2002). Cleavage of P21/Waf1 by proteinase 3, a myeloid specific serine protease, potentiates cell proliferation. *J. Biol. Chem. 26*, 26.

Woitas, R.P. and Morioka, T. (1996). Influence of isoelectric point on glomerular deposition of antibodies and immune complexes. *Nephron 74*, 713–719.

Wong, D., Phelps, R.G., and Turner, A.N. (2001). The Goodpasture antigen is expressed in the human thymus. *Kidney Int. 60*, 1777–1783.

Wu, J., Arends, J., Borillo, J., Zhou, C., Merszei, J., McMahon, J., and Lou, Y.H. (2004). A self T cell epitope induces autoantibody response: mechanism for production of antibodies to diverse glomerular basement membrane antigens. *J. Immunol. 172*, 4567–4574.

Wu, J., Borillo, J., Glass, W.F., Hicks, J., Ou, C.N., and Lou, Y.H. (2003). T-cell epitope of alpha3 chain of type IV collagen induces severe glomerulonephritis. *Kidney Int. 64*, 1292–1301.

Wu, J., Edberg, J.C., Redecha, P.B., Bansal, V., Guyre, P.M., Coleman, K., Salmon, J., and Kimberly, R.P. (1997). A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest. 100*, 1059–1070.

Xenocostas, A., Jothy, S., Collins, B., Loertscher, R., and Levy, M. (1999). Anti-glomerular basement membrane glomerulonephritis after extracorporeal shock wave lithotripsy. *Am. J. Kidney Dis. 33*, 128–132.

Xie, C., Liang, Z., Chang, S., and Mohan, C. (2003). Use of a novel elution regimen reveals the dominance of polyreactive antinuclear autoantibodies in lupus kidneys. *Arthritis Rheum. 48*, 2343–2352.

Yang, J.J., Pendergraft, W.F., Alcorta, D.A., Nachman, P.H., Hogan, S.L., Thomas, R.P., Sullivan, P., Jennette, J.C., Falk, R.J., and Preston, G.A. (2004). Circumvention of normal constraints on granule protein gene expression in peripheral blood neutrophils and monocytes of patients with antineutrophil cytoplasmic autoantibody-associated glomerulonephritis. *J. Am. Soc. Nephrol. 15*, 2103–2114.

Yang, J.J., Preston, G.A., Alcorta, D.A., Waga, I., Munger, W.E., Hogan, S.L., Sekura, S.B., Phillips, B.D., Thomas, R.P., Jennette, J.C., and Falk, R.J. (2002). Expression profile of leukocyte genes activated by antineutrophil cytoplasmic autoantibodies (ANCA). *Kidney Int. 62*, 1638–1649.

Yang, J.J., Preston, G.A., Pendergraft, W.F., Segelmark, M., Heeringa, P., Hogan, S.L., Jennette, J.C., and Falk, R.J. (2001). Internalization of proteinase 3 is concomitant with endothelial cell apoptosis and internalization of myeloperoxidase with generation of intracellular oxidants. *Am. J. Pathol. 158*, 581–592.

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*, 1604–1608.

CHAPTER

60

Orchitis and Male Infertility

LIVIA LUSTIG*

**Centro de Investigaciones en Reproducción, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*

KENNETH S. K. TUNG†

†Department of Pathology, University of Virginia, Charlottesville, Virginia, USA

HISTORIC BACKGROUND

The demonstration that the sera of animals sensitized with testicular homogenate or semen were able to immobilize spermatozoa was first reported over 100 years ago. Pioneered by Voisin (Voisin et al., 1951) and Freund (Freund et al., 1953), the first experimental model of autoimmune orchitis, induced by active immunization with homologous testicular antigens and adjuvant, was developed. Between 1970 and 1980, most research focused on the study of antibodies to sperm antigens as a cause of male or female infertility, independent of orchitis, and a major effort was made to standardize the methods of detecting sperm antibodies. Later, research interest shifted to defining the nature of testis-specific autoantigens. As yet, no well-characterized sperm antigen has been identified as a target in sperm autoimmunity or as a suitable candidate antigen in male gamete contraceptive vaccine development.

In this chapter, we review current knowledge on clinical male infertility, with emphasis on autoimmune orchitis and its pathogenetic mechanism.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Male infertility associated with immunologic mechanisms may result from the presence of sperm antibodies or autoimmune disease of the testis and its excurrent ducts. In the first case, antibodies, once generated, may result in infertility by a variety of mechanisms, mainly disturbances in sperm transport or disruptions in gamete interaction. In the second case, immunopathologic damage of the testis (and its excurrent ducts) occurs through T-cell-mediated mechanisms triggered by antigens or pathogens that disrupt testicular immunoprivilege.

Male Infertility Associated with Sperm Antibodies

Male infertility may result from the binding of antisperm antibodies (ASAs) to ejaculated sperm. Clinically, ASAs are found in 3–12% of men with infertility, compared to 0–2% of the general male population [reviewed by Turek and Lipshultz (1994)]. The ASAs present in local secretions of the genital tract rather than serum are associated with infertility [reviewed by Marshburn and Kutteh (1994)].

Importantly, data from *in vitro* fertilization programs indicate that a reduced fertilization rate is associated with IgA and IgG antibody binding to the head region of sperm.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Many potentially important human and animal sperm molecules [reviewed by Frayne and Hall (1999) and Bohring and Krause (2003)] have been identified by means of serum antibodies from infertile patients or monoclonal antibodies that affect different fertilization events—motility, cervical mucus penetration, acrosome reaction, zona binding, zona penetration, oolema binding, and pronucleous formation.

Analysis of the cognate antigens of the ASAs involved in the process of fertilization improves the identification of immunogenic proteins that are candidates for immune contraception.

Obstruction or injury of the male reproductive tract is associated with ASA formation, since leakage of large quantities of sperm antigens can induce antibody production. Sperm antibodies attendant to vasectomy may be responsible for infertility in some patients following vasovasostomy. Indeed, ASAs are common in patients with congenital absence of the vas deferens associated with cystic fibrosis (Box 60.1).

Male Infertility Associated with Autoimmune Orchitis

Autoimmune orchitis is well documented in spontaneously infertile animals and in experimental models of autoimmune orchitis. Human autoimmune orchitis is represented by the granulomatous orchitis of noninfectious origin, aspermatogenesis associated with testicular immune complex deposits, and orchitis associated with systemic viral infections.

Clinically, granulomatous orchitis causes mild testicular pain or swelling. The main challenge is to distinguish it from a tumor; sonographic studies and biopsy may help the diagnosis. The histopathology of the testis shows granuloma formation as an accumulation of histiocytes, macrophages, and epithelioid and multinucleated giant cells, associated with loss of germ cells.

Box 60.1
Human pathologies or conditions associated with antisperm antibodies

Testis
- Granulomatous orchitis
- Orchitis associated with viral infections (mainly mumps virus and HIV) and other infectious agents
- Orchitis associated with cryptorchidism or tumor
- Orchitis associated with trauma, torsion, or biopsy

Vas deferens
- Vasectomy
- Vasectomy reversal
- Agenesia of vas deferens

Autoimmune orchitis can be accompanied by epididymitis and vasitis, although genetic evidence suggests that each can occur as an independent entity (Roper et al., 1998). As in orchitis, spermatic granulomas of noninfectious origin can occur in the epididymis and the vas deferens as an autoimmune response to the host's own extravasated spermatozoa, inducing local immunoglobulin production and accumulation of T lymphocytes (Saravanamuthu et al., 1991). Sperm granuloma are commonly seen in men who have had a vasectomy. However, most cases of orchiepididymitis are due to infection [reviewed by Chan and Schlegel (2002) and Delavierre (2003)] and the main route is retrograde propagation from the vas deferens. Epididymitis can also be iatrogenic, a complication of treatment with amiodarone a drug used for cardiac arrhythmias, and although the exact mechanism of this disease is unknown, the lymphocyte infiltration present in a sterile microenvironment suggests an autoimmune phenomenon.

Two studies in the 1980s (Salomon et al., 1982; Lehman et al., 1987) on testis biopsies from infertile men described deposits of IgG and/or complement (C3) or immunocomplexes in the basement membranes (BM) of seminiferous tubules and damage of the germinal epithelium. Although the evidence for immune complex-induced orchitis is not yet confirmed, the alterations are consistent with the observation in the infertile mink (Tung et al., 1981) and in rats and guinea pigs passively immunized with antibodies to BM antigens (Denduchis and Lustig, 1981; Lustig et al., 2000).

Focal orchitis, presumably due to an autoimmune mechanism, is also observed following testicular damage induced by trauma, physical agents, torsion, biopsy or tumor. Histologic evidence of interstitial and/or intratubular inflammatory cells accompanied by loss of germ cells has been described: 4.8% of testicular biopsies from 166 men with azoospermia or oligozoospermia had focal or general leukocyte infiltration (Suominen and Söderström, 1982).

Nistal et al. (2002) evaluated the focal orchitis present in cryptorchidic testes of men not previously treated for cryptorchidism or antecedents of infectious diseases, and detected focal lymphoid infiltrates in the interstitium and/or seminiferous tubules associated with lesion of the germinal epithelium in 44% of 50 patients. The authors suggest that an alteration in the blood–testis barrier (BTB) allows antigens to enter the testicular interstitium and stimulate an autoimmune process.

Most reported cases of spontaneous autoimmune orchitis are associated with an infectious disease, usually following a viral infection. The pathogens are thought to induce orchitis by disrupting the mechanisms of testicular immuno-

privilege rather than through a direct cytopathologic effect. The best-known orchitogenic viruses in humans are the mumps virus and human immunodeficiency virus (HIV). The symptoms of mumps in most prepubertal boys are limited to infectious parotitis; however, 5–37% of adults with mumps infection also develop orchitis. Lymphocytic infiltration followed by oligozoospermia with tubular atrophy and fibrosis contribute to transient or permanent subfertility or infertility. The mumps virus does not appear to induce germ cell transformation and may not be directly spermatogenotoxic. However, mumps virus replicates in Leydig cells *in vitro*, inhibits testosterone secretion, and induces production of the interferon (IFN)-γ-induced protein 10, a chemokine that recruits leukocytes (Le Goffic et al., 2002; Mouchel et al., 2002).

Acquired immune deficiency syndrome (AIDS) patients often suffer from orchitis, hypogonadism, oligozoospermia or azoospermia. It has been suggested that reduction in testosterone levels in AIDS patients may result from a reduction in Leydig cell number or of their function. For example, alteration of the hypothalamic–pituitary–gonadal axis can be caused by proinflammatory cytokines [reviewed by Dejucq and Jégou, (2001)]. Kierszenbaum (1995) speculates that the uncommon perivascular accumulation of $CD4^+/HIV^+$ cells and cytokine production could affect the integrity of the BTB and favor the development of autoimmune orchitis. For example, antibodies against sperm antigens have been reported in AIDS patients.

The autoimmune component of orchitis associated with infections was investigated in several experimental models, including infection with myxoma virus (Fountain et al., 1997) and Sendai virus, which is closely related to mumps virus (Melaine et al., 2003). In mice unilaterally infected with *Listeria monocytogenes*, the contralateral testis developed orchitis without detectable microorganisms, providing evidence for an autoimmune component in infectious orchitis (Mukasa et al., 1995). This was supported by disease transfer to uninfected recipients by $CD4^+$ T cells from infected donors. It was further shown that the γδ T cells reduced αβ pathogenic T-cell function through cytokines, mainly IL-10 and TGF-β (Mukasa et al., 1998).

TESTICULAR IMMUNOREGULATION

Testicular antigens expressed in haploid germ cells appear after puberty when immunocompetence is already established. The ability of the testis to tolerate these autoantigens as well as to survive in the testicular interstitium of transplanted foreign tissue has led to the consideration that the testis is an immunoprivileged organ. The mechanisms of testis autoimmune disease prevention are described in Box 60.2 (Tung et al., 2000).

Box 60.2
Mechanisms of gonadal autoimmune disease prevention

Systemic immunoregulation (systemic tolerance mechanism)

- Regulation of pathogenic self-reactive T cells by regulatory T cells (Takahashi et al., 1998; Thornton and Shevach, 1998)

Regional immunoregulation (testis immunoprivilege)

- Blood–testis barrier (incomplete immunologic barrier that limits the access of germ cell antigens to the immune cells and antibodies) (Yule et al., 1988; Pelletier, 2001)
- Presence of regulatory T-cells in the testicular interstitium (Mukasa et al., 1995; 1998)
- Secretion of multiple immunosuppressor factors by macrophages, Sertoli and Leydig cells (De Cesaris et al., 1992; Tung et al., 2000; Hedger, 2002)

ANIMAL MODELS AND PATHOGENIC MECHANISMS

Studies on experimental autoimmune orchitis (EAO) have helped elucidate disease mechanisms as well as the systemic regulation that normally prevents disease.

Animal Models

EAO can be elicited by three approaches: 1) immunization with testis antigens with or without adjuvants; 2) passive transfer of testis-sensitized lymphocytes; and 3) manipulation of the normal immune system, such as by thymectomy at day 3 after birth (d3TX) or the transfer of defined T-cell populations from normal inbred mice to syngeneic athymic nu/nu mice recipients. In addition, autoimmune orchitis occurs spontaneously in the dark mink, beagle dog, and aging rat.

EAO is elicited by immunization with testis antigen in complete Freund adjuvant (CFA) in guinea pigs (Voisin et al., 1951) or with testis antigen in CFA and *Bordetella pertussis* toxin in mice (Kohno et al., 1983) or rats (Doncel et al., 1989), or by repeated subcutaneous injections of viable testicular cells, without adjuvants in mice (Sakamoto et al., 1985; Itoh et al., 1991a). Tung et al. (1994) have given a detailed description of the protocols for testicular autoimmune disease.

Pathogenic autoimmune responses to antigens in the male gonad are directed to haploid germ cells in the testis,

including spermatozoa. Although numerous EAO-inducing testis proteins have been isolated, their identity and function are only partially known [reviewed by Tung et al. (2000)].

d3TX or other manipulations that deplete regulatory T cells (Tregs; $CD4^+CD25^+$) also induce EAO, and the fact the disease is suppressed by normal T cells indicates that, although potentially orchitogenic $CD4^+$ T cells exist in normal mice, their action is prevented by natural Tregs. The activation of these cells by endogenous antigens or their molecular mimic, or defective immunoregulation can elicit autoimmune response and tissue injury (Tung et al., 2000).

Pathogenetic Mechanisms

Cellular Mechanisms

EAO is characterized by an interstitial lymphomononuclear cell infiltrate and damage of the seminiferous tubules. The histopathologic distribution of the initiation of inflammation differs between active versus passive EAO: in subcapsular seminiferous tubules far from the rete testis and in straight tubules adjacent to the rete testis (a region more permeable to circulating IgG), respectively (Figure 60.1). Passive EAO experiments in which testis or sperm antigen-specific T-cells that have been activated *in vitro* transfer

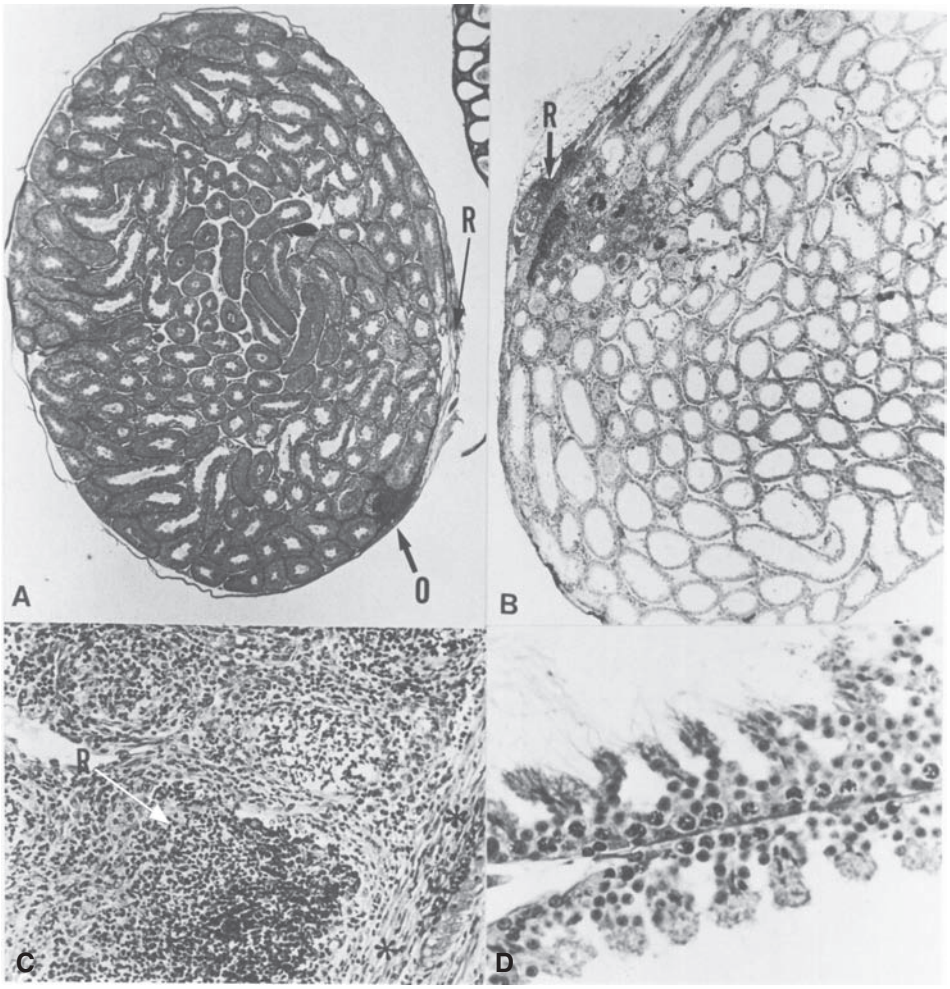


FIGURE 60.1 Predominant histopathologic lesions from mice with *A*, actively induced experimental autoimmune orchitis (active EAO) and *B–D*, from recipients of testis-sensitized lymphocytes (passive EAO). *A*, A common histopathologic finding in active EAO is found under the testicular capsule (arrow), away from the rete testis (R). O, orchitis. Original magnification ×50. *B*, In passive EAO, inflammation in the regions of the straight tubules, adjacent to the rete testis (R), is common, and this leads to severe dilation of seminiferous tubules. Original magnification ×50. *C*, In passive EAO, inflammation in the rete testis (R) is severe and obstructs the lumen. Original magnification ×200. The asterisk shows the capsule adjacent to the rete testis. *D*, In passive EAO, dilated seminiferous tubules have attenuated but intact germinal epithelium, without evidence of orchitis. Original magnification ×400.

Reproduced from Tung et al. (1987), with permission.

severe orchitis and vasitis to syngeneic euthymic mice (Tung et al., 2000) indicate that pathogenic autoreactive T cells against highly specific testis peptides are responsible for the disease. All orchitogenic T-cell clones express CD4, which recognizes peptides in association with MHC class II molecules present in testicular macrophages and dendritic cells, mainly around straight tubules (Tung et al., 1987). Then, orchitis spreads to peripheral seminiferous tubules. T cells produce interleukin (IL)-2 and interferon (IFN)-γ, but not IL-4, which is typical of type 1 helper (Th1) $CD4^+$ T cells. The germ cells undergo apoptosis, and the CD95-CD178 (Fas–Fas L) and tumor necrosis factor (TNF)-α–TNF receptor (TNFR)1 systems are involved in this process (Suescun et al., 2003; Theas et al., 2003). Moreover, the *in vivo* injection of antibodies to TNF-α markedly reduced the severity of EAO in mice transferred with pathogenic T-cell clones (Yule and Tung, 1993).

Humoral Mechanisms

Although autoantibodies to sperm antigens or immune complexes can be detected in EAO, antibody response alone is not sufficient to cause tissue injury. However, the autoantibody binding to a tissue antigen can cause a redistribution of T-cell-mediated inflammation.

GENETICS

Genetic analysis of inbred mouse strains indicates that susceptibility or resistance to autoimmune orchitis is polygenic and strongly influenced by both H2-linked and non-H2-linked genes (Person et al., 1992). The histamine H1 receptor (Hrh1) was definitively identified as the first "shared" non-MHC-linked gene controlling susceptibility to autoimmune orchitis and encephalomyelitis (Ma et al., 2002). These results provided a mechanistic basis for the genetic role of *Bordetella pertussis*-induced histamine sensitization (Bphs)/Hrh1 in eliciting pathogenic T cells during the induction phase of autoimmune disease. Nine chromosomal regions have been linked to EAO and many of these loci are found in regions that govern susceptibility to other autoimmune disease, such as experimental autoimmune encephalitis and type 1 diabetes of nonobese diabetic mice. Microsatellite analysis has mapped distinct chromosomal regions that encode susceptibility to orchitis, epididymitis, and vasitis in EAO, demonstrating the independent regulation of susceptibility to these pathologies (Roper et al., 1998).

Autoimmune orchitis is a component of the autoimmune polyglandular syndromes (APS) that exist as two major clinical entities, APS I and APS II, which differ in age of presentation, characteristic patterns of disease combinations, and modes of inheritance (see Chapter 38). Gonadal failure is more frequently associated with APS I than with APS II.

Recently, two novel immune mechanisms have been identified in patients with APS. APS I patients have a point mutation in the *AIRE* gene (21q23.3), which encodes a transcription factor of unknown function (The Finnish–German APECED Consortium, 1997; Pitkanen and Peterson, 2003). Mice with the knock-out *AIRE* gene spontaneously develop APS (Anderson et al., 2002). Because the *AIRE* protein, expressed in a thymic medullary epithelial cell, influences ectopic expression of peripheral tissue antigens in this thymic cell lineage, a dysfunctional *AIRE* can result in defective central T-cell tolerance (Liston et al., 2003). A second group of APS II patients has an intact *AIRE* gene, but a defective $CD4^+CD25^+$ Treg function (Kriegel et al., 2004).

TREATMENT AND OUTCOME

In patients with high levels of ASAs, corticosteroid treatment has been used to reduce antibody titers, but a direct correlation between ASA titers and fertility has not been observed (Marshburn and Kutteh, 1994).

In recent years new drugs have been used to prevent testicular autoimmunity in mice. Ablake et al. (2002) demonstrated that deoxyspergualin is capable of suppressing alloimmune and autoimmune reactions in experimental models. The authors suggested that the drug may interfere with the function of $CD4^+$ Th1 cells by altering autoantigen presentation and/or by inhibiting T-cell production of IFN-γ. Itoh et al. (1998) demonstrated that a single injection of anti-IFN-γ monoclonal antibody may successfully downregulate testicular autoimmunity in mice with EAO.

More recently, Ito et al. (2004) suggested agents such as α-melanocyte-stimulating hormone (MSH), insulin growth factor (IGF)-1, TGF-β1 or FK506 as promising candidates for immunoprivilege restoration and for suppressing MHC I expression in different autoimmune entities, like mumps autoimmune orchitis.

In assisted reproduction, intracytoplasmic sperm injection (ICSI) is the primary choice of treatment for immunologic infertility, especially when ASAs are located in/on the sperm head [reviewed by Lombardo et al. (2001)].

CONCLUDING REMARKS

Human testicular autoimmune diseases remain incompletely defined and should be a major focus of future research. Nevertheless, research based on models of autoimmune orchitis has contributed significantly to our understanding of the interactions between immune cells and somatic and germ cells of the testis, as well as the overall

concepts of autoimmune disease pathogenesis. The $CD4^+CD25^+$ Tregs play a central role in the control of immune reactivity. New targets for specific therapies may be developed by identifying the mechanisms by which regulatory cells function and the intracellular signaling cascades activated during autoimmune pathology. In addition, the recent finding of a single gene (*AIRE*) defect causing a systemic human autoimmune polyglandular syndrome provides an exciting tool for exploring molecular therapy for these autoimmune diseases.

References

Ablake, M., Itoh, M., Kaneko, T., IIimura, A., Nakamura, Y., Meroni, P.L., and Nicoletti, F. (2002). Short-term prophylaxis with deoxyspergualin prevents testicular autoimmunity in mice. *Eur. J. Pharmacol. 450*, 209–212.

Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J, von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science 298*, 1395–1401.

Bohring, C. and Krause, W. (2003). Immune infertility: towards a better understanding of sperm (auto)-immunity. The value of proteomic analysis. *Hum. Reprod. 18*, 915–924.

Chan, P.T. and Schlegel, P.N. (2002). Inflammatory conditions of the male excurrent ductal system. Part I. *J. Androl. 23*, 453–460.

De Cesaris, P., Filippini A., Cervelli, C., Riccioli, A., Muci S., Starace, G., Stefanini, M., and Ziparo, E. (1992). Immunosuppressive molecules produced by Sertoli cells cultured *in vitro*: Biological effects on lymphocytes. *Biochem. Biophys. Res. Commun. 186*, 1639–1646.

Dejucq, N. and Jégou, B. (2001). Viruses in the mammalian male genital tract and their effects on the reproductive system. *Microb. Mol. Biol. Rev. 65*, 208–231.

Delavierre, D. (2003). Orchi-épididymites. *Annales d'Urologie 37*, 322–338.

Denduchis, B. and Lustig, L. (1981). Experimental orchitis induced in rats by an antiserum to a noncollagen fraction of seminiferous tubule basement membrane. In G. Frajese, E. S. F. Hafez, C. Conti, and A. Fabbrini (eds), *Oligozoospermia: Recent Progress in Andrology* (pp. 207–218). New York: Raven Press.

Doncel, G.F., Di Paola, J.A., and Lustig, L. (1989). Sequential study of the histopathology and cellular and humoral immune response during the development of an autoimmune orchitis in Wistar rats. *Am. J. Reprod. Immunol. 20*, 44–51.

Fountain, S., Holland, M.K., Hinds, L.A., Janssens, P.A., and Kerr, P.J. (1997). Interstitial orchitis with impaired steroidogenesis and spermatogenesis in the testes of rabbits infected with an attenuated strain of myxoma virus. *J. Reprod. Fertil. 110*, 161–169.

Frayne, J. and Hall, L. (1999). The potential use of sperm antigens as targets for immunocontraception: past, present and future. *J. Reprod. Immunol. 43*, 1–33.

Freund, J., Lipton, M.M., and Thompson, G.E. (1953). Aspermatogenesis in guinea pig induced by testicular tissue and adjuvant. *J. Exp. Med. 97*, 711–725.

Hedger, M.P. (2002). Macrophages and the immune responsiveness of the testis. *J. Reprod. Immunol. 57*, 19–34.

Ito, T., Ito, N., Betterman, A., Tokura, Y., Takigawa, M., and Paus, R. (2004). Collapse and restoration of MHC class I dependent immune privilege. *Am. J. Pathol. 164*, 623–634.

Itoh, M., Hiramine, C., and Hojo, K. (1991a). A new murine model of autoimmune orchitis induced by immunization with viable syngeneic germ cells alone. I. Immunological and histological studies. *Clin. Exp. Immunol. 83*, 137–142.

Itoh, M., Mukasa, A., Tokunaga, Y., Hiramine, C., and Hojo, K. (1991b). New experimental model for adoptive transfer of murine autoimmune orchitis. *Andrologia 23*, 415–420.

Itoh, M., Yano, A., Xie, Q., Iwahashi, K., Takeuchi Y., Meroni, P.L., and Nicoletti, P. (1998). Essential pathogenic role for endogenous interferon-gamma (IFN-γ) during disease onset phase of murine experimental autoimmune orchitis. I. *In vivo* studies. *Clin. Exp. Immunol. 111*, 513–520.

Kierszenbaum, A.L. (1995). Testicular disease and the biology of HIV infection in the male. In P. L. Kimmel, J. S. Berns, and J. H. Stein (eds), *Renal and Urological Aspects of HIV Infection* (pp. 307–328). New York: Churchill Livingstone.

Kohno, S., Munoz, J.A., Williams, T.M., Teuscher, C., Bernard, C.C.A., and Tung, K. S.K. (1983). Immunopathology of murine experimental allergic orchitis. *J. Immunol. 130*, 2675–2682.

Kriegel, M.A, Lohmann, T., Gabler, C., Blank, N., Kalden, J.R., and Lorenz, H.M. (2004). Defective suppressor function of human CD4+CD25+ regulatory T cells in autoimmune polyglandular syndrome Type II. *J. Exp. Med. 199*, 1285–1291.

Le Goffic, R., Mouchel, T., Aubry, F., Patard, J.J., Ruffault, A., Jégou, B., and Samson, M. (2002). Production of the chemokines monocyte chemotactic protein-1, regulated on activation normal T cell expressed and secreted protein, growth-related oncogene, and interferon-γ-inducible protein-10 is induced by the Sendai virus in human and rat testicular cells. *Endocrinology 143*, 1434–1440.

Lehmann, D., Temminch, D., Da Rugna, D., Leibundgut, B., Sulmoni, A., and Muller, H.J. (1987). Role of immunological factors in male infertility: Immunohistochemical and serological evidence. *Lab. Invest. 57*, 21–28.

Liston, A., Lesage, S., Wilson, J., Peltonen, L., and Goodnow, C.C. (2003). Aire regulates negative selection of organ-specific T cells. *Nat. Immunol. 4*, 350–354.

Lombardo, F., Gandini, L., Dondero, F., and Lenzi, A. (2001). Immunology and immunopathology of the male genital tract. Antisperm immunity in natural and assisted reproduction. *Hum. Reprod. Update 7*, 450–456

Lustig, L., Denduchis, B., Ponzio, R., Lauzon, M., and Pelletier, R.M. (2000). Passive immunization with anti-laminin immunoglobulin G modifies the integrity of the seminiferous epithelium and induces arrest of spermatogenesis in the guinea pig. *Biol. Reprod. 62*, 1505–1514.

Ma R.Z., Gao J., Meeker N.D., Fillmore P.D., Tung K.S.K., Watanabe T., Zachary J.F., Offner H., Blankenhorn E.P., and Teuscher C. (2002). Identification of *Bphs,* an autoimmune disease locus, as histamine receptor H1. *Science 297*, 620–623.

Marshburn, P.B. and Kutteh, W.H. (1994). The role of antisperm antibody in infertility. *Fertil. Steril. 5*, 799–811.

Melaine, N., Ruffault, A., Dejucq-Rainsford, N., and Jégou, B. (2003). Experimental inoculation of the adult rat testis with Sendai virus: effect on testicular morphology and leukocyte population. *Hum. Reprod. 18*, 1574–1579.

Mouchel, T., Le Goffic, R., Patard, J.J., and Samson, M. (2002). Le virus ourlien et l'orchite: vers une approche physiopathologique. *Progr. Urol. 12*, 124–128.

Mukasa, A., Hiromatsu, K., Matsuzaki, G., O'Brien, R., Born, W., and Nomoto, K. (1995). Bacterial infection of the testis leading to autoaggressive immunity triggers apparently opposed responses of αβ and γδ T cells. *J. Immunol. 155*, 2047–2056.

Mukasa, A., Yoshida, H., Kobayashi, N., Matsuzaki, G., and Nomoto, K. (1998). γδ T cells in infection-induced and autoimmune-induced testicular inflammation. *Immunology 95*, 395–401.

Nistal, M., Riestra, M.L., and Paniagua, R. (2002). Focal orchitis in undescended testes. *Arch. Pathol. Lab. Med. 126*, 64–69.

Pelletier, R.M. (2001). The tight junctions in the testis, epididymis, and vas deferens. In M. Cereijido and J. Anderson (eds), *Tight Junctions* (pp. 599–628). Boca Raton: CRC Press.

Person, P.L., Snoek, M., Demant, P., Woodward, S.R., and Teuscher, C. (1992). The immunogenetics of susceptibility and resistance to murine experimental allergic orchitis. *Reg. Immunol. 4*, 284–297.

Pitkanen, J. and Peterson, P. (2003). Autoimmune regulator: from loss of function to autoimmunity. *Genes Immun. 4*, 12–21.

Roper, R.J., Doerge, R.W., Call, S.B., Tung, S.K., Hickey, W.F., and Teuscher, C. (1998). Autoimmune orchitis, epididymitis, and vasitis are immunogenetically distinct lesions. *Am. J. Pathol. 152*, 1337–1345.

Sakamoto, H., Himeno, K., Sanui, H., Yoshida, S., and Nomoto, K. (1985). Experimental allergic orchitis in mice. I. A new model induced by immunization without adjuvants. *Clin. Immunol. Immunopathol. 37*, 360–368.

Salomon, F., Saremaslani, P.P., Jakob, M., and Hedinger, C.F. (1982). Immune complex orchitis in infertile men. *Lab. Invest. 47*, 555–567.

Saravanamu, V., Foster, R.A., Ladds, P.W., and Gorrell, M.D. (1991). T and B lymphocyte subsets in spermatic granulomas in five rams. *Vet. Pathol. 28*, 482–491.

Suescun, M.O., Rival, C., Theas, M.S., Calandra, R.S., and Lustig, L. (2003). Involvement of tumor necrosis factor-alpha in the pathogenesis of autoimmune orchitis in rats. *Biol. Reprod. 68*, 2114–2121.

Suominen, J. and Söderström, K.O. (1982). Lymphocyte infiltration in human testicular biopsies. *Int. J. Androl. 5*, 461–466.

Takahashi, T., Kuniyasu, Y., Toda, M., Sagakuchi, N., Itoh, M., Iwata, M., Shimizu, J., and Sakaguchi, S. (1998). Immunologic self-tolerance maintained by CD25+ CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol. 10*, 1969–1980.

Theas, M.S., Rival, C., and Lustig, L. (2003). Germ cell apoptosis in autoimmune orchitis: involvement of the Fas-Fas L system. *Am. J. Reprod. Immunol. 50*, 166–176.

The Finnish-German APECED Consortium (1997). An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PhD-type zinc-finger domains. *Nat. Genet. 17*, 393–398.

Thornton, A.M. and Shevach, E.M. (1998). $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J. Exp. Med. 188*, 287–296.

Tung, K.S.K., Ellis, L., Teuscher, C., Meng, A., Blaustein, J.C., Kohno, S., and Howell, R. (1981). The black mink (*Mustela vison*): A natural model of immunologic male infertility. *J. Exp. Med. 154*, 1016–1032.

Tung, K.S.K., Yule, T.D., Mahi-Brown, C.A., and Listrom, M.B. (1987). Distribution of histopathology and Ia positive cells in actively-induced and adoptively-transferred experimental autoimmune orchitis. *J. Immunol. 138*, 752–759.

Tung, K.S.K., Taguchi, O., and Teuscher, C. (1994). Testicular and ovarian autoimmune diseases. In I. R. Cohen and A. Miller (eds), *Autoimmune Disease Models* (pp. 267–290). San Diego: Academic Press.

Tung, K.S.K., Fusi, F., and Teuscher, C. (2000). Autoimmune disease of the spermatozoa, ovary and testis. In A. N. Theofilopoulos and C. A. Bona (eds), *The Molecular Pathology of Autoimmune Diseases* (pp. 1031–1045). New York: Taylor & Francis.

Turek, P.J. and Lipshultz, L.I. (1994). Immunologic infertility. *Urol. Clin. North Am. 21*, 447–468.

Voisin, G.A., Delauney, A., and Barber, M. (1951). Sur les lesions tésticulaires provoqueés chez les cobayes par iso-et autosensibilisation. *Ann. Inst. Pasteur (Paris) 81*, 48–63.

Yule, T.D., Montoya G.D., Russell, L.D., Williams, T.M., and Tung, K.S.K. (1988). Autoantigenic germ cells exist outside the blood testis barrier. *J. Immunol. 141*, 1161–1167.

Yule, T.D. and Tung, K.S.K. (1993). Experimental autoimmune orchitis induced by testis and sperm antigen-specific T cell clones: An important pathogenic cytokine is tumor necrosis factor. *Endocrinology 113*, 1098–1107.

CHAPTER

61

Oophoritis

JUDITH L. LUBORSKY*
Departments of Pharmacology and Obstetrics and Gynecology, Rush University Medical Center, Chicago, Illinois

KENNETH S. K. TUNG†
†*Department of Pathology, University of Virginia, Charlottesville, Virginia, USA*

HISTORIC FEATURES

The concept of autoimmune ovarian disease (AOD), or oophoritis, was suggested over 35 years ago. Irvine observed that Addison's disease was associated with a higher than expected occurrence of premature ovarian failure (POF, or premature menopause before age 40), and steroid cell autoantibodies to adrenal and ovary (Irvine et al., 1968). Independently, antibodies to oocyte cytoplasm were detected in women with infertility (Vallotton and Forbes, 1966). Until recently, studies of the autoimmune basis of POF were more common than studies of AOD in infertility. Interestingly, an animal model of autoimmune ovarian failure, based on mice thymectomized on neonatal day 3, was reported at about the same time (Nishizuka and Sakakura, 1969).

AOD has been identified primarily by indirect and circumstantial evidence according to the criteria summarized by Rose and Bona (1993). Circumstantial evidence for AOD includes lymphocytic infiltration of ovarian follicles in women with POF, frequent association of POF and infertility with other autoimmune diseases, and a tendency of POF to be familial. Indirect evidence for an autoimmune disease of the ovary includes the association of ovarian autoantibodies with POF and some types of infertility [reviewed in Luborsky (2002b)].

CLINICAL, PATHOLOGIC AND EPIDEMIOLOGIC FACTORS

Clinical Features

AOD usually presents as POF or infertility (Figure 61.1; Table 61.1), although these phenotypes represent heterogeneous etiologies.

Etiologies of POF include induction by radiation or chemotherapy (Chiarelli et al., 1999), environmental factors (Vermeulen, 1993; Cooper et al., 1999; Silbergeld and Flaws, 1999), genetic defects and chromosomal deletions (Davis, 1996; Hoek et al., 1997), and unexplained POF. POF is empirically defined as ovarian failure before the age of 40 (Cramer and Xu, 1996; WHO, 1996; Hoek et al., 1997). Like natural menopause around the age of 50, premature menopause is identified by cessation of menstrual cycles for 1 year, accompanied by elevated follicle-stimulating hormone (FSH) and reduced estradiol levels in blood (WHO, 1996). AOD is associated with POF of unknown or iatrogenic etiology (Luborsky, 2002b).

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

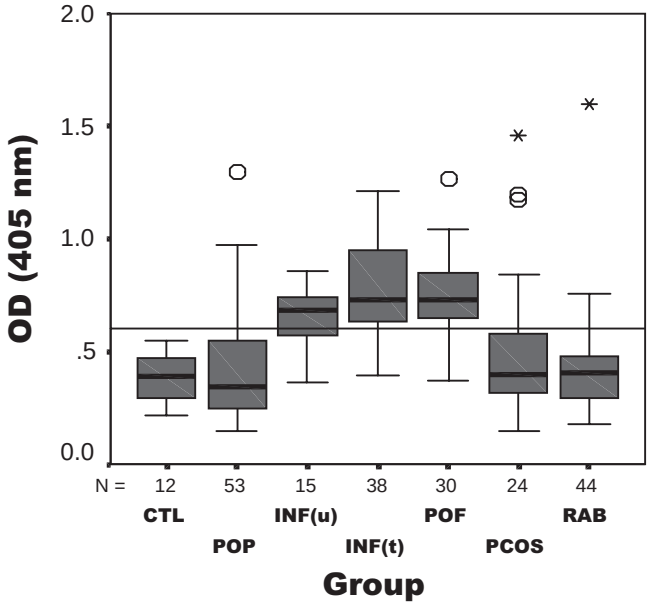


FIGURE 61.1 Association of ovarian autoantibodies with premature menopause and unexplained infertility. The optical density (OD) values obtained by ovarian antibody assay and the corresponding number of positive values are shown. Immunoassay results for ovarian antibodies in different groups of women are shown as a box and whiskers plot of OD values. The horizontal line represents the control mean plus two standard deviations. Values above the line are positive. CTL, control women with normal menstrual cycles or natural menopause and no diagnosed autoimmune disease; INF(u), unexplained infertility and no prior hormone stimulation; INF(t), unexplained infertility and at least two cycles of in-vitro fertilization; PCOS, polycystic ovary syndrome; POF, premature ovarian failure; POP, women aged 18–45 years from a blood bank (anonymous); RAB, recurrent fetal loss.

TABLE 61.1 Association of ovarian antibodies with premature menopause and unexplained infertility. Prevalence of ovarian antibodies in same groups as in Figure 61.1, and the corresponding significance compared with controls

Group	Prevalence of ovarian antibodies (%) (*P*)
Control (CTL)	Reference
Blood bank (POP)	17 (NS)
Unexplained infertility [INF(u)]	33 (<0.001)
Unexplained infertility [INF(t)]	61 (<0.001)
Premature ovarian failure (POF)	53 (<0.001)
Polycystic ovary syndrome (POS)	25 (NS)
Recurrent fetal loss (RAB)	18 (NS)

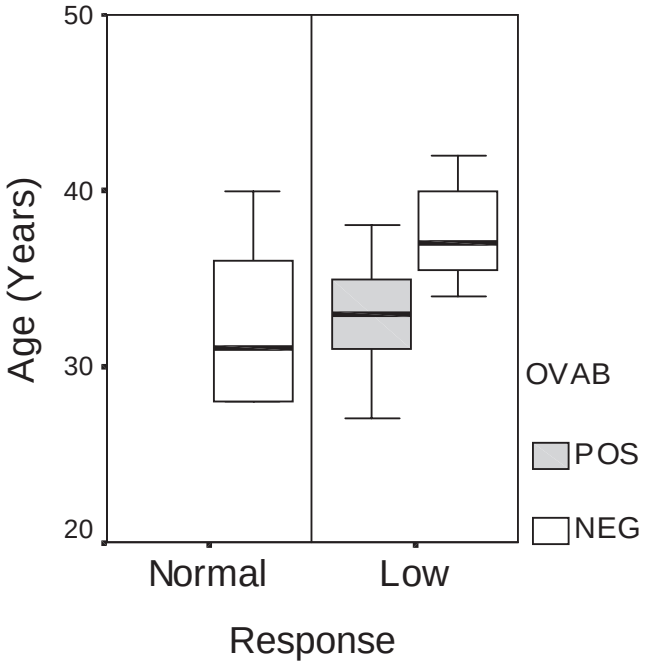


FIGURE 61.2 Ovarian autoantibodies discriminate between low estrogen responses associated with autoimmunity and those associated with aging. Age and estradiol responses are compared in patients with infertility, undergoing hormone stimulation during in-vitro fertilization treatment. Ovarian antibodies (OVAB) were associated with lower than expected estradiol responses to follicle-stimulating hormone (FSH). Data are displayed as a box and whiskers plot of the response to FSH, defined as a ratio of the peak estradiol/dose of FSH. The median, range (whiskers), 25th–75th percentile (box), and outliers (circles) are shown. Normal responders were 32.5 ± 4.6 years old, low responders without ovarian antibodies (NEG) were 37.1 ± 3.8 years old ($P = 0.013$ compared to normal responders), and low responders with ovarian antibodies (POS) were 33.4 ± 4.2 years old ($P = 0.6$ compared to normal responders). This observation suggests that although some poor responses are associated with ovarian failure associated with early stages of natural menopause, other poor responses are associated with an autoimmune process that occurs at a younger age.

Reproduced from Luborsky et al. (2002b), with permission.

Infertility is the inability of a couple to conceive for at least 1 year during unprotected intercourse (WHO, 1992). Of the diagnostic categories of infertility, ovarian autoimmunity is primarily associated with unexplained infertility (Luborsky, 2002b). Unexplained infertility is identified by exclusion, after normal results for semen analysis, postcoital test, ovulation (luteal-phase progesterone), and tubal patency are obtained in a standard clinical evaluation (Forti and Krausz, 1998).

Women with infertility and ovarian autoantibodies tend to have lower than expected estradiol responses to gonadotropin hormone stimulation (i.e., "poor responders") (Meyer et al., 1990) and lower pregnancy rates following infertility treatment (Luborsky and Pong, 2000). Poor responders with ovarian antibodies are younger than poor responders without ovarian antibodies (Luborsky et al., 2002b) (Figure 61.2). This suggests that although some poor responses are associated with early stages of the menopause progression and reduced number of functional follicles, others are associated with an autoimmune process.

In addition to the risk of other autoimmune diseases (Hoek et al., 1997), such as polyglandular syndrome (Baker, 1997), POF is associated with a risk of osteoporosis (Anasti et al., 1998; Luborsky et al., 2002b) and cancer-related mortality (Cooper and Sandler, 1998) that is higher than for menopause. However, there is no information on the relative risk of osteoporosis or other health factors specifically in women with autoimmune POF.

Pathology

Evidence of ovarian pathology is derived from ovarian biopsy. The ovary contains a pool of follicles, which are the functional units of the ovary. A group of primordial follicles are recruited each cycle, and under the influence of hormones, grow and differentiate. In women with POF, histologic features of ovarian inflammation range from extreme cellular destruction with no evidence of primordial follicles, to follicles with lymphocyte invasion, often seen as "cysts" in existing follicles, to normal follicles with no obvious inflammation (Russell et al., 1982; Fox, 1992). Lymphocytic infiltrates tend to be associated with growing and maturing follicles rather than primordial follicles in humans (Rebar and Connolly, 1990). Similar variations in ovarian structure have been confirmed by ultrasound of women with POF (Conway et al., 1996; Kalantaridou and Nelson, 2000). Because there is no rationale for biopsies in women with infertility, there is little information on the pathology of ovarian autoimmunity associated with infertility.

Epidemiology

There are no epidemiologic studies of AOD. The prevalence of POF in population studies is about 1% based on elevated FSH (Coulam et al., 1986) or cessation of menstrual cycles before the age of 40 (Cramer and Xu, 1996). In a multiethnic population study, POF occurred at a general prevalence of 1.1% and varied by ethnic group, with a lower prevalence among Japanese women (Luborsky et al., 2002a).

Using autoantibodies as an indicator, about 40–70% of women with POF have AOD, or about one to two million women in the US (Luborsky, 2002a). This suggests that AOD is not as rare as Addison's disease, myasthenia gravis or systemic lupus erythematosus (SLE), and may rank with moderately prevalent autoimmune diseases such as type 1 diabetes (insulin-independent diabetes mellitus), pernicious anemia, and multiple sclerosis (Jacobson et al., 1997).

It is difficult to determine the prevalence of AOD in infertility since study group definitions may differ, and different antibody tests are often used. In general, about 10% of women experience infertility and about 20% are classified as unexplained (WHO, 1992; Collins, 1995). Ovarian antibodies are detected in 30–60% of cases of unexplained infertility (Luborsky et al., 1999; 2000a).

AUTOIMMUNE FEATURES

Autoantibodies

Several types of ovary-specific autoantibodies have been reported, including those to microsomal antigens, granulosa cells, thecal cells, zona pellucida (ZP), and oocytes [reviewed in Luborsky (2002b)]. Ovarian antibodies are detected in serum of both POF and infertility patients by immunohistochemistry (Cameron et al., 1988; Muechler et al., 1991; Belvisi et al., 1993; Betterle et al., 1993; Horejsi et al., 2000), enzyme immunoassay (Sontag et al., 1982; Luborsky et al., 1990; Meyer et al., 1990; Barbarino-Monnier et al., 1991; Wheatcroft et al., 1994; 1997a; Fenichel et al., 1997; Wheatcroft and Weetman, 1997), immunoprecipitation (Coulam and Ryan, 1979) and Western blot (Wheatcroft et al., 1997a). The prevalence of ovarian autoantibodies reported in different studies varies depending on the test and study group.

ZP antibodies are detected in serum of women with POF and infertility by immunohistochemistry (Shivers and Dunbar, 1977; Buckshee and Mhaskar, 1985; Damewood et al., 1986; Mikulikova et al., 1989; Mantzavinos et al., 1993; Smith and Hosid, 1994), enzyme immunoassay (EIA) (Singh and Mhaskar, 1985; Hovav et al., 1994; Ulcova-Gallova and Mardesic, 1996; Ivanova et al., 1999), and passive hemagglutination (Kamada et al., 1992; Mantzavinos et al., 1993; Ulcova-Gallova and Mardesic, 1996). Antibodies to oocyte cytoplasm detected by immunohistochemistry and EIA were also reported (Damewood et al., 1986; Luborsky et al., 1990; Meyer et al., 1990; Horejsi et al., 2000). The typical prevalence of ZP or oocyte antibodies ranges from 30% to 60%, depending on the test format and study group definition.

Autoantibodies to ovarian antigens, ZP, and oocytes also are detected in follicular fluid obtained at oocyte retrieval during controlled ovarian hyperstimulation of infertility patients undergoing in-vitro fertilization (IVF) (Mantzavinos et al., 1993; Papale et al., 1994; Horejsi et al., 2000; Luborsky et al., 2000a). Interestingly, the content of ZP antibodies among follicles is heterogeneous, with some follicles showing no detectable antibody (Papale et al., 1994).

Cellular Immunity

In women with POF, profiles of total T-cell subtypes also appear to be shifted in favor of CD4 subtypes, along with an overall increase in total $CD8^+$ and $CD4^+$ lymphocytes (Miyake et al., 1987; Huang et al., 1996; Lin et al., 1998). The ratio of $CD4^+/CD8^+$ cells is increased in both infertility

and POF compared to normal control women (van Kasteren et al., 2000). Other markers of immune activation are also altered, e.g., B cells (Hoek et al., 1993), interleukin-2 receptor (IL2-R) expression (Nelson et al., 1991), major histocompatibility complex (MHC) class II expression on granulosa cells (Hill et al., 1990; Giglio et al., 1994), and complement activity (Wheatcroft et al., 1997a) are increased, and tumor necrosis factor (TNF)-α (Naz et al., 1995), natural killer (NK) cells (Hoek et al., 1993), and antigen presentation and surveillance are decreased (Arif et al., 1999; Yan et al., 2000). Interestingly, some immune parameters are reversed by estrogen therapy (Ho et al., 1988; 1993; Hoek et al., 1993).

Faustman and colleagues showed that diabetes, SLE, Sjögren syndrome, rheumatoid arthritis, myasthenia gravis, as well as POF, are associated with a reduced density of conformationally correct HLA class I proteins (Faustman et al., 1991; Fu et al., 1993; Li et al., 1995; Yan et al., 2000). This suggests that autoimmunity is associated with a reduced ability to present peptide fragments of antigens and to maintain peripheral tolerance. About 70% of POF cases are associated with abnormal antigen processing (Yan et al., 2000), which is similar to our estimate that 69% of POF is autoimmune, based on autoantibody detection (Luborsky et al., 1990).

GENETIC FEATURES

In general, the genetics and risk factors for all autoimmune disease expression remain to be fully elucidated. Autoimmune diseases tend to run in families and thus there is a genetic component. However, autoimmunity is not expressed clinically in all twin pairs (Faustman et al., 1991; Li et al., 1995; Fu et al., 1998), suggesting that secondary factors have a role in inducing autoimmune disease in susceptible individuals.

There is little information on the genetic component of AOD. Genetic studies of POF have not differentiated autoimmune from nonautoimmune POF (Bione et al., 1998; Bondy et al., 1998; Christin-Maitre et al., 1998; Laml et al., 2002). In general, POF has a genetic component since it is familial as well as sporadic (Cramer et al., 1995). A preliminary report of an association between POF, antibodies to 3-β hydroxy steroid dehydrogenase and a distinctive HLA-DQ molecule suggested that presentation of antigenic peptides to T lymphocytes by HLA-DQ molecules with Asp57-β chains is important in the pathogenesis of autoimmune POF (Arif et al., 1999). The autoimmune regulator gene (AIRE) in polyglandular syndrome type I has been identified but it is unclear if this is related to ovarian failure (Ruan et al., 1999).

POF is also associated with GALT (galactose-1-phosphate uridyl transferase) polymorphism (Cramer et al., 1989; 1994; Cooper et al., 1994), fragile X syndrome (Partington et al., 1996; Kenneson et al., 1997; Uzielli et al., 1999; Marozzi et al., 2000) and inhibin gene defects (Laml et al., 2002). Inhibin is a product of ovarian granulosa cells and regulates (inhibits) pituitary FSH. A Finnish study reported that defects in the FSH receptor were associated with POF (Aittomaki et al., 1995; 1996), although subsequent studies in other countries have not confirmed this finding (Whitney et al., 1995; da Fonte Kohek et al., 1998; Layman et al., 1998; Conway et al., 1999; Layman, 1999; Takakura et al., 2001), suggesting a founder effect in Finland. The relationship of these genetic defects to AOD was not investigated.

ENVIRONMENTAL INFLUENCES

There is little information on environmental influences that might trigger AOD, but based on twin studies it is likely that there are environmental triggers. Smoking and some toxins reduce the age at menopause (Silbergeld and Flaws, 1999) and contribute to infertility (Hruska et al., 2000), but a relationship to autoimmunity has not been shown. Galactose consumption and altered galactose metabolism have been associated with POF (Santoro, 2001).

ANIMAL MODELS

The pathogenesis of AOD has been investigated in several murine models. Three of these are particularly informative: 1) AOD induced by immunization with a murine ZP3 peptide (pZP3) that contains both T-cell epitopes and a native B-cell epitope (Rhim et al., 1992) (Figure 61.3); 2) AOD that develops spontaneously in mice thymectomized on day 3 after birth (d3TX) (Nishizuka and Sakakura, 1969); and 3) AOD in neonatal mice triggered by maternal antibody to murine ZP3 (Setiady et al., 2003).

An autoimmune disorder of the ovaries is induced when regulatory $CD25^+$ T cells are depleted by d3TX. In these mice, endogenous ovarian antigens spontaneously stimulate pathogenic T-cell responses within 2–3 weeks, and this leads to ovarian inflammation, followed rapidly by ovarian atrophy and development of oocyte antibodies. An important cytoplasmic oocyte autoantigen was identified by Tong and Nelson (Tong and Nelson, 1999; Tong et al., 2000; 2002) by screening an ovarian cDNA library with antiserum from the thymectomized mouse model. The antigen is an oocyte-specific molecule that functions in the two-cell embryo post-fertilization. Although the mouse protein, identified as MATER (maternal antigen that embryos require) and its human homolog are highly conserved, it is not clear if the MATER antigen is relevant to human ovarian autoimmune disease. One of the *aod* genetic loci (*aod* 5),

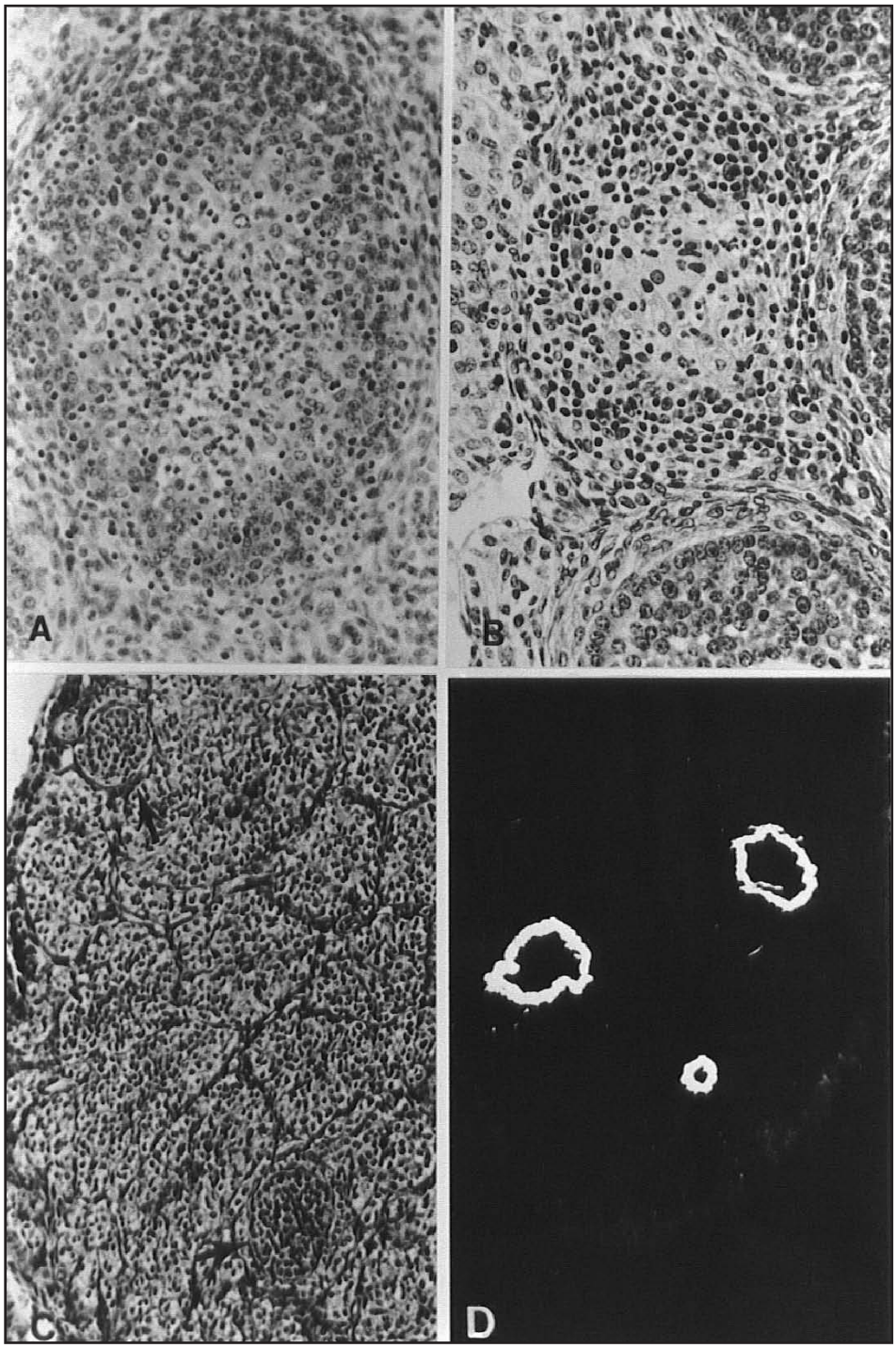


FIGURE 61.3 Histopathology of experimental autoimmune oophoritis induced by immunization with the ZP3 (330–342) peptide in complete Freund adjuvant. *A*, Inflammatory cells have replaced the oocytes in the center of a Graffian follicle; many lymphoid cells can be seen amid granulosa cells. Original magnification, ×100. *B*, A granuloma is found in the interstitial region. Original magnification, ×200. *C*, In severe oophoritis, all oocytes disappear; inflammation is not present, and the ovary is atrophic. Original magnification, ×100. *D*, Ovaries with autoimmune oophoritis show in-vivo binding of IgG antibody to the zona pellucida, as demonstrated by direct immunofluorescence. Original magnification, ×100.

Reproduced from Rhim et al. (1992), with permission.

mentioned below, has been mapped to the region that encodes MATER (Roper et al., 2003).

AOD also can be induced by stimulation by exogenous antigens through the mechanism of T-cell epitope molecular mimicry. Thus, immunization with foreign or unrelated self-peptides with partial sharing of amino acid residues appears critical for T-cell induction (Luo et al., 1993; Garza and Tung, 1995). At least one infectious agent has been implicated in the pathogenesis of AOD. The enteric nematode (the rodent pinworm) operates as a cofactor and, together with a nonimmunogenic stimulus from ovarian pZP3, elicits AOD in neonatal mice. Infected mice are imprinted with long-term memory for the autoantigen and mount a rapid and intense autoimmune response when challenged at a later date (Agersborg et al., 2001). The $CD4^+$ type 1 helper T cell (Th1) is the major pathogenic T cell, requiring both CD28 and CD40 ligand costimulatory pathways for T- and B-cell activation (Griggs et al., 1996). In addition, there is evidence that $CD4^+$ Th2 cells also elicit AOD that result in eosinophilic oophoritis, with autoreactive T cells producing IL-4 and IL-5, but no interferon (IFN)-γ (Agersborg et al., 2001). In human ovarian autoimmune disease, mononuclear cells are the dominant infiltrating cells, although some patients had dominant eosinophilic inflammation (Lewis, 1993).

An unexpected mechanism of autoantibody induction and an unexpected action of autoantibody in tissue inflammation were uncovered during the study of AOD induced by pZP3 in adult mice. Ovarian antibodies are stimulated spontaneously and rapidly by endogenous ovarian antigens in mice in which T cells for ovarian antigen have been activated (Lou et al., 1996; Bagavant et al., 1999). Thus, mice immunized with a pZP3 that lacks native B-cell epitopes develop IgG ZP3 antibodies that recognize distant B-cell epitopes of ZP3, and the antibodies do not cross-react with the pZP3 immunogen. The antibody response is abrogated in mice with ovarian ablation. Thus, autoreactive B cells to ovarian antigens are not tolerized but are primed by endogenous antigens. This discovery, made in parallel with studies on diversification of autoantibody responses to lupus autoantigens, provides the critical evidence that endogenous antigens can drive a diversified antibody response. Secondly, although ZP antibodies do not cause AOD, they influence the distribution of AOD induced by $CD4^+$ T cells. Atretic follicles in the ovarian interstitial space are the prime targets for pathogenic T cells, and T-cell-mediated interstitial oophoritis is compatible with normal ovarian function (Bagavant et al., 1999). In the presence of ZP antibody, the T-cell–mediated inflammation is "retargeted" or shifted to the ovarian follicles, leading to ovarian atrophy (Lou et al., 2000).

A recent model of neonatal AOD was induced by maternal antibody to ZP3 in the progeny. The antibody forms immune complexes in neonatal ovaries, which activate innate cells, including NK cells. Interestingly, immune complexes are also processed by antigen-presenting cells (APCs) and stimulate a *de novo* pathogenic T-cell response that causes severe oophoritis and atrophy (Setiady et al., 2003; 2004). Importantly, the same antibody does not cause ovarian disease in adult mice, and this is due to the presence of competent $CD4^+CD25^+$ regulatory T cells (Tregs) in the adults.

Finally, the pathogenesis of AOD has been investigated by mapping disease-associated genetic loci. Studies on genetic loci that influence AOD induced by d3TX have uncovered six non-H2 chromosomal loci associated with ovarian inflammation, autoantibody induction, and ovarian atrophy (Wardell et al., 1995; Teuscher et al., 1996; Roper et al., 2003).

PATHOGENIC MECHANISMS

Pathogenic mechanisms associated with AOD in humans are not well studied. There is some evidence for cytotoxic mechanisms. Cytotoxic T-cell subsets (CD8 and NK cells) are elevated in the peripheral circulation in POF (Giglio et al., 1994) and complement breakdown product C3a is elevated in women with unexplained infertility and elevated FSH (Wheatcroft et al., 1997b).

Direct actions of autoantibodies on ovarian function were sought but not found. Antibodies from women with POF did not alter gonadotropin action in vitro (van Weissenbruch et al., 1991; Anasti et al., 1995; Lambert et al., 1996), although FSH-induced granulosa cell division and growth were blocked by immunoglobulin from POF sera in one study (van Weissenbruch et al., 1991). Antibodies to partially purified luteinizing hormone (LH) receptor in POF and infertility were reported (Austin et al., 1979; Moncayo et al., 1989). However, stimulating or inhibiting antibodies to gonadotropin receptors were not detected in serum from POF patients tested in functional assays with primary cultures (Wheatcroft et al., 1994; Lambert et al., 1996) or cell lines transfected with recombinant receptor (Anasti et al., 1995).

The majority of reports suggest antibodies to ZP are associated with altered fertilization [reviewed in Luborsky (2002b)]. Antibodies from POF sera were reported to block oocyte maturation and alter Ca^{2+} channel flux (Hotsuliak and Ianchii, 1999). There is relatively little information on the identity of specific oocyte or ZP autoantigens.

IMMUNOLOGIC MARKERS IN DIAGNOSIS

Ovarian autoantibodies are the main diagnostic indicator of AOD. Antibodies are detected by immunoassay or immunohistochemistry, and results may vary slightly

depending on the antigen preparation and assay conditions. There are currently few commercial assays or routine services for detecting ovarian antibodies. Current research is directed at identifying the specific target antigens that will lead to routine commercial tests.

Potential specific antigens have been investigated. The enzymes involved in steroid hormone biosynthesis in ovary and adrenal were likely candidates. The major adrenal enzyme involved in cortisol biosynthesis, 21-hydroxylase (CYP21), was identified in isolated Addison's disease and with polyglandular syndrome (Peterson and Krohn, 1994; Song et al., 1994; Arif et al., 1996; 1999; 2001; Chen et al., 1996; Peterson et al., 1997; Seissler et al., 1999; de Carmo Silva et al., 2000). It was suggested that 17-α hydroxylase (CYP17), the comparable enzyme in the ovary responsible for conversion of progesterone to androgens as precursors to estrogen, is a major antigen in POF. However, consistent with the rare occurrence of steroid cell antibodies that cross-react with steroidogenic tissues (adrenal, placenta, Leydig cells, and granulosa cells) in isolated POF, antibodies to recombinant CYP21 and CYP17, and side-chain cleavage (CYP11A1) enzymes were not major antigens in isolated POF (Chen et al., 1996; Tanaka et al., 1997; Reimand et al., 2000). Similarly, we found that about 20% of infertility sera react with CYP17 (Luborsky, 2002a), suggesting it is a possible antigen, but it does not account for the majority of anti-ovarian antibodies.

Gonadotropin receptors were also investigated as a potential antibody target. There is no clear evidence for anti-FSH or anti-LH receptor antibodies. Other receptors involved in regulation of ovarian cell growth and function, such as those for various growth factors, have not been investigated.

TREATMENT AND OUTCOME

There is no standardized treatment specifically for AOD. Women with infertility may seek assisted reproductive methods. These methods are sometimes successful. However, reduced fertilization (Mantzavinos et al., 1993; Papale et al., 1994; Ivanova et al., 1999), poor response to gonadotropin stimulation (Hovav et al., 1994), and decreased pregnancy rates (Unlu et al., 1990; Kamada et al., 1992) were associated with ZP antibodies in all but one study (Curtis et al., 1991). Likewise, ovarian antibodies were associated with poor response to gonadotropin (Meyer et al., 1990; Luborsky et al., 2002b), and reduced pregnancy rates (Barbarino-Monnier et al., 1991; Horejsi et al., 2000; Luborsky and Pong, 2000).

The heterogeneity of follicles with respect to ZP antibodies (Papale et al., 1994) may explain the partial success of infertility treatment, since there appear to be some "normal" follicles despite the presence of autoimmunity. Fertilization was reduced or absent in follicles with ZP antibodies, and was normal in follicles without autoantibodies within the same ovary.

Spontaneous reversal of POF, or reversal of POF during or after estrogen therapy, or "intermittent" POF have been reported (Alper and Garner, 1985; Alper et al., 1986; Boyers et al., 1988; Letterie et al., 1990; Blumenfeld et al., 1993; Fenichel et al., 1997). Conway et al. (1996) showed by ultrasound that 60% of women had follicular structure. Some women with POF exhibit intermittent estradiol increases (Boyers et al., 1988), mature follicles (Nelson et al., 1994), and evidence of follicular activity by ultrasound (Conway et al., 1996). Interestingly, Fenichel et al (1997) reported that four of 46 women with POF spontaneously conceived during estrogen therapy and of the four, three had ovarian antibodies.

Low-dose corticosteroid treatment has been used to suppress the autoimmune reaction in POF or infertility. Most reports of successful treatment are anecdotal. Success with low-dose corticosteroid treatment of POF with autoimmunity confirmed by ovarian biopsy (Sedmak et al., 1987; Kalantaridou et al., 1999) or ovarian antibody (Rabinowe, 1986; Luborsky et al., 1990) has been reported. Because follicles are recruited at least 1–2 months before the growth and activation cycle, it may require several months for re-expression of normal follicle function after treatment. Two of our POF patients with ovarian antibodies spontaneously resumed menstrual cycles and became pregnant 2 months after corticosteroid immunosuppression with no exogenous hormone stimulation (Luborsky et al., 1990). Similarly, hormone stimulation of patients with AOD and infertility 1–2 months after immunosuppression was successful (Rabinowe, 1986; Barbarino-Monnier et al., 1995; Geva et al., 1999). Controlled clinical trials have not been reported and there are concerns about the side effects of immunosuppressants (Kalantaridou et al., 1999).

FUTURE PROSPECTS

The next significant advance in characterizing AOD will be definitive identification of the specific antigens and development of standardized tests based on use of specific antigens. This will improve clinical diagnosis and permit more precise studies of disease pathogenesis, health risks associated with ovarian autoimmunity, and genetic and environmental factors associated with disease susceptibility.

References

Agersborg, S.S., Garza, K.M., and Tung, K.S. (2001). Intestinal parasitism terminates self tolerance and enhances neonatal induction of autoimmune disease and memory. *Eur. J. Immunol. 31*, 851–859.

Aittomaki, K., Lucena, J.L., Pakarinen, P., Sistonen, P., Tapanainen, J., Gromoll, J., Kaskikari, R., Sankila, E.M., Lehvaslaiho, H., and Engel, A.R. (1995). Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell 82*, 959–968.

Aittomaki, K., Herva, R., Stenman, U.H., Juntunen, K., Ylostalo, P., Hovatta, O., and de la Chapelle, A. (1996). Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J. Clin. Endocrinol. Metab. 81*, 3722–3726.

Alper, M.M. and Garner, P.R. (1985). Premature ovarian failure: its relationship to autoimmune disease. *Obstet. Gynecol. 66*, 27–30.

Alper, M.M., Jolly, E.E., and Garner, P.R. (1986). Pregnancies after premature ovarian failure. *Obstet. Gynecol. 67*, 59S–62S.

Anasti, J., Flack, M., Froehlich, J., and Nelson, L. (1995). The use of human recombinant gonadotropin receptors to search for immunoglobulin G-mediated premature ovarian failure. *J. Clin. Endocrinol. Metab. 80*, 824–828.

Anasti, J.N., Kalantaridou, S.N., Kimzey, L.M., Defensor, R.A., and Nelson, L.M. (1998). Bone loss in young women with karyotypically normal spontaneous premature ovarian failure. *Obstet. Gynecol. 91*, 12–15.

Arif, S., Vallian, S., Farzaneh, F., Zanone, M., James, S., Pietropaolo, M., Hettiarachchi, S., Vergani, D., Conway, G., and Peakman, M. (1996). Identification of 3 beta-hydroxysteroid dehydrogenase as a novel target of steroid cell autoantibodies: association of autoantibodies with endocrine autoimmune disease. *J. Clin. Endocrinol. Metab. 81*, 4439–4445.

Arif, S., Underhill, J.A., Donaldson, P., Conway, G.S., and Peakman, M. (1999). Human leukocyte antigen-DQB1* genotypes encoding aspartate at position 57 are associated with 3beta-hydroxysteroid dehydrogenase autoimmunity in premature ovarian failure. *J. Clin. Endocrinol. Metab. 84*, 1056–1060.

Arif, S., Varela-Calvino, R., Conway, G.S., and Peakman, M. (2001). 3 beta hydroxysteroid dehydrogenase autoantibodies in patients with idiopathic premature ovarian failure target N- and C-terminal epitopes. *J. Clin. Endocrinol. Metab. 86*, 5892–5897.

Austin, G., Coulam, C., and Ryan, R. (1979). A search for antibodies to luteinizing hormone receptors in premature ovarian failure. *Mayo Clin. Proc. 54*, 394–400.

Bagavant, H., Adams, S., Terranova, P., Chang, A., Kraemer, F.W., Lou, Y., Kasai, K., Luo, A.M., and Tung, K.S. (1999). Autoimmune ovarian inflammation triggered by proinflammatory (Th1) T cells is compatible with normal ovarian function in mice. *Biol. Reprod. 61*, 635–642.

Baker, J.J. (1997). Autoimmune endocrine disease. *JAMA 278*, 1931–1937.

Barbarino-Monnier, P., Gobert, B., Guillet-Rosso, F., Bene, M.C., Landes, P. and Faure, G. (1991). Antiovary antibodies, repeated attempts, and outcome of in vitro fertilization. *Fertil. Steril. 56*, 928–932.

Barbarino-Monnier, P., Gobert, B., Guillet-May, F., Bene, M.C., Barbarino, A., Foliguet, B., and Faure, G.C. (1995).Ovarian autoimmunity and corticotherapy in an in-vitro fertilization attempt. *Hum. Reprod. 10*, 2006–2007.

Belvisi, L., Bombelli, F., Sironi, L., and Doldi, N. (1993). Organ-specific autoimmunity in patients with premature ovarian failure. *J. Endocrinol. Invest. 16*, 889–892.

Betterle, C., Rossi, A., Dalla Pria, S., Artifoni, A., Pedini, B., Gavasso, S., and Caretto, A. (1993). Premature ovarian failure: autoimmunity and natural history. *Clin. Endocrinol. (Oxf.) 39*, 35–43.

Bione, S., Sala, C., Manzini, C., Arrigo, G., Zuffardi, O., Banfi, S., Borsani, G., Jonveaux, P., Philippe, C., Zuccotti, M., Ballabio, A., and Toniolo, D. (1998) A human homologue of the *Drosophila melanogaster* diaphanous gene is disrupted in a patient with premature ovarian failure: evidence for conserved function in oogenesis and implications for human sterility. *Am. J. Hum. Genet. 62*, 533–541.

Blumenfeld, Z., Halachmi, S., Peretz, B.A., Shmuel, Z., Golan, D., Makler, A., and Brandes, J.M. (1993). Premature ovarian failure—the prognostic application of autoimmunity on conception after ovulation induction. *Fertil. Steril. 59*, 750–755.

Bondy, C.A., Nelson, L.M., and Kalantaridou, S.N. (1998). The genetic origins of ovarian failure. *J. Womens Health 7*, 1225–1229.

Boyers, S.P., Luborsky, J.L., and DeCherney, A.H. (1988). Usefulness of serial measurements of serum FSH, LH and estradiol in patients with premature ovarian failure. *Fertil. Steril. 50*, 408–412.

Buckshee, K. and Mhaskar, A. (1985). Status of autoantibodies to zona pellucida in human reproduction. *Int. J. Fertil. 30*, 13–17.

Cameron, I.T., O'Shea, F.C., Rolland, J.M., Hughes, E.G., de Kretser, D.M., and Healy, D.L. (1988). Occult ovarian failure: a syndrome of infertility, regular menses, and elevated follicle-stimulating hormone concentrations. *J. Clin. Endocrinol. Metab. 67*, 1190–1194.

Chen, S., Sawicka, J., Betterle, C., Powell, M., Prentice, L., Volpato, M., Rees Smith, B., and Furmaniak, J. (1996). Autoantibodies to steroidogenic enzymes in autoimmune polyglandular syndrome, Addison's disease, and premature ovarian failure. *J. Clin. Endocrinol. Metab. 81*, 1871–1876.

Chiarelli, A.M., Marrett, L.D., and Darlington, G. (1999). Early menopause and infertility in females after treatment for childhood cancer diagnosed in 1964–1988 in Ontario, Canada. *Am. J. Epidemiol. 150*, 245–254.

Christin-Maitre, S., Vasseur, C., Portnoi, M.F., and Bouchard, P. (1998). Genes and premature ovarian failure. *Mol. Cell. Endocrinol. 145*, 75–80.

Collins, J. (1995) Unexplained infertility. In W. Keye, R. Chang, R. Rebar and M. R. Soules (eds), *Infertility: Evaluation and Treatment* (pp. 249–262). Philadelphia: WB Saunders.

Conway, G.S., Kaltsas, G., Patel, A., Davies, M.C., and Jacobs, H.S. (1996). Characterization of idiopathic premature ovarian failure. *Fertil. Steril. 65*, 337–341.

Conway, G.S., Conway, E., Walker, C., Hoppner, W., Gromoll, J., and Simoni, M. (1999) Mutation screening and isoform prevalence of the follicle stimulating hormone receptor gene in women with premature ovarian failure, resistant ovary syndrome and polycystic ovary syndrome. *Clin. Endocrinol. (Oxf.) 51*, 97–99.

Cooper, G.S., Hulka, B.S., Baird, D.D., Savitz, D.A., Hughes, C.L., Jr., Weinberg, C.R., Coleman, R.A., and Shields, J.M. (1994). Galactose consumption, metabolism, and follicle-stimulating hormone concentrations in women of late reproductive age. *Fertil. Steril. 62*, 1168–1175.

Cooper, G.S. and Sandler, D.P. (1998). Age at natural menopause and mortality. *Ann. Epidemiol. 8*, 229–235.

Cooper, G.S., Sandler, D.P., and Bohlig, M. (1999). Active and passive smoking and the occurrence of natural menopause. *Epidemiology 10*, 771–773.

Coulam, C. and Ryan, R. (1979). Premature menopause I. Etiology. *Am. J. Obstet. Gynecol. 133*, 639–645.

Coulam, C., Adamson, S., and Annegers, J. (1986). Incidence of premature ovarian failure. *Obstet. Gynecol. 67*, 604–606.

Cramer, D. and Xu, H. (1996). Predicting age at menopause. *Maturitas 23*, 319–326.

Cramer, D. W., Harlow, B.L., Barbieri, R.L., and Ng, W.G. (1989). Galactose-1-phosphate uridyl transferase activity associated with age at menopause and reproductive history. *Fertil. Steril. 51*, 609–615.

Cramer, D.W., Barbieri, R.L., Xu, H., and Reichardt, J.K. (1994). Determinants of basal follicle-stimulating hormone levels in premenopausal women. *J. Clin. Endocrinol. Metab. 79*, 1105–1109.

Cramer, D.W., Xu, H., and Harlow, B.L. (1995). Family history as a predictor of early menopause. *Fertil. Steril. 64*, 740–745.

Curtis, P., Burford, G., Amso, N., Keith, E., and Shaw, R. (1991). Assessment of the relevance of zona pellucida antibodies in serum and cervical mucus in patients who have fertilization failure during in vitro fertilization. *Fertil. Steril. 56*, 1124–1127.

da Fonte Kohek, M.B., Batista, M.C., Russell, A.J., Vass, K., Giacaglia, L.R., Mendonca, B.B., and Latronico, A.C. (1998). No evidence of the

inactivating mutation (C566T) in the follicle-stimulating hormone receptor gene in Brazilian women with premature ovarian failure. *Fertil. Steril. 70*, 565–567.

Damewood, M.D., Zacur, H. A., Hoffman, G.J., and Rock, J.A. (1986). Circulating antiovarian antibodies in premature ovarian failure. *Obstet. Gynecol. 68*, 850–854.

Davis, S. (1996). Premature ovarian failure. *Maturitas 23*, 1–8.

de Carmo Silva, R., Kater, C.E., Dib, S.A., Laureti, S., Forini, F., Cosentino, A., and Falorni, A. (2000). Autoantibodies against recombinant human steroidogenic enzymes 21-hydroxylase, side-chain cleavage and 17alpha-hydroxylase in Addison's disease and autoimmune polyendocrine syndrome type III. *Eur. J. Endocrinol. 142*, 187–194.

Faustman, D., Li, X.P., Lin, H.Y., Fu, Y.E., Eisenbarth, G., Avruch, J., and Guo, J. (1991). Linkage of faulty major histocompatibility complex class I to autoimmune diabetes. *Science 254*, 1756–1761.

Fenichel, P., Sosset, C., Barbarino-Monnier, P., Gobert, B., Hieronimus, S., Bene, M., and Harter, M. (1997). Prevalence, specificity and significance of ovarian antibodies during spontaneous premature ovarian failure. *Hum. Reprod. 12*, 2623–2628.

Forti, G. and Krausz, C. (1998). Evaluation and treatment of the infertile couple. *Fertil. Steril. 83*, 4177–4188.

Fox, H. (1992). The pathology of premature ovarian failure. *J. Pathol. 167*, 357–363.

Fu, Y., Nathan, D.M., Li, F., Li, X., and Faustman, D.L. (1993). Defective major histocompatibility complex class I expression on lymphoid cells in autoimmunity. *J. Clin. Invest. 91*, 2301–2307.

Fu, Y., Yan, G., Shi, L., and Faustman, D. (1998). Antigen processing and autoimmunity. Evaluation of mRNA abundance and function of HLA-linked genes. *Ann. N. Y. Acad. Sci. 842*, 138–155.

Garza, K.M. and Tung, K.S. (1995). Frequency of molecular mimicry among T cell peptides as the basis for autoimmune disease and autoantibody induction. *J. Immunol. 155*, 5444–5448.

Geva, E., Fait, G., Lerner-Geva, L., Lessing, J.B., Swartz, T., Wolman, I., Daniel, Y., and Amit, A. (1999). The possible role of antiovary antibodies in repeated in vitro fertilization failures. *Am. J. Reprod. Immunol. 42*, 292–296.

Giglio, T., Imro, M.A., Filaci, G., Scudeletti, M., Puppo, F., De Cecco, L., Indiveri, F., and Costantini, S. (1994). Immune cell circulating subsets are affected by gonadal function. *Life Sci. 54*, 1305–1312.

Griggs, N.D., Agersborg, S.S., Noelle, R.J., Ledbetter, J.A., Linsley, P.S., and Tung, K.S. (1996). The relative contribution of the CD28 and gp39 costimulatory pathways in the clonal expansion and pathogenic acquisition of self-reactive T cells. *J. Exp. Med. 183*, 801–810.

Hill, J.A., Welch, W.R., Faris, H.M., and Anderson, D.J. (1990). Induction of class II major histocompatibility complex antigen expression in human granulosa cells by interferon gamma: a potential mechanism contributing to autoimmune ovarian failure. *Am. J. Obstet. Gynecol. 162*, 534–540.

Ho, P.C., Tang, G.W., Fu, K.H., Fan, M.C., and Lawton, J.W. (1988). Immunologic studies in patients with premature ovarian failure. *Obstet. Gynecol. 71*, 622–626.

Ho, P.C., Tang, G.W., and Lawton, J.W. (1993). Lymphocyte subsets and serum immunoglobulins in patients with premature ovarian failure before and after oestrogen replacement. *Hum. Reprod. 8*, 714–716.

Hoek, A., van Kasteren, Y., de Haan-Meulman, M., Schoemaker, J., and Drexhage, H. A. (1993). Dysfunction of monocytes and dendritic cells in patients with premature ovarian failure. *Am. J. Reprod. Immunol. 30*, 207–217.

Hoek, A., Schoemaker, J., and Drexhage, H.A. (1997). Premature ovarian failure and ovarian autoimmunity. *Endocrinol. Rev. 18*, 107–134.

Horejsi, J., Martinek, J., Novakova, D., Madar, J. and Brandejska, M. (2000). Autoimmune antiovarian antibodies and their impact on the success of an IVF/ET program. *Ann. N. Y. Acad. Sci. 900*, 351–356.

Hotsuliak, I. and Ianchii, R.I. (1999). Antibodies depress the meiotic oocyte maturation of mice in vitro. *Fiziol. Zh. 45*, 28–34.

Hovav, Y., Almagor, M., Benbenishti, D., Margalioth, E.J., Kafka, I., and Yaffe, H. (1994). Immunity to zona pellucida in women with low response to ovarian stimulation, in unexplained infertility and after multiple IVF attempts. *Hum. Reprod. 9*, 643–645.

Hruska, K.S., Furth, P.A., Seifer, D.B., Sharara, F.I., and Flaws, J.A. (2000). Environmental factors in infertility. *Clin. Obstet. Gynecol. 43*, 821–829.

Huang, Q., Liu, Q. and Wu, J. (1996). Determinations of antiovarian antibodies and cellular immunity functions in patients with premature ovarian failure. *Chung Hua Fu Chan Ko Tsa Chih 31*, 603–605.

Irvine, W.J., Chan, M., Scarth, L., Kolb, F.O., Hartog, M., Bayliss, R., and Drury, M.I. (1968). Immunological aspects of premature ovarian failure associated with idiopathic Addison's disease. *Lancet ii*, 883–887.

Ivanova, M., Djarkova, T., Mollova, M., Petrov, M., Tikhomirova, T., and Dakhno, F. (1999). Zona pellucida autoantibodies in women undergoing ART. *Folia Biol. 45*, 59–62.

Jacobson, D., Gange, S., Rose, N., and Graham, N. (1997). Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol. 84*, 223–243.

Kalantaridou, S.N. and Nelson, L.M. (2000). Premature ovarian failure is not premature menopause. *Ann. N. Y. Acad. Sci. 900*, 393–402.

Kalantaridou, S.N., Braddock, D.T., Patronas, N.J., and Nelson, L.M. (1999) 'Treatment of autoimmune premature ovarian failure. *Hum. Reprod. 14*, 1777–1782.

Kamada, M., Daitoh, T., Mori, K., Maeda, N., Hirano, K., Irahara, M., Aono, T., and Mori, T. (1992). Etiological implication of autoantibodies to zona pellucida in human female infertility'. *Am. J. Reprod. Immunol. 28*, 104–109.

Kenneson, A., Cramer, D.W., and Warren, S.T. (1997). Fragile X premutations are not a major cause of early menopause. *Am. J. Hum. Genet. 61*, 1362–1369.

Lambert, A., Weetman, A., McLoughlin, J., Wardle, C., Sunderland, J., Wheatcroft, N., Anobile, C. and Robertson, W. (1996). A search for immunoglobulins inhibiting gonadal cell steroidogenesis in premature ovarian failure. *Hum. Reprod. 11*, 1871–1876.

Laml, T., Preyer, O., Umek, W., Hengstschlager, M., and Hanzal, H. (2002). Genetic disorders in premature ovarian failure. *Hum. Reprod. Update 8*, 483–491.

Layman, L. (1999). Mutations in human gonadotropin genes and their physiologic significance in puberty and reproduction. *Fertil. Steril. 71*, 201–218.

Layman, L.C., Amde, S., Cohen, D.P., Jin, M., and Xie, J. (1998). The Finnish follicle-stimulating hormone receptor gene mutation is rare in North American women with 46, XX ovarian failure. *Fertil. Steril. 69*, 300–302.

Letterie, G.S., Bork, M.D., and Miyazawa, K. (1990). Intermittent ovarian failure. A report of two cases. *J. Reprod. Med. 35*, 731–733.

Lewis, J. (1993). Eosinophilic perifolliculitis: a variant of autoimmune oophoritis? *Int. J. Gynecol. Pathol. 12*, 360–364.

Li, F., Linan, M.J., Stein, M.C., and Faustman, D.L. (1995). Reduced expression of peptide-loaded HLA class I molecules on multiple sclerosis lymphocytes. *Ann. Neurol. 38*, 147–154.

Lin, J., Yan, J., and Lin, Q. (1998). Determination of cellular and humoral immunity in mice of autoimmune ovarian failure. *Chung Hua Fu Chan Ko Tsa Chih 33*, 735–736.

Lou, Y.H., McElveen, M.F., Garza, K.M., and Tung, K.S. (1996). Rapid induction of autoantibodies by endogenous ovarian antigens and activated T cells: implication in autoimmune disease pathogenesis and B cell tolerance. *J. Immunol. 156*, 3535–3540.

Lou, Y.H., Park, K.K., Agersborg, S., Alard, P., and Tung, K.S. (2000). Retargeting T cell-mediated inflammation: a new perspective on autoantibody action. *J. Immunol. 164*, 5251–5257.

Luborsky, J. (2002a). *Test for Ovarian Autoimmunity by Detecting Autoantibodies to CYP17*. Chicago: Rush University.

Luborsky, J.L. (2002b). Ovarian autoimmune disease and ovarian autoantibodies: a review. *J. Womens Health Gender Based Med. 11*, 585–599.

Luborsky, J. and Pong, R. (2000). Pregnancy outcome and ovarian autoimmunity in infertility patients undergoing controlled ovarian hyperstimulation. *Am. J. Reprod. Immunol. 44*, 261–265.

Luborsky, J.L., Visintin, I., Boyers, S., Asare, T., Caldwell, B., and DeCherney, A. (1990). Ovarian antibodies detected by immobilized antigen immunoassay in patients with premature ovarian failure. *J. Clin. Endocrinol. Metab. 70*, 69–75.

Luborsky, J., Llanes, B., Davies, S., Binor, Z., Radwanska, E., and Pong, R. (1999). Ovarian autoimmunity: greater prevalence of autoantibodies in POF and unexplained infertility than in the general population. *Clin. Immunol. 90*, 368–374.

Luborsky, J., Roussev, R. and Coulam, C. (2000a). Ovarian antibodies, FSH and inhibin are independent markers associated with unexplained infertility. *Hum. Reprod. 15*, 1046–1051.

Luborsky, J., Thiruppathi, P., Rivnay, B., Roussev, R., and Coulam, C. (2000b). Distinction between ovarian autoimmunity and endocrine mechanisms of low ovarian responses to gonadotropin. Second International Conference on Experimental and Clinical Reproductive Immunobiology, Amsterdam, The Netherlands.

Luborsky, J., Meyer, P., Sowers, M., Santoro, N., and Gold, E. (2002a). Premature menopause in a multi-ethnic population study of the menopause transition. *Hum. Reprod. 18*, 199–206.

Luborsky, J., Thiruppathi, P., Rivnay, B., Roussev, R., Coulam, C., and Radwanska, E. (2002b). Evidence for different etiologies of low estradiol response to FSH: age related accelerated luteinization of follicles or presence of ovarian autoantibodies. *Hum. Reprod. 17*, 2641–2649.

Luo, A.M., Garza, K.M., Hunt, D., and Tung, K.S. (1993). Antigen mimicry in autoimmune disease sharing of amino acid residues critical for pathogenic T cell activation. *J. Clin. Invest. 92*, 2117–2123.

Mantzavinos, T., Dalamanga, N., Hassiakos, D., Dimitriadou, F., Konidaris, S., and Zourlas, P.A. (1993). Assessment of autoantibodies to the zona pellucida in serum and follicular fluid in in-vitro fertilization patients. *Clin. Exp. Obstet. Gynecol. 20*, 111–115.

Marozzi, A., Vegetti, W., Manfredini, E., Tibiletti, M.G., Testa, G., Crosignani, P.G., Ginelli, E., Meneveri, R., and Dalpra, L. (2000). Association between idiopathic premature ovarian failure and fragile X premutation. *Hum. Reprod. 15*, 197–202.

Meyer, W., Lavy, G., DeCherney, A., Visintin, I., Economy, K., and Luborsky, J. (1990). Evidence of gonadal and gonadotropin antibodies in women with a suboptimal ovarian response to exogenous gonadotropin. *Obstet. Gynecol. 75*, 795–799.

Mikulikova, L., Veselsky, L., Cerny, V., Martinek, J., Malbohan, I., and Fialova, L. (1989). Immunofluorescence detection of porcine anti-zona pellucida antibodies in sera of infertile women. *Acta Univ. Carol. [Med] (Praha) 35*, 63–68.

Miyake, T., Sato, Y., and Takeuchi, S. (1987). Implications of circulating autoantibodies and peripheral blood lymphocyte subsets for the genesis of premature ovarian failure. *J. Reprod. Immunol. 12*, 163–171.

Moncayo, H., Moncayo, R., Benz, R., Wolf, A., and Lauritzen, C. (1989). Ovarian failure and autoimmunity. Detection of autoantibodies directed against both the unoccupied luteinizing hormone/human chorionic gonadotropin receptor and the hormone-receptor complex of bovine corpus luteum. *J. Clin. Invest. 84*, 1857–1865.

Muechler, E.K., Huang, K.E., and Schenk, E. (1991). Autoimmunity in premature ovarian failure. *Int. J. Fertil. 36*, 99–103.

Naz, R.K., Thurston, D., and Santoro, N. (1995). Circulating tumor necrosis factor (TNF)-alpha in normally cycling women and patients with premature ovarian failure and polycystic ovaries. *Am. J. Reprod. Immunol. 34*, 170–175.

Nelson, L.M., Kimzey, L.M., Merriam, G.R., and Fleisher, T.A. (1991). Increased peripheral T lymphocyte activation in patients with karyotypically normal spontaneous premature ovarian failure. *Fertil. Steril. 55*, 1082–1087.

Nelson, L.M., Anasti, J.N., Kimzey, L.M., Defensor, R.A., Lipetz, K.J., White, B.J., Shawker, T.H., and Merino, M.J. (1994). Development of luteinized graafian follicles in patients with karyotypically normal spontaneous premature ovarian failure. *J. Clin. Endocrinol. Metab. 79*, 1470–1475.

Nishizuka, Y. and Sakakura, T. (1969). Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science 166*, 753–755.

Papale, M.L., Grillo, A., Leonardi, E., Giuffrida, G., Palumbo, M., and Palumbo, G. (1994). Assessment of the relevance of zona pellucida antibodies in follicular fluid of in-vitro fertilization (IVF) patients. *Hum. Reprod. 9*, 1827–1831.

Partington, M.W., Moore, D.Y., and Turner, G.M. (1996). Confirmation of early menopause in fragile X carriers. *Am. J. Med. Genet. 64*, 370–372.

Peterson, P. and Krohn, K. (1994). Mapping of B cell epitopes on steroid 17 alpha-hydroxylase, an autoantigen in autoimmune polyglandular syndrome type I. *Clin. Exp. Immunol. 98*, 104–109.

Peterson, P., Uibo, R., Peranen, J., and Krohn, K. (1997). Immunoprecipitation of steroidogenic enzyme autoantigens with autoimmune polyglandular syndrome type I (APS I) sera; further evidence for independent humoral immunity to P450c17 and P450c21. *Clin. Exp. Immunol. 107*, 335–340.

Rabinowe, S.L. (1986). Lymphocyte dysfunction in autoimmune oophoritis/resumption of menses with corticosteroids. *Am. J. Med. 81*, 347–350.

Rebar, R. and Connolly, H. (1990). Clinical features of young women with hypergonadotropic amenorrhea. *Fertil. Steril. 53*, 804–900.

Reimand, K., Peterson, P., Hyoty, H., Uibo, R., Cooke, I., Weetman, A.P., and Krohn, K. J. (2000). 3beta-hydroxysteroid dehydrogenase autoantibodies are rare in premature ovarian failure. *J. Clin. Endocrinol. Metab. 85*, 2324–2326.

Rhim, S., Millar, S., Robey, F., Luo, A., Lou, Y., Yule, T., Allen, P., Dean, J., and Tung, K. (1992). Autoimmune disease of the ovary induced by a ZP3 peptide from the mouse zona pellucida. *J. Clin. Invest. 89*, 28–35.

Roper, R.J., McAllister, R.D., Biggins, J.E., Michael, S.D., Min, S.H., Tung, K.S., Call, S.B., Gao, J., and Teuscher, C. (2003). Aod1 controlling day 3 thymectomy-induced autoimmune ovarian dysgenesis in mice encompasses two linked quantitative trait loci with opposing allelic effects on disease susceptibility. *J. Immunol. 170*, 5886–5891.

Rose, N.R. and Bona, C. (1993). Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol. Today 14*, 426–430.

Ruan, Q.G., Wang, C.Y., Shi, J.D., and She, J.X. (1999). Expression and alternative splicing of the mouse autoimmune regulator gene (Aire). *J. Autoimmun. 13*, 307–313.

Russell, P., Bannatyne, P., Shearman, R.P., Fraser, I.S., and Corbett, P. (1982). Premature hypergonadotropic ovarian failure: clinicopathological study of 19 cases. *Int. J. Gynecol. Pathol. 1*, 185–201.

Santoro, N. (2001). Research on the mechanisms of premature ovarian failure. *J. Soc. Gynecol. Investig. 8*, S10–12.

Sedmak, D., Hart, W., and Tubbs, R. (1987). Autoimmune oophoritis: a histopathologic study of involved ovaries with immunologic characterization of mononuclear cell infiltrate. *Int. J. Gynecol. Pathol. 6*, 73–77.

Seissler, J., Schott, M., Steinbrenner, H., Peterson, P., and Scherbaum, W. (1999). Autoantibodies to adrenal cytochrome P450 antigens in isolated Addison's disease and autoimmune polyendocrine syndrome type II. *Exp. Clin. Endocrinol. Diabetes 107*, 208–213.

Setiady, Y., Pramoonjago, P., and Tung, K. (2004). Requirements of NK cells and proinflammatory cytokines in T cell-dependent neonatal autoimmune ovarian disease triggered by immune complex. *J. Immunol. 173*, 1051–1058.

Setiady, Y.Y., Samy, E.T., and Tung, K.S. (2003). Maternal autoantibody triggers de novo T cell-mediated neonatal autoimmune disease. *J. Immunol. 170*, 4656–4664.

Shivers, C. and Dunbar, B. (1977). Autoantibodies to zona pellucida: a possible cause for infertility in women. *Science 197*, 1082–1084.

Silbergeld, E.K. and Flaws, J.A. (1999). Chemicals and menopause: effects on age at menopause and on health status in the postmenopausal period. *J. Womens Health 8*, 227–234.

Singh, J. and Mhaskar, A. (1985). Enzyme-linked immunosorbent determination of autoantibodies to zona pellucida as a possible cause of infertility in women. *J. Immunol. Methods 79*, 133–141.

Smith, S. and Hosid, S. (1994). Premature ovarian failure associated with autoantibodies to the zona pellucida. *Int. J. Fertil. Menopausal Stud. 39*, 316–319.

Song, Y., Connor, E.L., Muir, A., She, J.X., Zorovich, B., Debrovanesian, D., and Maclaren, N. (1994). Autoantibody epitope mapping of 21-hydroxylase antigen in autoimmune Addison's disease. *J. Clin. Endocrinol. Metabol 78*, 1108–1112.

Sontag, W., Sikorski, R., and Kapec, E. (1982). Antibodies of ovarian antigens in different diseases of the ovary. *Am. J. Reprod. Immunol. 2*, 58–63.

Takakura, K., Takebayashi, K., Wang, H.Q., Kimura, F., Kasahara, K., and Noda, Y. (2001). Follicle-stimulating hormone receptor gene mutations are rare in Japanese women with premature ovarian failure and polycystic ovary syndrome. *Fertil. Steril. 75*, 207–209.

Tanaka, H., Perez, M.S., Powell, M., Sanders, J.F., Sawicka, J., Chen, S., Prentice, L., Asawa, T., Betterle, C., Volpato, M., Smith, B.R., and Furmaniak, J. (1997). Steroid 21-hydroxylase autoantibodies: measurements with a new immunoprecipitation assay. *J. Clin. Endocrinol. Metab. 82*, 1440–1446.

Teuscher, C., Wardell, B.B., Lunceford, J.K., Michael, S.D., and Tung, K.S. (1996). Aod2, the locus controlling development of atrophy in neonatal thymectomy-induced autoimmune ovarian dysgenesis, co-localizes with Il2, Fgfb, and Idd3. *J. Exp. Med. 183*, 631–637.

Tong, Z.B. and Nelson, L.M. (1999). A mouse gene encoding an oocyte antigen associated with autoimmune premature ovarian failure. *Endocrinology 140*, 3720–3726.

Tong, Z.B., Gold, L., Pfeifer, K.E., Dorward, H., Lee, E., Bondy, C.A., Dean, J., and Nelson, L.M. (2000). Mater, a maternal effect gene required for early embryonic development in mice. *Nat. Genet. 26*, 267–268.

Tong, Z.B., Bondy, C.A., Zhou, J., and Nelson, L.M. (2002). A human homologue of mouse Mater, a maternal effect gene essential for early embryonic development. *Hum. Reprod. 17*, 903–911.

Ulcova-Gallova, Z. and Mardesic, T. (1996). Does in vitro fertilization (IVF) influence the levels of sperm and zona pellucida (ZP) antibodies in infertile women? *Am. J. Reprod. Immunol. 36*, 216–219.

Unlu, C., Bohmer, S., and Maas, D. (1990). Immunological factors in extracorporeal fertilization. The role of antibodies against spermatozoa and zona pellucida antigen. *Fortschr. Med. 108*, 450–453.

Uzielli, M.L., Guarducci, S., Lapi, E., Cecconi, A., Ricci, U., Ricotti, G., Biondi, C., Scarselli, B., Vieri, F., Scarnato, P., Gori, F., and Sereni, A. (1999). Premature ovarian failure (POF) and fragile X premutation females: from POF to fragile X carrier identification, from fragile X carrier diagnosis to POF association data. *Am. J. Med. Genet. 84*, 300–303.

Vallotton, M.B. and Forbes, A.P. (1966). Antibodies to cytoplasm of ova. *Lancet 2*, 264–265.

van Kasteren, Y.M., von Blomberg, M., Hoek, A., de Koning, C., Lambalk, N., van Montfrans, J., Kuik, J., and Schoemaker, J. (2000). Incipient ovarian failure and premature ovarian failure show the same immunological profile. *Am. J. Reprod. Immunol. 43*, 359–366.

van Weissenbruch, M., Hoek, A., van Vliet-Bleeker, I., Schoemaker, J., and Drexhage, H. (1991). Evidence for existence of immunoglobulins that block ovarian granulosa cell growth in vitro. A putative role in resistant ovary syndrome. *J. Clin. Endocrinol. Metab. 73*, 360–367.

Vermeulen, A. (1993). Environment, human reproduction, menopause, and andropause. *Environ. Health Perspect. 101*(Suppl 2), 91–100.

Wardell, B.B., Michael, S.D., Tung, K.S., Todd, J.A., Blankenhorn, E.P., McEntee, K., Sudweeks, J.D., Hansen, W.K., Meeker, N.D., and Griffith, J.S. (1995). Aod1, the immunoregulatory locus controlling abrogation of tolerance in neonatal thymectomy-induced autoimmune ovarian dysgenesis, maps to mouse chromosome 16. *Proc. Natl. Acad. Sci. USA 92*, 4758–4762.

Wheatcroft, N. and Weetman, A.P. (1997). Is premature ovarian failure an autoimmune disease? *Autoimmunity 25*, 157–165.

Wheatcroft, N., Toogood, A., Li, T., Cooke, I., and Weetman, A. (1994). Detection of antibodies to ovarian antigens in women with premature ovarian failure. *Clin. Exp. Immunol. 96*, 122–128.

Wheatcroft, N.J., Salt, C., Milford-Ward, A., Cooke, I.D., and Weetman, A.P. (1997a). Identification of ovarian antibodies by immunofluorescence, enzyme-linked immunosorbent assay or immunoblotting in premature ovarian failure. *Hum. Reprod. 12*, 2617–2622.

Wheatcroft, N.J., Rogers, C.A., Metcalfe, R.A., Lenton, E.A., Cooke, I.D., and Weetman, A.P. (1997b). Is subclinical ovarian failure an autoimmune disease? *Hum. Reprod. 12*, 244–249.

Whitney, E.A., Layman, L.C., Chan, P.J., Lee, A., Peak, D.B., and McDonough, P.G. (1995). The follicle-stimulating hormone receptor gene is polymorphic in premature ovarian failure and normal controls. *Fertil. Steril. 64*, 518–524.

WHO (1992). *Recent Advances in Medically Assisted Reproduction*. WHO Technical Report Series 820 (pp. 1). Geneva: World Health Organization.

WHO (1996). *Research on Menopause in the 1990s.* WHO Technical Report Series 866 (pp. 1–79). Geneva: World Health Organization.

Yan, G., Schoenfeld, D., Penney, C., Hurxthal, K., Taylor, A., and Faustman, D. (2000). Identification of premature ovarian failure patients with underlying autoimmunity. *J Women Health Gender-Based Med. 9*, 275–287.

CHAPTER

62

Rheumatic Heart Disease

LUIZA GUILHERME*† AND JORGE E. KALIL*†‡

*Heart Institute (InCor), School of Medicine, University of São Paulo; †Institute for Immunology Investigation, Millenium Institute; and ‡Clinical Immunology and Allergy, Department of Clinical Medicine, University of São Paulo, School of Medicine, São Paulo, Brazil

HISTORIC BACKGROUND

The relationship between rheumatism and heart disease was first observed by Pitcairn in 1788. It was subsequently described in a textbook approximately 10 years later by Baillie. Since then, several major contributions to the association between acute rheumatism and heart disease have indicated that rheumatic fever (RF) is primarily a disease of children (Baggenstone and Titus, 1968). Bouillaud (1840) made an important remark, "rheumatic fever licks the joint but bites the heart." Cheadle's observation in 1889 that the disease was more frequent in families suggested a genetic pattern of susceptibility. An etiologic agent responsible for RF and thus for rheumatic heart disease (RHD) was sought by several investigators in the period between 1900 and 1940. Many viruses and bacteria were suspected to be the cause of RF. In 1930, Coburn focused on the β-hemolytic *Streptococcus pyogenes* as the possible causative agent of RF (Baggenstone and Titus, 1968).

CLINICAL, PATHOLOGIC AND EPIDEMIOLOGIC FEATURES

The clinical signs and symptoms of RF are the same throughout the world. In the 1950s Jones established the major criteria for diagnosing initial attacks of RF—polyarthritis, carditis, and chorea (Special Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young of the American Heart Association, 1992). These criteria remain useful today, despite small periodic changes. Arthritis is one of the earliest and most common features of the disease, present in 60–80% of patients. It usually affects the peripheral large joints; small joints and the axial skeleton are rarely involved. Knees, ankles, elbows, and wrists are most frequently affected. The arthritis is usually migratory and very painful. Carditis, the most serious manifestation of the disease, occurs a few weeks after group A streptococcal infection, and usually presents as a pancarditis. Endocarditis is the most serious sequel and frequently leads to chronic RHD. Mitral and aortic regurgitation are the most common events caused by valvulitis. Sydenham chorea is characterized by involuntary movements, especially of the face and limbs, muscular weakness, and disturbances of speech, gait, and voluntary movements. It is usually a delayed manifestation, and is often the

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

sole manifestation of acute RF. Other manifestations, such as subcutaneous nodules and erythema marginatum, can also occur during episodes of RF and are characterized by nodules on the surface of the joints and skin lesions, respectively.

RF is a sequel of throat infection by *S. pyogenes*, affecting 3–4% of untreated children. RHD develops 4–8 weeks (or later) after group A streptococci infection in 30–45% of individuals with RF.

Studies by Rebecca Lancefield (1941) classified streptococci groups by serology based on their cell wall polysaccharides (groups A, B, C, F and G). The *S. pyogenes*, or group A streptococci, cell wall is composed of carbohydrates, such as N-acetyl β-D-glucosamine linked to a polymeric rhamnose backbone. Group A streptococci contain M, T, and R surface proteins and lipoteichoic acid (LTA), which is involved in bacterial adherence to throat epithelial cells. The M protein, which extends from the cell wall, is composed of two polypeptide chains of approximately 450 amino acid residues, in an α-helical coiled-coil configuration. The N-terminal portion presents antigenic variations but has high homology except for the first 11 amino acid residues that define the different serotypes, of which 120 have been identified to date. The C-terminal half is conserved and contains multiple repeat regions (Fischeti, 1991).

Some of the 120 different serotypes of group A streptococci have been consistently found to be more frequently associated with RF, whereas others are more often associated with acute glomerulonephritis. These serotypes or strains are called rheumatogenic and nephritogenic, respectively (Stollerman, 1997).

The M protein is the most important bacterial antigenic structure and shares structural homology with α-helical coiled-coil human proteins like cardiac myosin, tropomyosin, keratin, laminin, vimentin, and several valvular proteins. The pathogenic mechanisms involved in the development of RF and RHD are not fully understood. It is considered that the molecular mimicry mechanism is responsible for the cross-reaction between streptococcal antigens and human tissue proteins, mainly heart tissue proteins in susceptible individuals. It is now clear that the disease is mediated by both humoral and cellular immune responses and that the cellular arm of the immune response is more closely involved in the development of RHD.

The incidence of RF in developed countries has declined over the past decades. However, the prevalence in developing areas is still high, having been estimated as up to 24 in 1000 individuals (Stollerman, 1998). Data from the World Health Organization (WHO) have shown that 25–40% of cardiovascular diseases in developing countries are due to RF. RF occurs most frequently among children and adolescents between the ages of 5 and 18 years, coinciding with the age distribution of the highest prevalence of streptococcal infections. It occurs equally in males and females and it is more prevalent among groups of low socioeconomic status.

AUTOIMMUNE FEATURES

The pathogenesis of RHD depends on several host factors that mediate a pathologic autoimmune response triggered by a defensive immune response against *S. pyogenes*. Genetic predisposition is one of the leading factors contributing to the development of autoimmunity. Several HLA class II alleles have been associated with the development of humoral or cellular-mediated diseases and are considered as genetic markers. One explanation for the association of autoimmune diseases, such as RHD, with some HLA class II alleles could be the fact that these molecules expressed on the surface of antigen-presenting cells (APCs), e.g., macrophages, dendritic cells, and B lymphocytes, trigger activation of some T-cell populations upon specific self-antigen stimulation, leading to autoimmune reactions.

In both acute and chronic RHD, mononuclear cells (macrophages, T and B lymphocytes) infiltrate the heart (Raizada et al, 1983; Kemeny et al, 1989). T and B lymphocytes are able to react against self-antigens through molecular mimicry (Guilherme et al, 1995; Cunningham, 2000, respectively). It has recently been shown that the presence of cross-reactive antibodies in the heart leads to initial tissue inflammation through the action of complement, and to the destruction of myocardial muscle fibers and the valvular endothelial surface. T-cell infiltration takes place through the activated valvular endothelium and the upregulation of certain adhesion molecules, such as VCAM-1, promotes lymphocyte adhesion to the endothelium (Cunningham, 2003).

Furthermore, in acute rheumatic carditis, Aschoff bodies, the pathognomonic sign of the disease, develop in the myocardium and/or endocardium. The Aschoff body is a granulomatous lesion containing both T and B lymphocytes, macrophages, Anitschkow cells, multinucleated cells, and polymorphonuclear leukocytes (Virmani et al, 1999). It is interesting to note that different inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-2 have been found, depending on the developmental phase of the Aschoff body (Fraser et al., 1997). These cytokines and others such as interferon (IFN)-γ play a role in the amplification and maintenance of rheumatic heart lesions.

T-cell recognition is mediated by the T-cell receptor (TCR). Combinations of genes for the TCR generate around 10^{18} possible receptors. Both receptor chains have three regions, designated the complementarity-determining regions CDR1, CDR2, CDR3; CDR1 and CDR2 of the TCR interact with major histocompatibility complex (MHC) molecules. The peptide side chains of the MHC–peptide

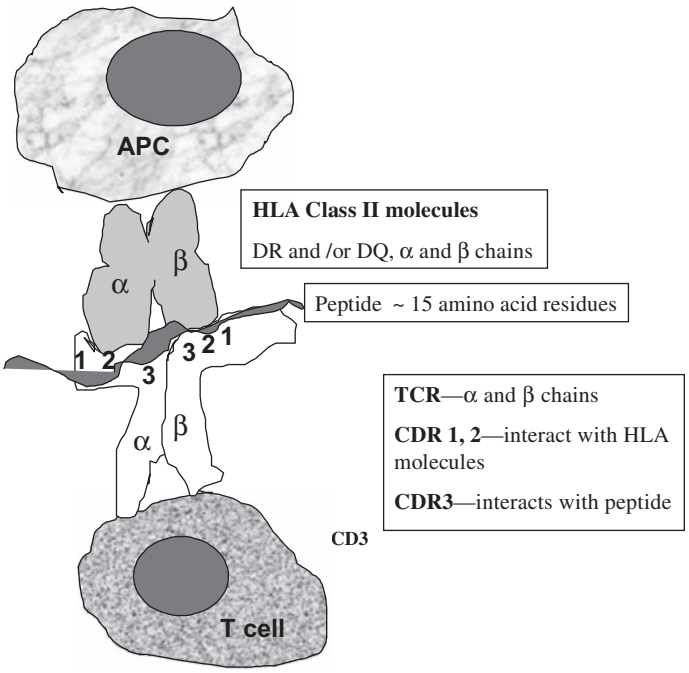


FIGURE 62.1 Trimolecular complex: T-cell receptor (TCR), MHC class II molecules, and immunogenic peptide interactions. Antigen-presenting cells (APCs), such as monocytes in the blood, macrophages in the tissue, dendritic cells, and B lymphocytes take up microbial antigens or self-antigens from damaged tissue, intracellular proteins, generating different peptides by enzymatic degradation. Peptides combined with MHC molecules are presented to $CD4^+$ or $CD8^+$ T lymphocytes, depending on the nature of the protein (extra- or intra-cellular, respectively). Interactions between amino acid residues take place. Complementarity-determining regions (CDRs) 1 and 2, in the α and β chains of the TCR, interact with MHC molecules. CDR3 interacts with the peptide. The peptide interacts only with certain predefined residues of the MHC molecules. In the case of rheumatic heart disease, streptococcal and self-antigens are presented mainly to $CD4^+$ T cells by HLA class II molecules (DR and DQ).

complex on the surface of APCs interact most closely with the hypervariable region of the TCR (CDR3) encoded by the V–D–J region on the TCR β chain. The interactions of HLA class II molecules (mainly DR and DQ) and antigen (microbial or self-antigens) drive the T-cell response (Figure 62.1) and can lead to pathologic or physiologic autoimmunity, as defined by Cohen (2001).

GENETIC FEATURES

RF/RHD occurs only in a select group of individuals, and certain genes appear to be responsible for the disease. Several genetic markers and some molecules involved in the control of immune responses have been studied. Thus, Patarroyo et al. (1979) described an alloantigen on the surface of B cells, designated 883, which was present in high frequency in RF patients. The alloantigen 883 is probably related to the HLA class II molecules. A monoclonal antibody, named D8/17, was produced against B cells from RF patients bearing the 883 alloantigen; however, this monoclonal antibody recognizes another antigen with variable levels of expression on the surface of B cells of RF patients (10–20%). The D8/17 antigen was present in increased frequency in RF patients (90–95%) (Zabriskie et al.,1985).

Association between different HLA class II antigens and RF/RHD has been found in several populations (Table 62.1). HLA-DR7 was the allele most consistently associated with RF (Guilherme et al., 1991; Ozkan et al., 1993; Weidebach et al., 1994, Guédez et al., 1999; Visanteiner et al., 2000; Stanevicha et al., 2003). The association of DR7 with different DQ-B or DQ-A alleles seems to be associated with the development of multiple valvular lesions (MVLs) or mitral valve regurgitation (MVR) in RHD patients (Guédez et al., 1999; Stanevicha et al., 2003). HLA-DR53, another HLA class II molecule, is in linkage disequilibrium with HLA-DR4, -DR7, and -DR9. This allele strongly associated with RF/RHD in two studies with mulatto Brazilian patients (Guilherme et al., 1991; Weidebach et al., 1994), but not in Brazilian white patients (Visanteiner et al., 2000). Although

TABLE 62.1 HLA class II and rheumatic fever (RF)/rheumatic heart disease (RHD)

Country	Population	HLA	Clinical picture	References
South Africa	African	DR1, DR6	RF/RHD	Maharaj et al. (1987)
Brazil	Mulatto	DR1	Sydenham chorea	Donadi et al. (2000)
USA	American black	DR2	RF/RHD	Anastasiou-Nana et al. (1986)
USA	American white	DR4, DR9	RF/RHD	Anastasiou-Nana et al. (1986), Ayoub et al. (1986)
Saudi Arabia	Arabians	DR4	RF/RHD	Rajapakse et al. (1987)
India	Indian	DR3. DQW2	RF/RHD	Jhinghan et al. (1986), Taneja et al. (1989)
Turkey	Turkish	DR11	RF/RHD	Olmez et al. (1992)
Turkey	Turkish	DR3, DR7	RHD	Ozkan et al. (1993)
Brazil	Mulatto	DR7, DR53	RF/RHD	Guilherme et al. (1991)
Brazil	Mulatto	Allogenotype Taq1 DRβ 13,81 kb[†]	RF/RHD	Weidebach et al. (1994)
Brazil	White	DR7	RF/RHD	Visentainer et al. (2000)
Egypt[‡]	Egyptian	DRB1*0701 DQA1*0201	RHD–MVR	Guédez et al. (1999)
		DRB1*13 DQA1*0501, DQA1*0301	RHD	
Latvia[‡]	Latvian	DRB1*0701 DQB1*0302	RF/RHD–MVL	Stanevicha et al. (2003)
		DRB10701 DQB1*0401	RF/MVR and Sydenham chorea	
Japan[‡]	Japanese	DQA1*0104, DQB1*05031	RHD–mitral stenosis	Koyanagi et al. (1996)
Mexico[‡]	Mestizo	DRBq*1602, DQA1*0501, DQB1*0301	RHD	Hernandez-Pacheco et al. (2003)

[†]Defined by RFLP study, 13.81 kb fragment corresponds to the HLA DR53 antigen.
[‡]Studies from Egypt, Japan, Latvia and Mexico employed molecular methods (PCR).
MVL, multivalvular lesions; MVR, mitral valve regurgitation.

DR53 had not been described in previous studies, DR4 and DR9 were associated with RF in American white patients and Arabian patients (Ayoub et al., 1986; Rajapkase et al., 1987), whereas in Egyptian and Latvian patients, DR7 was associated with the disease (Guédez et al., 1999; Stanevicha et al, 2003) (see Table 62.1). In Japanese RHD patients susceptibility to mitral stenosis seems to be in part controlled by the HLA-DQA gene or by genes in close disequilibrium linkage with HLA-DQA*0104 and DQB1*05031 (Koyanagi et al., 1996). HLA-DQA*0501 DQB*0301 with DRB1*1601 (DR2) was associated with RHD in a Mexican Mestizo population (Hernandez-Pacheco et al., 2003) (see Table 62.1). The differences in HLA class II alleles associated with the RF/RHD in different populations are probably due to the ability of these HLA molecules to present various streptococcal epitopes.

ENVIRONMENTAL INFLUENCES

Low socioeconomic status is a marker of regions where RF and RHD are still prevalent. Overcrowding, poor living conditions, and reduced access to healthcare facilitate the spread of streptococcal infections and the development of the disease in individuals with a genetic predisposition. Cold and/or humid climates and high altitude may also increase the prevalence of the disease (Jarvis, 2003).

PATHOGENIC MECHANISMS

The pathogenic mechanisms involved in the development of RF/RHD are not fully understood. However, RF/RHD is the most convincing example of molecular mimicry in

human pathologic autoimmunity, given the cross-reactions between streptococcal antigens and human tissue proteins, mainly heart tissue proteins, which follow throat infection by *S. pyogenes* in susceptible individuals.

RHD is mediated by both humoral and cellular immune responses, but data described in the past 20 years by us and others define RHD as an essentially T-cell-mediated disease. The presence of human and animal antibodies against streptococcal antigens and human heart tissue proteins were described more than 50 years ago, but their role in the development of heart lesions was unknown. Several studies conducted by the Cunningham group showed that cross-reactive antibodies could bind to the endothelial surface, leading to inflammation, cellular infiltration, and valve scarring (Galvin et al., 2000). The upregulation of the adhesion molecule VCAM-1 after binding of cross-reactive antibodies to the valvular endothelium facilitates cellular infiltration (Roberts et al., 2001). These data definitively established the role of the heart tissue cross-reactive antibodies in the early stages of inflammation and T-cell infiltration in RHD lesions.

We shall first describe the major humoral features, followed by the cellular data that have contributed to the current knowledge of autoimmune reactions in RHD, considered to be the human model *par excellence* for post-infection autoimmune diseases.

Humoral Immune Response

Heart-reactive antibodies were first described by Calveti (1945). Kaplan and Suchy (1964) demonstrated the presence of streptococcal and anti-heart cross-reactive antibodies in sera of animals immunized with streptococcal cell wall products, and in sera from RF and RHD patients. They coined the term "biology mimicry" to explain the mechanism for the observed cross-reactivity between human and streptococcal antigens. Kaplan (Kaplan and Svec, 1964) also found immunoglobulins and complement bound to the myocardium of patients with acute RF. After this initial work, several studies were conducted using sera from immunized animals and humans, or monoclonal antibodies, demonstrating the presence of cross-reactive antibodies to streptococcal antigens and human proteins. Among human proteins, cardiac myosin is one of the most studied and seems to be a major cross-reactive antigen [reviewed by Cunningham (2000)].

We have recently analyzed the humoral response against overlapping peptides of the N-terminal portion of the M5 protein and we have identified 11 immunodominant epitopes that are recognized by patients with mild RHD, most with associated Sydenham chorea (Faé et al., 2004). Antibodies from patients with severe RHD recognized five N-terminal epitopes. Only two epitopes, M5(83–103) and M5(181–200), were recognized by antibodies from mild and severe RHD patients (Figure 62.2).

Group A streptococcal antigens, such as hyaluronic acid, M protein N-terminal epitopes, cell wall components (rhamonose, N-acetyl-glucosamine, and peptidoglycan–polysaccharide complexes), and pyrogenic toxins cross-reacted with human proteins, such as laminin, keratin, actin, vimentin, myosin, and tropomyosin [reviewed by Cunnimgham (2000)].

Cellular Immune Response

Studies of the cellular arm of the immune response began in the early 1970s. In light of the important role played by T cells in RF, studies have shown increased numbers of $CD4^+$ T cells in human peripheral blood when compared with normal individuals (Bathia et al, 1989). Also, peripheral T cells are able to recognize streptococcal cell wall and tissue antigens when assessed by proliferation assay of tritiated thymidine incorporation (Read et al., 1974; Gray et al., 1981). The cytotoxic activity of $CD8^+$ T cells from normal peripheral blood towards immortalized human heart cells was also described (Dale et al., 1981). A predominance of $CD4^+$ T cells in rheumatic heart lesions was the first evidence for $CD4^+$ T-cell involvement in RHD (Raizada et al, 1983). The isolation of T cells from heart valves of patients with RF led Yoshinaga et al. (1995) to compare the reactivity of phytohemagglutinin (PHA)-stimulated T-cell lines from heart valve specimens and peripheral blood lymphocytes; such T cells recognized cell wall and membrane streptococcal antigens, but failed to react with the M protein, myosin or other mammalian cytoskeletal proteins.

We have shown the significance of molecular mimicry between β-hemolytic streptococci and heart tissue by analysis of the T-cell repertoire leading to local tissue damage in RHD. Thus, infiltrating T-cell clones from heart lesions of RHD patients with severe RHD do recognize M protein peptides and heart tissue-derived proteins, and there are three M5 immunodominant regions (residues 1–25, 81–103, and 163–177) that cross-react with several heart protein fractions, mainly those derived from valvular tissue, with molecular masses of 95–150, 43–63, and 30–43 kDa (Guilherme et al., 1995). Employing a proteomics approach, we characterized a number of mitral valve proteins identified by molecular weight and isoelectric point. Four valve-derived proteins with molecular masses ranging between 52 and 79 kDa and different isoelectric points cross-reacted with the M5 immunodominant peptides and were recognized by proliferation assay with intralesional T-cell clones from patients with severe RHD (Faé et al., 2005). Figure 62.3 illustrates rheumatic heart lesions and shows the predominance of $CD4^+$ T cells in lesion sites, as well as streptococcal and heart tissue-derived protein reactivity with intralesional $CD4^+$ T-cell clones.

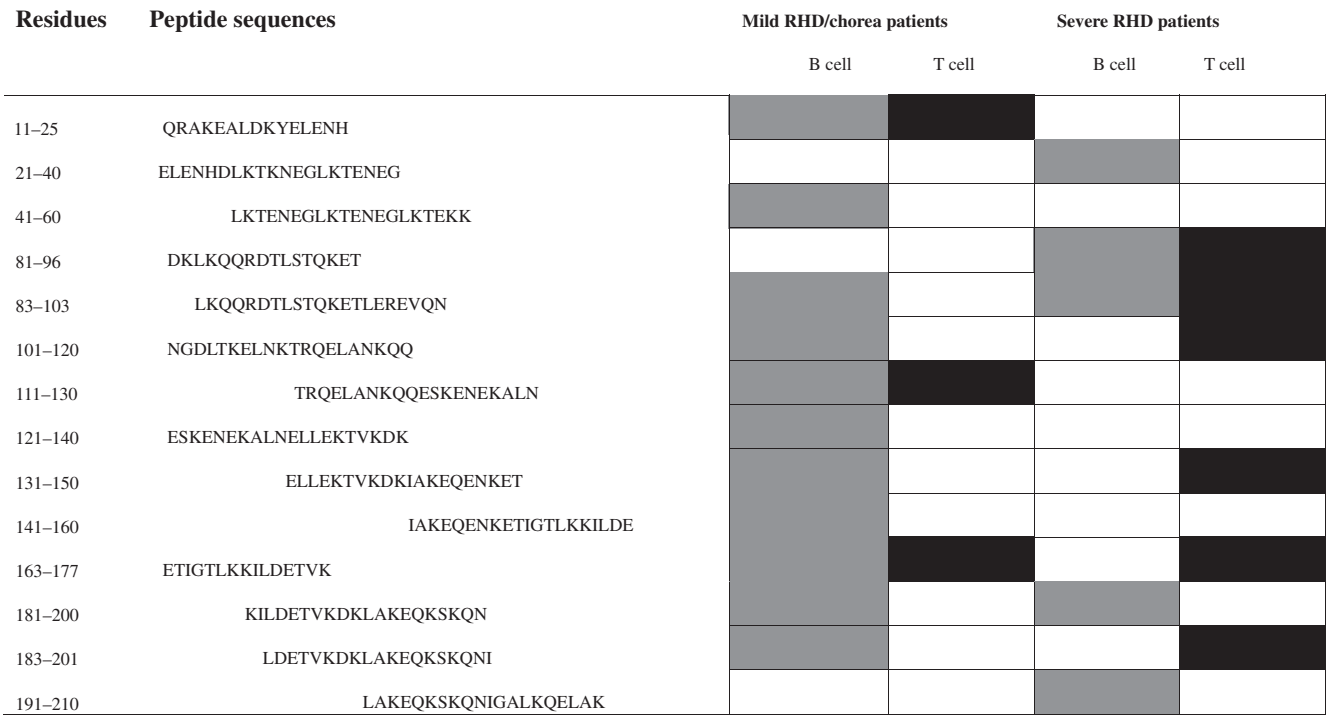


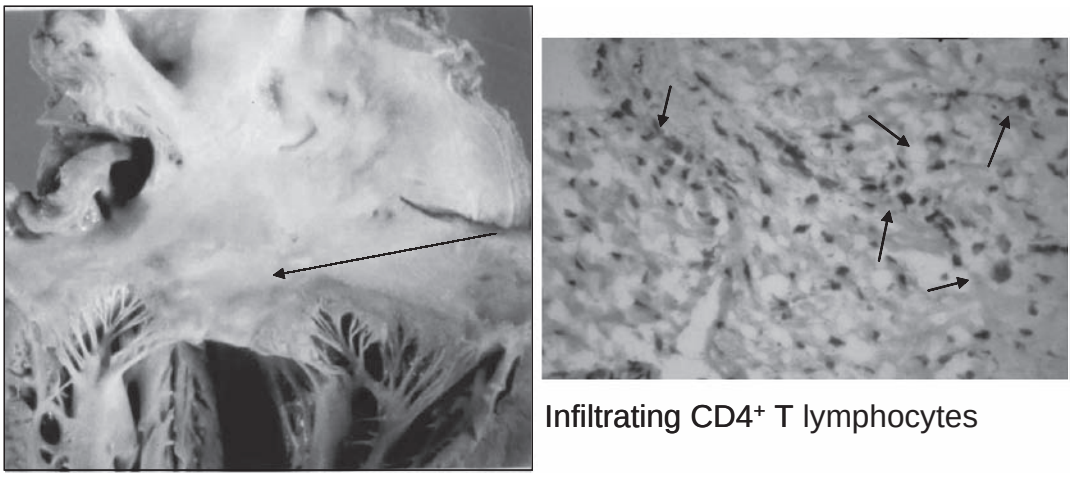
FIGURE 62.2 Immunodominant epitopes of the streptococcal N-terminal region of the M5 protein recognized by peripheral T and B lymphocytes in rheumatic heart disease (RHD) and Sydenham chorea patients. Peptide sequences M5(1–25), M5(81–96), M5(83–103), and M5(163–177) were based on the sequence of the M5 protein published by Manjula et al. (1984). For other peptides the sequences were taken from Miller et al. (1988). Gray boxes, B lymphocyte reactivity; black boxes, T lymphocyte reactivity.

Peripheral blood T lymphocytes from RF/RHD patients also recognize M5 peptides and valve proteins. The M5 epitopes that were preferentially recognized by T lymphocytes from patients with RF presenting mild carditis and/or Sydenham chorea (M5 epitopes 11–25 and 111–130) differed from those recognized by patients with severe RHD (M5 epitopes 81–96, 83–103, 101–120, 131-150, and 183-201). Both groups of patients recognized the M5(163–177) epitope (see Figure 62.2).

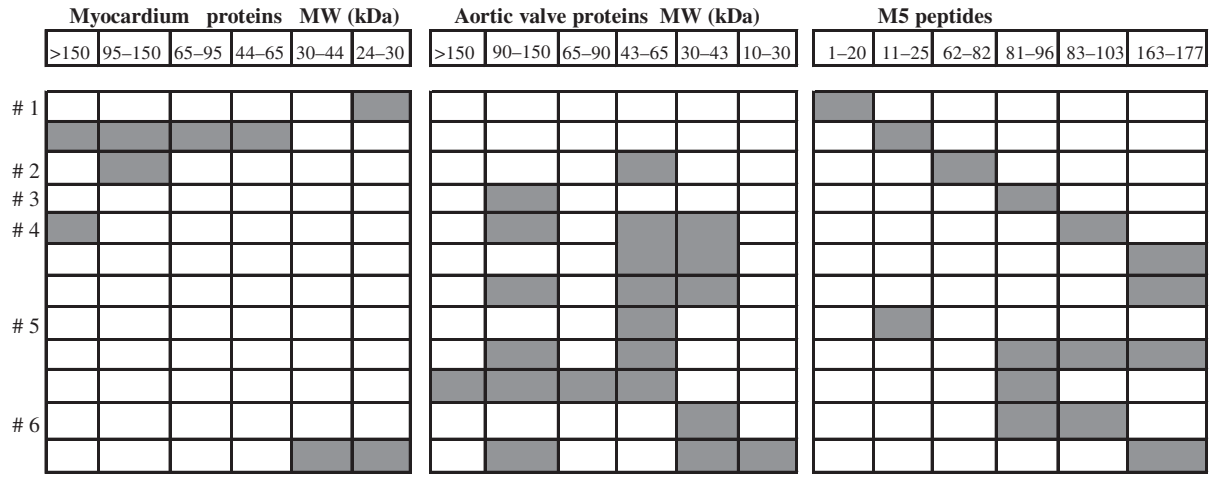
The M5(81–96) epitope is included in the M5(81–103) epitope, which is an immunodominant region recognized by intralesional T cells, and was preferentially recognized in patients with severe RHD and expressing HLA-DR7^{+}DR53^{+} (Guilherme et al, 2001). These data suggest a role for the HLA class II molecules DR7 DR53 in presenting the streptococcal immunodominant peptide to the TCR, and show the significance of the propensity of DR7 to cause severe RHD in Brazilian patients, and probably in other populations in which this class II allele is also associated with RHD and the development of valvular lesions (see Table 62.1). Several other valve-derived proteins were also recognized by peripheral blood T lymphocytes, including vimentin and other cytoskeleton proteins.

Myosin/M5 protein cross-reactive T-cell epitopes were investigated in mice immunized with intact cardiac myosin (Cunningham et al., 1997). Lymph-node T cells were tested by proliferation assays against overlapping M5 peptides, and seven regions, termed NT4/5/6, B1B2, B2, B2B3A, and B3A, were predominantly recognized. The NT5/6 and B1B2/B2 regions aligned with the M5 regions previously identified by our group, namely M5(81–103) and M5(163–177), respectively (Table 62.2). Robinson et al. (1991) obtained lymph node T-cell clones from mice immunized with recombinant M5 protein that recognized M5 epitopes; however, of the M5 epitopes recognized by the mouse T-cell clones, only one region, M5 (1–35), could be aligned with the M5 (1–25) region recognized by human heart-infiltrating T-cell clones (see Table 62.2).

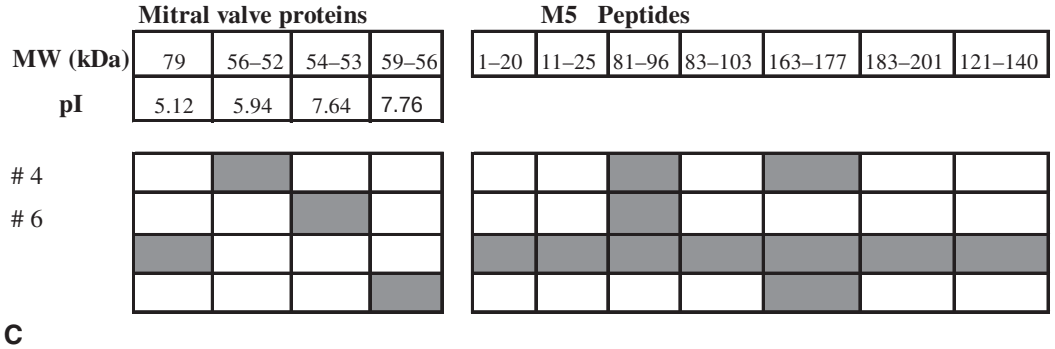
Superantigens are proteins that polyclonally activate T cells through an MHC class II-dependent, but haplotype-unrestricted, mechanism. Proliferative responses to superantigens are limited to T cells expressing a particular TCR-BV gene, but are independent of the antigen specificity of the TCR. The M protein plays an important role in host antistreptococcal immune response and for this reason a putative superantigenic property was investigated in the 1990s. A superantigenic effect of streptococcal M5 protein preparations (pepsin-cleaved fragment, pepM5) for normal human T cells expressing TCR-BV2, BV4, and BV8 was found (Kotb et al., 1990; Tomai et al., 1990; Watanabe-



A



B



C

FIGURE 62.3 Intralesional T-cell reactivity against heart tissue proteins and streptococcal peptides. *A*, Left: mitral valve from a rheumatic heart disease patient showing verrucae lesions (arrow). Right: immunohistochemistry of mitral valve lesions showing infiltrating $CD4^+$ T lymphocytes (arrows). Magnification: 200×. *B* and *C*, Reactivity of $CD4^+$ T-cell clones obtained from heart lesions of six patients. *B*, Twelve T-cell clones (patients 1–6) and *C*, four T-cell clones (patients 4 and 6) showing cross-reactivity between heart tissue proteins identified by molecular weight (MW) and M5 peptides, and MW, isoelectric point (pI), and M5 peptides, respectively.

TABLE 62.2 Murine immunodominant T-cell epitopes of the N-terminal portion of the streptococcal M5 protein

M5 epitope*	Sequence	Reference
1–35	AVTRGTINDPQRAKEALDKYELENHDLKTKNEGLK	Robinson et al. (1991)
40–58 (NT4)	GLKTENEGLKTENEGLKTE	Cunningham et al. (1997)
59–76 (NTS)	KKEHEAENDKLKQQRDTL	
72–89 (NT6)	QRDTLSTQKETLEREVQN	
137–154 (B1B2)	VKDKIAKEQENKETIGTL	
150–167 (B2)	TIGTLKKILDETVKDKIA	
163–180 (B2B3A)	KDKIAKEQENKETIGTLK	
(B3A)	IGTLKKILDETVKDKLAK	

*M5(1–35) were recognized by lymph node cells and T-cell clones from BALB/c mice, and aligned with M5(1–25) recognized by human peripheral T cells and heart-infiltrating T-cell clones from rheumatic heart disease patients. NT5/6 and B1B2/B2 shared sequences with the M5(81–103) and M5(163–177) immunodominant regions recognized by human T cells.

Onishi et al., 1994). However, the superantigenic effect was later discounted by other studies that showed that the superantigenicity of pepM1 and pepM5 was due to contamination with pyrogenic exotoxins, which themselves had a potent superantigen effect on BV2-bearing human T cells (Fleischeir et al., 1992; Li et al., 1997).

We analyzed TCR-BV usage in peripheral blood and heart-infiltrating T-cell lines from patients with severe RHD, looking for oligoclonal β chain expansions in line with antigen-driven immune responses. Our results showed the expansion of several BV families with oligoclonal profiles among heart-infiltrating T cells, favoring the absence of superantigenicity of M proteins in RHD patients (Guilherme et al., 2000).

Cytokines

The cytokine pattern produced by helper T (Th) cells in response to a given antigen is crucial in driving the humoral or cellular immune response.

RF exhibits different clinical features, such as arthritis, chorea, carditis, erythema marginatum, and subcutaneous nodules. Each may involve particular autoantigens as targets of pathologic autoimmunity. Arthritis and mild RHD, frequently associated with chorea, are partly due to a pathologic autoimmune reaction, probably mediated by Th2-type cytokines and leading to an exaggerated humoral response, as reported by several authors [reviewed by Cunningham (2000)]. On the other hand, severe RHD is mediated mainly by T lymphocytes by a delayed-hypersensitivity type reaction, leading to local inflammation, as mentioned above.

The evaluation of proinflammatory cytokine levels produced by peripheral blood and tonsillar mononuclear cells after streptococcal antigen and pokeweed mitogen stimulation from RF/RHD patients without congestive heart failure showed different patterns with peripheral blood mononuclear cells (PBMCs) or tonsillar cells. TNF-α, IL-1, and IL-2 were overproduced by PBMCs but underproduced by tonsillar mononuclear cells (Miller et al., 1989). Increased production of IL-2 in patients with acute RF or active RHD has been reported, with these patients also showing high numbers of $CD4^+CD25^+$ T cells, suggesting expansion of activated $CD4^+$ T cells in peripheral blood during the active phase of the disease (Morris et al., 1993).

In heart lesions, during the acute phase of RHD, the production of IL-1, TNF-α, and IL-2 correlated with the progression of the Aschoff nodule (Fraser et al., 1997). Recently, we showed that intralesional mononuclear cells from heart lesions predominantly secrete IFN-γ and TNF-α in both acute RF and chronic RHD, with low IL-4 production (Guilherme et al, 2004). The predominant Th1-type cytokine produced mainly by $CD4^+$ T cells infiltrating valvular tissue could mediate the severe RHD valve lesions, and an ability of myocardial infiltrating cells to produce regulatory cytokines could contribute to a milder degree of myocardial damage in RHD.

ANIMAL MODELS

In the 1980s it was demonstrated that macrophages of mice infected with group A streptococcus extracts could induce heart lesions when transferred to syngeneic recipients, as well as a specific response to syngeneic heart extracts *in vitro*; this model suggested a key role for macrophages, involving the selection of antigens for presentation to the TCR (Dos Reis et al., 1982). It has been shown, by using M protein synthetic peptides, that immunization with certain peptides could induce inflammatory heart disease in mice (Huber and Cunningham, 1996).

Recently, injection of M6 recombinant protein in Lewis rats was shown to induce myocarditis and inflammatory valvular heart lesions similar to those seen in RHD. A lymph

node $CD4^+$ T-cell line obtained from a rat immunized with M6 recombinant protein responded to the M6 recombinant protein and cardiac myosin by a proliferation assay (Quinn et al., 2001). Following their experiments in animal models, the same group characterized different segments of cardiac myosin capable of inducing myocarditis or valvulitis (Galvin et al., 2002); they described cardiac myosin S2 region epitopes which induced autoimmune myocarditis associated with an upregulation of inflammatory cytokine production in Lewis rats, but without induction of valvulitis (Li et al, 2004).

IMMUNOLOGIC MARKERS IN DIAGNOSIS

Acute-phase reactants and particularly C-reactive protein are useful for diagnosis of acute RF, for exclusion of other diseases, and for monitoring inflammatory activity (Binotto et al., 2002). Elevated or rising levels of antistreptolysin O (ASO) are used to document a previous infection with group A streptococcus, there being a remarkable response during the acute phase of RF. The specificity of this test is 93% when ASO levels are above 960 IU/ml. The detection of autoantibodies and T-cell reactivity of patients with RF and RHD in the sera and peripheral blood, respectively, against streptococcal M protein could help to evaluate the autoimmune responses, but are not currently used for diagnosis.

TREATMENT AND OUTCOME

Prevention of initial attacks of RF requires the eradication of group A streptococcus from the pharynx. Antibiotic therapy is recommended. Benzathine penicillin G is the antibiotic of choice for primary and secondary prevention of RF. In cases of penicillin allergy, other antibiotics should be used as recommended (Dajani et al, 1995). During episodes of arthritis, salicylates continue to be the first-line treatment, but other nonsteroidal anti-inflammatory drugs can also be used. Moderate-to-severe carditis is usually an indication for cortiscoteroids, although efficacy in reducing sequelae has not been proven.

CONCLUDING REMARKS AND FUTURE PROSPECTS

RF/RHD is the most convincing example of molecular mimicry in which the response against *S. pyogenes* triggers autoimmune reactions with human tissues. The development of RHD involves a complex interplay of autoimmune reactions in which several major factors are implicated. $CD4^+$ T lymphocytes are the prime effectors of heart lesions, and display a degenerate pattern of antigen recognition. Several self-antigens, such as vimentin, myosin, and other mitral valve-derived proteins, are recognized by reason of molecular mimicry of streptococcal-immunodominant peptides, particularly in individuals with a genetic predisposition. Some HLA class II alleles have been shown to be genetic markers of susceptibility to RF, particularly HLA-DR7 which is associated with RHD in several countries with populations of different genetic backgrounds. Furthermore, DR7 when combined with certain HLA-DQ alleles seems to be associated with the development of multiple valvular lesions in RHD patients. Autoimmune reactions are mediated mainly by heart-infiltrating $CD4^+$ T lymphocytes; however, a few $CD8^+$ T cells also infiltrate the heart lesions. Th1-type cytokines seem to predominate in heart lesions, especially in valvar lesions. In addition, the deficiencies in IL-4-producing cells in valvular tissue may contribute to the progression of the lesions.

All this information creates a new picture of the development of RHD, opening new possibilities for immunotherapy. Molecular knowledge of the autoimmune reactions mediated by intralesional T cells will certainly aid in the choice of streptococcal-protective epitopes for the construction of an effective and safe vaccine.

References

Anastasiou-Nana, M., Anderson, J.L., Carquist, J.F., and Nana, J.N. (1986). HLA DR typing and lymphocyte subset evaluation in rheumatic heart disease: a search for immune response factors. *Am. Heart J. 112*, 992–997.

Ayoub, E.M., Barrett, D.J., MacLaren, N.K., and Krischen, J.P. (1986). Association of class II histocompatibility leukocyte antigens with rheumatic fever. *J. Clin. Invest. 77*, 2019–2026.

Baggenstone, A.H. and Titus, J.L. (1968). Rheumatic and collagen disorders of the heart. In C. Ch. Thomas (ed.), *Pathology of the Heart and Blood Vessels* (pp 649–722). Illinois: Springerfield.

Bathia, R., Narula, J., Reddy, K.S., Koicha, M., Malaviya, A.N., Pothineni, R.B., Tandon, R., and Bathia, M.L. (1989). Lymphocyte subsets in acute rheumatic fever and rheumatic heart disease. *Clin. Cardiol. 12*, 34–38.

Binotto, M.A., Guilherme, L., and Tanaka, A.C. (2002). Rheumatic fever: Images. *Paediatr. Cardiol. 11*, 1–22.

Calveti, P.A. (1945). Autoantibodies in rheumatic fever. *Proc. Soc. Exp. Biol. Med. 60*, 379–381.

Cohen, I.R. (2001). Antigenic mimicry, clonal selection and autoimmunity. *J. Autoimmun. 16*, 337–340.

Cunningham, M.W. (2000). Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev. 13*, 470–511.

Cunningham, M.W. (2003). Autoimmunity and molecular mimicry in the pathogenesis of post-streptococcal heart disease. *Frontiers Biosci. 8*(Suppl), 533–543.

Cunningham, M.W., Antone, S.M., Smart, M., Liu, R., and Kosanke, S. (1997). Molecular analysis of human cardiac myosin-cross-reactive B and T-cell epitopes of the group A streptococcal M5 protein. *Infect. Immun. 65*, 3913–3923.

Dajani, A., Taubert, K., Ferrieri, P., Peter, G., and Shulman, S. (1995). Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. Committee on

Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, the American Heart Association. *Pediatrics 96*, 758–764.

Dale, J.B., Simpson, W.A., Ofek, I., and Beachey, E. (1981). Blastogenic responses of human lymphocytes to structurally defined polypeptide fragments of streptococcal M protein. *J. Immunol. 126*, 1499–1505.

Donadi, E.A., Smith, A.G., Louzada-Junior, P., Voltarelli, J.C., and Nepom, G.T. (2000). HLA class I and class II profiles of patients presenting RF with Sydenham's chorea. *J. Neurol. 247*, 122–128.

Dos Reis, G.A., Gaspar, M.I.C., and Barcinski, M.A. (1982). Immune recognition in the streptococcal carditis of mice: the role of macrophages in the generation of heart-reactive lymphocytes. *J. Immunol. 128*, 1514–1521.

Faé, K., Kalil, J., Toubert, A., and Guilherme, L. (2004) Heart infiltrating T cell clones from a rheumatic heart disease patient display a common TCR usage and a degenerate antigen recognition pattern. *Mol Immunol. 40*, 1129–1135.

Faé, K.C., Oshiro, S.E., Toubert, A., Charron, D., Kalil, J., and Guilherme, L. (2005) How an autoimmune reaction triggered by molecular mimicry between streptococcal M protein and cardiac tissue proteins leads to heart lesions in rheumatic heart disease. *J. Autoimmun 24*, 101–109.

Fishetti, V. (1991). Streptococcal M protein. *Sci. Am. 264*, 32–39.

Fleischer, B., Schmidt, K.H., Gerlach, D., and Kohler, W. (1992). Separation of T cell stimulating activity from streptococcal M protein. *Infect. Immun. 1*, 767–772.

Fraser, W.J., Haffejee, Z., Jankelow, D., Wadee, A., and Cooper, K. (1997). Rheumatic Aschoff nodules revisited. II. Cytokine expression corroborates recently proposed sequential stages. *Histopathology 31*, 460–464.

Galvin, J.E., Hemric, M.E., Ward, K., and Cunningham, M. (2000). Cytotoxic monoclonal antibody from rheumatic carditis reacts with human endothelium: implications in rheumatic heart disease. *J. Clin. Invest. 106*, 217–224.

Galvin, J.E., Hemric, M.E., Kosanke, S.D., Factor, S.M., Quinn, A., and Cunningham, M. (2002). Induction of myocarditis and valvulitis in Lewis rats by different epitopes of cardiac myosin and its implications in rheumatic carditis. *Am. J. Pathol. 160*, 297–306.

Gray, E.D., Wannamaker, L.W., Ayoub, E. and El Kholy, A. (1981). Cellular immune responses to extracellular streptococcal products in rheumatic heart disease. *J. Clin. Invest. 68*, 665–671.

Guédez, Y., Kotby, A., El-Demellaway, M., Galal, A., Thomson, G., Zaher, S., Kassem, S., and Kotb, M. (1999). HLA class II associations with rheumatic heart disease are more evident and consistent among clinically homogeneous patients. *Circulation 99*, 2784–2790.

Guilherme, L., Weidebach, W., Kiss, M.H., Snitcowsky, R., and Kalil, J. (1991). Association of human leukocyte class II antigens with rheumatic fever or rheumatic heart disease in a Brazilian population. *Circulation 83*, 1995–1998.

Guilherme, L., Cunha-Neto, E., Coelho, V., Snitcowsky, R., Pomerantzeff, P.M.A., Assis, R.V., Pedra, F., Neumann, J., Goldberg, A., and Patarroyo, M.E. (1995). Human heart-infiltrating T-cell clones from rheumatic heart disease patients recognized both streptococcal and cardiac proteins. *Circulation 92*, 415–420.

Guilherme, L., Dulphy, N., Douay, C., Coelho, V., Cunha-Neto, E., Oshiro, S.E., Assis, R. V., Tanaka, A.C., Pomerantzeff, P.M., Charron, D., Toubert, A., and Kalil, J. (2000). Molecular evidence for antigen-driven immune responses in cardiac lesions of rheumatic heart disease patients. *Int. Immunol. 12*, 1063–1074.

Guilherme, L., Oshiro, S.E., Faé, K.C., Cunha-Neto, E., Renesto, G., Goldberg, A.C., Tanaka, A.C., Pomeranteff, P.M., Kiss, M.H., Silva, C., Guzman, F., Patarroyo, M.E., Southwood, S., Sette, A., and Kalil, J. (2001). T cell reactivty against streptococcal antigens in the periphery mirrors reactivity of heart infiltrating T lymphocytes in rheumatic heart disease patients. *Infect. Immunity. 69*, 5345–5351.

Guilherme, L., Cury, P., Demarchi, L.M., Coelho, V., Abel, L., Lopez, A.P., Oshiro, S.E., Aliotti, S., Cunha-Neto, E., Pomerantzeff, P.M., Tanaka, A.C., and Kalil, J. (2004). Rheumatic heart disease: pro-inflammatory cytokines play a role in the progression and maintenance of valvular lesions. *Am. Pathol. J. 165*, 1583–1591.

Hernandez-Pacheco, G., Aguilar-Garcia, J., Flores-Dominguez, C., Rodriguez-Perez, J.M., Perez-Hernandez, N., Alvaez-Leon, E., Reyes, P.A., and Vargas-Alcaron, G. (2003). MHC class II alleles in Mexican patients with rheumatic heart disease. *Int. J. Cardiol. 92*, 49–54.

Huber, A.S. and Cunningham, M.W. (1996). Streptococcal M protein peptide with similarity to myosin induces $CD4^+$ T cell-dependent myocarditis in $MRL/^{++}$ mice and induces partial tolerance against coxsackieviral myocarditis. *J. Immunol. 156*, 3528–3534.

Jarvis, N.T. (2003). Rheumatic fever. *Primary Care Update Obs/Gyns. 10*, 9–13.

Jhinghan, B., Mehra, N.K., Reddy, K.S., Taneja, V., Vaidya, M.C., and Bhatia, M.L. (1986). HLA, blood groups and secretor status in patients with established rheumatic fever and rheumatic heart disease. *Tissue Antigens 27*, 172–178.

Kaplan, M.H. and Svec, K.H. (1964). Immunologic relation of streptococcal antibody cross-reactive with heart tissue: Association with streptococcal infection, rheumatic fever and glomerulonephritis. *J. Exp. Med. 119*, 651–666.

Kaplan, M.H. and Suchy, M.L. (1964). Immunological relation of streptococcal and tissue antigens II. Cross-reactions antisera to mammalian heart tissue with cell wall constituent of certain strains of group A streptococci. *J. Exp. Med. 119*, 643–650.

Kemeny, E., Grieve, T., Marcus, R., Sareli, P., Zabriskie, J.B. (1989). Identification of mononuclear cells and T cell subsets in rheumatic valvulitis. *Clin. Immunol. Immunopathol. 52*, 225–237.

Kotb, M., Majumdar, G., Tomai, M., and Beachey, E.H. (1990). Accessory cell-independent stimulation of human T cells by streptococcal M protein superantigen. *J. Immunol. 145*, 1332–1336.

Koyanagi, T., Koga, Y., Nishi, H., Toshima, H., Sasazuki, T., Imaizumi, T., and Kimura, A. (1996). DNA typing of HLA class II genes in Japanese patients with rheumatic heart disease. *J. Mol. Cell. Cardiol. 28*, 1349–1353.

Lancefield, R.C. (1941). Specific relationships of cell composition to biologic activity of hemolytic streptococci. *Harvey Lectures 36*, 251–265.

Li, P.L.L., Tiedemann, R.E., Moffat, L.S., and Fraser, J.D. (1997). The superantigen streptococcal pyrogenic exotoxin C (SPE-C) exhibits a novel mode of action. *J. Exp. Med. 186*, 375–391.

Li, Y., Heuser, J.S., Kosanke, S.D., Hemric, M., and Cunningham, M.W. (2004). Cryptic epitope identified in rat and human cadiac myosin S2 region induces myocarditis in the Lewis rat. *J. Immunol. 172*, 3225–3234

Maharaj, B., Hammond, M.G., Appadoo, B., Leary, W.P., and Pudifin, D.J. (1987). HLA-A, B, DR and DQ antigens in black patients with severe chronic rheumatic heart disease. *Circulation 76*, 259–261.

Manjula, B.N., Acharya, A.S., Mische, M.S., Fairwell, T., and Fischetti, V.A. (1984). The complete amino acid sequence of a biologically active 197-residue fragment of M protein isolated from type 5 group A streptococci. *J. Biol. Chem. 259*, 3686–3693.

Miller, L.C., Gray, E.D., Beachey, E.H., and Kehoe, M.A. (1988). Antigenic variation among group A streptococcal M proteins: nucleotide sequence of the serotype 5 M protein gene and its relationship with genes encoding types 6 and 24 M proteins. *J. Biol. Chem. 263*, 5668–5673.

Miller, L.C., Gray, E.D., Mansour, M., Abdin, Z.H., Kamel, R., Zaher, S., and Regelmann, W.E. (1989). Cytokines and immunoglobulin in rheumatic heart disease: production by blood and tonsillar mononuclear cells. *J. Rheumatol. 16*, 1436–1442.

Morris, K., Mohan, C., Wahi, P.L., Anand, I.S., and Ganguly, N.K. (1993). Enhancement of IL-, IL-2 production and IL-2 receptor generation in patients with acute rheumatic fever and active rheumatic heart disease; a prospective study. *Clin. Exp. Immunol. 91*, 429–436.

Olmez, U., Turgay, M., Ozenirler, S., Tutkak, H., Duzgun, N., Duman, M., and Tokgoz, G. (1992). Association of HLA class I and class II antigens with rheumatic fever in a Turkish population. *Rheumatology 22*, 49–52.

Ozkan, M., Carin, M., Sonmez, G., Senocak, M., Ozdemir, M., and Yakut, C. (1993). HLA antigens in Turkish race with rheumatic heart disease. *Circulation 87*, 1974–1978.

Patarroyo, M.E., Winchester, R.J., Vejerano, A., Gibofsky, A., Chalem, F., Zabriskie, J.B., and Kunkel, H.G. (1979). Association of a B-cell alloantigen with susceptibility to rheumatic fever. *Nature 278*, 173–174.

Quinn, A., Kosanke, S.D., Fischetti, V.A., Factor, S.M., and Cunningham, M. (2001). Induction of autoimmune valvular heart disease by recombinant streptococcal M protein. *Infect. Immun. 69*, 4072–4078.

Raizada, V., Williams, R.C. Jr., Chopra, P., Gopinath, N., Prakash, K., Sharma, K.B., Cherian, K.M., Panday, S., Arora, R., Nigam, M., Zabriskia, J.B., and Husby, G. (1983). Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal anti-T cells antibodies. *Am. J. Med. 74*, 90–96.

Rajapakse, C.N.A., Halim, K., Al-Orainey, L., Al-Nozha, M., and Al-Aska, A.K. (1987). A genetic marker for rheumatic heart disease. *Br. Heart J. 58*, 659–662.

Read, S.E., Fischetti, V.A., Utermohlen, V., Falk, R.E., and Zabriskie, J.B. (1974). Cellular reactivity studies to streptococcal antigens. Migration inhibition studies in patients with streptococcal infections and rheumatic fever. *J. Clin. Invest. 54*, 439–450.

Roberts, S., Kosanke, S., Dunn, T.S., Jankelow, D., Duran, C.M.G., and Cunningham, M.W. (2001). Pathogenic mechanism in rheumatic carditis: Focus on valvular endothelium. *J. Infect. Dis. 183*, 507–511.

Robinson, J.H., Atherton, M.C., Goodacre, J.Á., Pinkney, M., Weightman, H., and Kehoe, M.A. (1991). Mapping T-cell epitopes in group A streptococcal type 5 M protein. *Infect. Immun. 59*, 4324–4331.

Special Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young of the American Heart Association. *JAMA 268*, 2069–2073.

Stanevicha, V., Eglite, J., Sochevs, A., Gardovska, D., Zavadska, D., and Shantere, R. (2003). HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. *Arthritis Res. Ther. 5*, 340–346.

Stollerman, G.H. (1997). Rheumatic fever. *Lancet 349*, 935–942.

Stollerman, G.H. (1998). The changing face of rheumatic fever in the 20th century. *J. Med. Microbiol. 47*, 1–3.

Taneja, V., Mehra, N.K., Reddy, K.S., Narula, J., Tandon, R., Vaidya, M.C., and Bhatia, M.L. (1989). HLA-DR/DQ antigens and reactivity to B cell alloantigen D8/17 in Indian patients with rheumatic heart disease. *Circulation 80*, 335–340.

Tomai, M., Kotb, M., Majumdar, G., and Beachey, E.H. (1990). Superantigenicity of streptococcal M protein. *J. Exp. Med. 172*, 359–362

Virmani, R., Farb, A., Burke, A.P., and Narula, J. (1999). Pathology of acute rheumatic carditis. In J. Narula, R. Virmani, K.S. Reddy, and R. Tandon (eds), *Rheumatic Fever* (pp 217–234). Washington: American Registry Pathology.

Visentainer, J.E., Pereira, F.C., Dalalio, M.M., Tsuneto, L.T., Donadio, P.R., and Moliterno, R.A. (2000). Association of HLA-DR7 with rheumatic fever in the Brazilian population. *J. Rheumatol. 27*, 1518–1520.

Watanabe-Ohnishi, R., Aelion, J., Legros, L., Tomai, M.A., Sokurenko, E.V., Newton, D., Takahara, J., Irino, S., Rashed, S., and Kotb, M. (1994). Characterization of unique human TCR Vb specificities for a family of streptococcal superantigens represented by rheumatogenic serotypes of M protein. *J. Immunol. 152*, 2066–2073.

Weidebach, W., Goldberg, A.C., Chiarella, J., Guilherme, L., Snitcowsky, R., Pileggi, F., and Kalil, J. (1994). HLA class II antigens in rheumatic fever: analysis of the DR locus by RFLP and oligotyping. *Hum. Immunol. 40*, 253–258.

Yoshinaga, M., Figueiroa, F., Wahid, M.R., Marcus, R.H., Suh, E., and Zabriskie, J.B. (1995). Antigenic specificity of lymphocytes isolated from valvular specimens of rheumatic fever patients. *J. Autoimmun. 8*, 601–613.

Zabriskie J.B., Lavenchy, D., Williams, R.C., Jr., Fu, S.M., Yeadon, C.A., Fotino, M., and Braun, D.G. (1985). Rheumatic fever-associated B cell alloantigen as identified by monoclonal antibodies. *Arthritis. Rheum. 28,* 1047–1051.

CHAPTER

63

Myocarditis and Dilated Cardiomyopathy

NOEL R. ROSE*

**Department of Pathology and W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins University Medical Institutions, Baltimore, Maryland*

KENNETH L. BAUGHMAN†

†Advanced Heart Disease Section, Division of Cardiovascular Medicine, Department of Medicine, Harvard Medical School and Brigham & Women's Hospital, Boston, Massachusetts, USA

HISTORIC BACKGROUND

The role of autoimmunity in cardiovascular disease has long been a topic of investigation in the clinic and the laboratory. Years of research effort were devoted to establishing a link between streptococcal infection and rheumatic heart disease on the basis of an autoimmune response (see Chapter 62). Chagas' disease is believed to be based on a cross-reaction of antibodies to *Trypanosoma cruzi* with myocardial or cardiac conductive tissue (Cunha-Neto et al., 2004). Finally, post-pericardiotomy syndrome and postmyocardial infarction syndrome are sometimes cited as instances of an autoimmune response instigated by damaged or necrotic tissue (Maisch et al., 1979). This chapter reviews the evidence linking autoimmunity with two important forms of heart disease, myocarditis and dilated cardiomyopathy. It must be stated, *ab initio*, that immunologic testing has so far not been effective in allowing a clear distinction between autoimmune and other etiologies of these diseases.

The classic description of myocarditis was given by Corvisart in 1812 [reference in Gravanis and Sternby (1991)], but for many years progress in studying the disease was impeded by the uncertainties of clinical diagnosis. Definitive diagnosis was dependent upon autopsy examination. Interest in the disease increased in recent years because of the availability of better, antemortem diagnostic tools, especially the endomyocardial biopsy, greater understanding of the role of cardiotropic viruses, and the potential of new modalities of therapy.

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Myocarditis

While myocarditis may be asymptomatic, the major features include arrhythmias (palpitations, dizziness, syncope, or sudden cardiac death), embolic events, congestive heart failure, or cardiogenic shock. These clinical findings can be supported by electrocardiographic changes, such as nonspecific ST-T wave abnormalities and atrial or ventricular arrhythmias. Two-dimensional echocardiography, a noninvasive way of evaluating heart size and function, may show normal ventricular size with thick walls and decreased contractility early in the illness or progressive heart enlargement with thinning of the muscle in chronic cases. Biventricular enlargement is seen in chronic cases. A pericardial rub may be detected, indicating pericardial irritation with or

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

without a pericardial effusion. As the disease progresses, gallop rhythms and signs of congestive heart failure appear. The patient may recall a recent viral illness with symptoms of malaise, chills and fever, upper respiratory or gastrointestinal symptoms, myalgia and chest pain. The majority of cases, however, cannot be traced back to an obvious preceding illness. Most patients with myocarditis present with either left ventricular failure or arrhythmias as their only clinical signs.

None of the clinical features described here is diagnostic of myocarditis. Before the widespread use of the endomyocardial biotome, the diagnosis could only be established with certainty by postmortem examination (Mason and O'Connell, 1989). Even now, most patients who are diagnosed clinically with myocarditis do not meet the strict pathologic standards of the disease, referred to as the Dallas criteria.

Studies of the occurrence of myocarditis in North America, Europe, and Japan have suggested that the incidence varies widely in different areas (Jacobson et al., 1997). Prevalence figures of 1.06, 3.5, 5.4, and as high as 10 have been reported in different series (Gravanis and Sternby, 1991). The reasons for these wide differences are not known, but may be related to the differing diagnostic criteria used or may reflect exposures to different types and strains of cardiotropic viruses, as well as genetic differences in the host populations. The 5-year survival of biopsy-proven myocarditis in adults is only 56% (Grogan et al., 1995). In pediatric populations, the prevalence and mortality are even higher. Noren et al. (1976) described histologic evidence of latent myocarditis in 4.2% of accidental deaths of children and 16.7% of unexplained, unexpected deaths, suggesting that unsuspected myocarditis may be a significant cause of unexpected death in children (Liberthson, 1996).

Felker et al. (2000) and McCarthy et al. (2000) have demonstrated that the prognosis of cardiomyopathy in patients with myocarditis is dependent on the clinical pathologic classification of their initial disorder. Patients with fulminant myocarditis, who do not die within the first 2 weeks, have a survival rate which is virtually identical to that of age-matched controls. Patients with subacute myocarditis who develop persistent left ventricular dysfunction have an outcome that is virtually identical to that of patients with idiopathic dilated cardiomyopathy. Patients with chronic persistent myocarditis, while they continue to have myocardial inflammation, have no deterioration in ventricular function and usually have a normal survival. Patients with chronic active myocarditis and who have ongoing inflammation and fibrosis develop a restrictive cardiomyopathy severe enough to require transplantation within 2–3 years (Lieberman et al., 1991). Patients with giant cell myocarditis (Cooper et al., 1997) develop rapidly progressive mildly dilated but severely hypofunctional cardiomyopathy and have a life-expectancy, without vigorous treatment, that is measured in months.

The so-called Dallas criteria, the consensus of a group of cardiovascular pathologists, led to better standardization of the histopathologic examination (Aretz et al., 1986). Active myocarditis requires the presence of mononuclear inflammation associated with adjacent myocyte damage (Figure 63.1). Myocyte damage can take the form of necrosis or myocyte vacuolization. The term borderline myocarditis is applied when an unequivocal diagnosis of myocarditis cannot be made either because the inflammatory infiltrate is too sparse or because the damage to the myocyte is not clearly demonstrable (Figure 63.2). The terminology used for subsequent biopsies is ongoing, resolving or resolved myocarditis. Ongoing myocarditis indicates persistent myocardial inflammation associated with myocyte damage. Resolving myocarditis resembles borderline myocarditis, but reparative fibrosis is evident. Resolved or healed myocarditis is diagnosed if no inflammatory infiltrate is seen.

The Dallas criteria also require that myocarditis must be distinguished from the histologic pattern of myocardial injury evident in ischemic heart disease. The inflammatory infiltrate in myocarditis is composed primarily of mononuclear cells, although in the more acute phases polymorphonuclear cell infiltration is common. A prominent eosinophilia is seen in hypersensitivity myocarditis.

The Dallas criteria, which have been used extensively for clinical trials, may be too insensitive to diagnose many patients with inflammatory heart disease. Utilizing the Dallas criteria even in patients who have died of myocarditis, and with five tissue samples, it is only possible to establish a histologic diagnosis of myocarditis in approximately

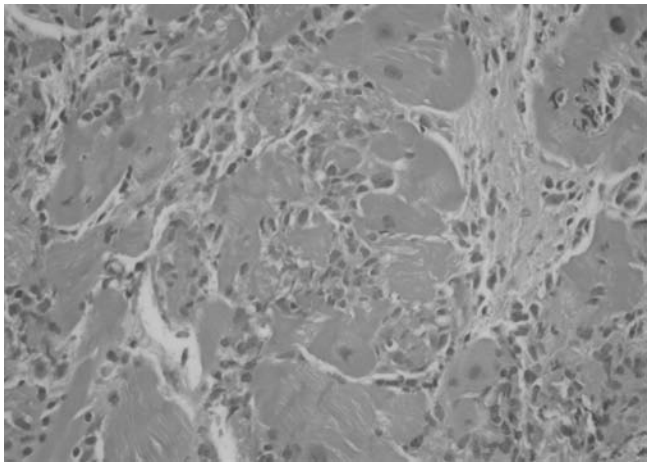
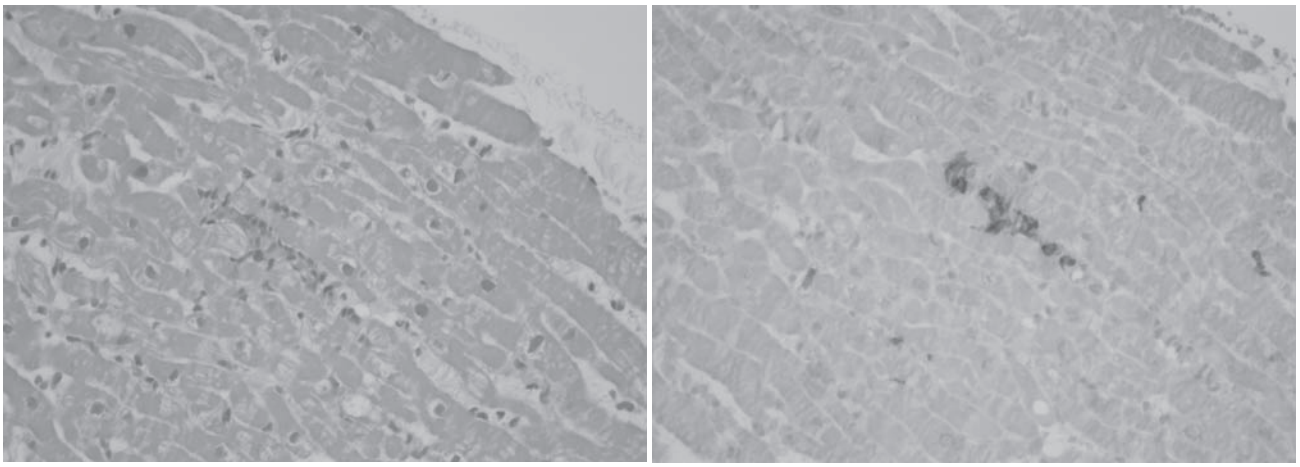


FIGURE 63.1 Lymphocytic myocarditis. There is a heavy infiltrate of large activated lymphocytes throughout the myocardium. Myocyte necrosis is noted in the middle of this image. Fibrosis is present on the right side of the image. (H&E 400×). See color plate section.



A B

FIGURE 63.2 Borderline myocarditis. *A*, A single cluster of perivascular lymphocytes is present. No myocardial damage is identified. (H&E 400×). *B*, A CD8 immunohistochemical stain highlights the infiltrating T lymphocytes. (H&E 400×). See color plate section.

54% of patients (Chow et al., 1989; Hauck et al., 1989). Additionally, in Towbin et al.'s (1994) study of children with suspected clinical myocarditis (67% of these patients had adenoviral infection), 13 of the 26 patients positive for viral nucleic acid by the polymerase chain reaction (PCR) did not display the histopathologic features that would allow the diagnosis of myocarditis to be established. Finally, Wojnicz et al. (2001) and Frustaci et al. (2003), utilizing upregulation of HLA by endomyocardial biopsy or the presence of antiheart antibodies as markers of an autoimmune response, identified patients with suspected myocarditis who appeared to respond to immunosuppressive therapy. It is anticipated that by utilizing other markers of immune activation it will be possible to identify a subpopulation of myocarditis patients who are likely to respond to immunosuppressive therapy.

There are several distinct histologic forms of myocarditis. Drug- or allergy-mediated myocarditis is characterized by an eosinophilic infiltrate. Fulminant myocarditis demonstrates intense infiltration in virtually all sections with marked myocyte necrosis (Figure 63.3). Chronic active myocarditis reveals ongoing myocardial inflammation associated with fibrosis and occasional giant cells (Lieberman et al., 1993). Giant-cell myocarditis is a rare but frequently fatal form of myocarditis (Figure 63.4) (Cooper et al., 1997). The victims are primarily young, healthy adults who die suddenly of heart failure or ventricular arrhythmia. Although cardiac transplantation may be curative, several instances of recurrent disease in the transplanted heart have been reported. Histologic findings in giant-cell myocarditis are diffuse myocardial necrosis with numerous multinucleated giant cells and a mixed inflammatory infiltrate of lymphocytes and macrophages. Collections of eosinophils are seen in some patients.

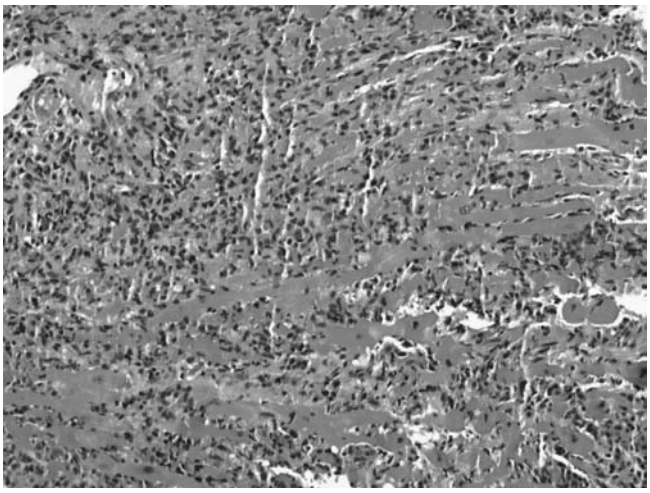


FIGURE 63.3 Fulminant myocarditis. The myocardium is replaced by a marked polymorphous inflammatory infiltrate composed predominantly of lymphocytes and macrophages with rarer eosinophils and neutrophils. Global myocyte injury and loss is noted. No giant cells are present. (H&E 100×). See color plate section.

Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is a chronic form of heart disease characterized by left and right ventricular dilatation and impaired contraction (Gravanis and Ansari, 1987). The clinical spectrum is broad, ranging from individuals with asymptomatic cardiomegaly to patients who present with severe congestive heart failure. Patients may also display symptoms or signs of arrhythmia or systemic embolization with or without congestive heart failure. Other signs include systemic or pulmonary venous congestion,

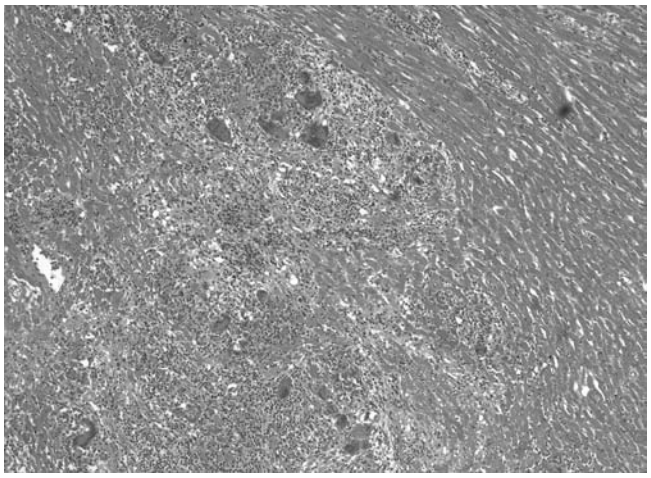


FIGURE 63.4 Giant cell myocarditis. The myocardium is infiltrated by a patchy and diffuse inflammatory infiltrate composed primarily of lymphocytes and macrophages. Multiple collections of giant cells are seen within the infiltrate along with eosinophils. There is significant injury and loss of myocytes in areas of inflammation, while adjacent myocardium is relatively uninvolved. (H&E 50×). See color plate section.

cardiomegaly, gallop rhythms, and mitral or tricuspid regurgitation. The diagnosis requires exclusion of heart failure due to other causes, such as coronary artery disease, toxic exposure, drug allergy, medication (adriamycin) effect or physical agent injury. Once heart failure is established in a patient with DCM, the expected outcome is poor, with a 5-year mortality of 46% (Grogan et al., 1995).

A large number of etiologic agents have been associated with DCM (Felker et al., 2000), including coronary artery disease, infections and metabolic, endocrinologic, and nutritional disturbances, as well as toxins and drugs. However, in approximately 50% of patients, no specific etiology can be identified. The same pathologic process probably results from a number of different etiologies.

In 1985, the prevalence of DCM in Olmsted County, Minnesota, was 36.5 in 100,000. African-American race and male gender were associated with increased risk (Cetta and Michels, 1995). The incidence of DCM in Malmö, Sweden, was reported to be 10 in 100,000 per year (Torp, 1981), and estimated in Great Britain at 0.7–7.5 in 100,000 per year, with a prevalence in 1985 of 8.3 cases in 100,000 (Williams and Olsen, 1985). In a nationwide study in Finland, the incidence of DCM among children and adolescents was 0.34 in 100,000 per year, with a prevalence in 1991 of 2.6 in 100,000. The number of new cases increased each year over the 10-year study period (Arola et al., 1997). Since a number of these patients utilize significant medical resources in their care or eventually require cardiac transplantation, there is a great need for early and definitive diagnosis.

It is still uncertain how many cases represent progression from myocarditis to DCM (Dec et al., 1985). In practice, endomyocardial biopsy is not routinely performed in patients with DCM, so that a possible link between these diseases is based mostly on relatively small case series and anecdotal case reports. For example, Kline and Saphir (1960) documented progressive myocardial failure and death in a series of patients within months to years after acute myocarditis. Miklozek et al. (1986) found that 12 of 16 patients diagnosed as having viral myocarditis had continued cardiac functional abnormalities. Abelmann (1984) reported that half of 16 patients had cardiac symptoms or physical evidence of persistent cardiac dysfunction after recovery from acute myocarditis. Most symptomatic relatives of DCM patients with left ventricular dysfunction have evidence of myocardial inflammation consistent with early or mild myocarditis (Mahon et al., 2002).

Like myocarditis, DCM may be associated with Coxsackievirus infection. In 50 infants and children with DCM, Ayuthya et al. (1974) found significantly increased titers of neutralizing antibody to Coxsackieviruses. Initially, Bowles et al. (1986) and Kandolf et al. (1987) demonstrated Coxsackievirus B-specific nucleic acid sequences in heart tissue of a small number of patients with DCM. Since then molecular testing has become increasingly sophisticated. Towbin et al. (1994) demonstrated that children with suspected myocarditis were PCR positive for viral etiologies on endomyocardial biopsy (26 of 38 samples from 34 patients). Additionally, the viral sequences demonstrated were more frequently adenoviral (15) than enteroviral (8).

In cases of DCM, the heart assumes a globular shape due to enlargement of all of the chambers, especially the left ventricle (Gravanis and Ansari, 1987). The histologic findings are generally nonspecific, and include myocardial cell hypertrophy and an increase in interstitial fibrous connective tissue. In areas of degeneration of the myocardial fibers, small clusters of lymphocytes may be seen. This finding may blur the distinction of DCM from myocarditis.

AUTOIMMUNE FEATURES AND IMMUNOLOGIC MARKERS

Circulating Antibodies

The ambiguities in diagnosing inflammatory disease of the heart muscle are evident. Although the availability of endomyocardial biopsy has helped to clarify the situation, many cases are still inconclusive. There is, at present, a need for noninvasive, inexpensive diagnostic procedures to distinguish autoimmune from other forms of inflammatory heart disease. Much evidence points to a significant role of autoimmunity in some animal models of heart muscle disease. It is logical, therefore, to propose that serologic tests, based on the demonstration of circulating autoantibodies to cardiac antigens, might be useful in the identifica-

tion of autoimmune forms of myocarditis and DCM in humans. The literature was recently reviewed by Caforio et al. (2002) and Rose and Cihakova (2004).

Immunofluorescence

Immunofluorescence tests utilize cardiac tissue of rat or human origin. Both frozen sections and isolated myocytes have been employed. Antibody generally localizes at the surface of the myocyte, giving a sarcolemmal or myolemmal pattern, or on the striations, producing a fibrillar pattern. Whether these two immunofluorescent patterns represent antibodies of different specificities is unclear, because both patterns can be seen in sera from mice immunized with purified cardiac myosin. A major problem in the interpretation of indirect immunofluorescence is the high prevalence of reactions obtained with sera from healthy control subjects. Maisch (1987) found that 91% of patients with myocarditis gave positive reactions with human or rat cardiocytes, but 31–35% of healthy controls showed similar reactions, although generally at lower levels. Neumann et al. (1990) used more conservative criteria, so that 59% of patients with biopsy-proven myocarditis were positive, as were 20% of patients with DCM. In contrast, none of the healthy controls and only 4% of patients with ischemic heart disease were positive in this test under the conditions used.

Western Immunoblot

The Western immunoblot is potentially more sensitive than immunofluorescence and is capable of identifying particular antigens recognized by heart-reactive antibodies. Neumann et al. (1990) detected heart-reactive antibodies in 48 of 103 samples from biopsy-proven myocarditis or DCM patients by immunofluorescence, whereas 97 of the 103 samples exhibited reactivity by Western immunoblotting. No single pattern of antigen reactivity was unique to patients with myocarditis or DCM, but myocarditis sera showed an elevated prevalence of antibody against myosin heavy chain, whereas cardiomyopathy sera exhibited a greater prevalence of reactivity against cardiac muscle actin. Many normal sera reacted with the same antigen, but generally in lower titers. A quantitative immunoassay, such as an enzyme-linked immunosorbent assay (ELISA) using purified human cardiac myosin heavy chain, therefore, is needed for clinical evaluation of these antibodies.

Immunoassay with Defined Antigens

Among the well-characterized antigens used for the study by immunoassay of antibodies in sera of patients with myocarditis and DCM are myosin (Neumann et al., 1990; Caforio et al., 1992), laminin (Wolff et al., 1989) and β1-adrenergic receptors (Limas et al., 1989), and the mitochondrial components, adenine nucleotide translocator (ANT) protein (Schultheiss et al., 1986) and branched-chain ketodehydrogenase (BCKD) (Ansari et al., 1988).

ANT facilitates the transport of ATP into the cytosol and the return of ADP to the inner mitochondrial space. Therefore, it plays a significant role in the energy metabolism of the myocardiocyte. Using a sensitive ELISA, Schultheiss et al. (1986) found that autoantibodies directed to ANT were significantly elevated in 24 of 32 patients with DCM. Control sera from patients with coronary heart disease, alcoholic cardiomyopathy, and hypertrophic obstructive cardiomyopathy were all within normal limits. Since there was less binding of the autoantibodies to the analogous ANT protein from the liver, there was a suggestion of specificity for the cardiac-specific isoform of ANT. Cloning and sequencing of the three isoforms of ANT confirmed the identity of the antigen and identified two protein sequences that react with sera from cardiomyopathy patients (Neckelmann et al., 1987).

IgG fractions of serum were found to inhibit nucleotide exchange in myocytes. Preabsorption of the antisera by isolated heart mitochondria reduced the inhibition of the ADP–ATP catalytic activity. In addition to showing that these antibodies are functionally effective, these experiments suggest that some determinants of the ANT protein are accessible on the surface of the myocardiocyte, possibly as epitopes shared with connexon or calcium channel molecules embedded in the sarcolemma (Morad et al., 1988; Schultheiss et al., 1988).

These results show that many patients with myocarditis and DCM develop autoantibodies to a number of cardiac constituents. Large-scale evaluation is necessary before it can be concluded that detection of any single antibody, or group of antibodies, is sufficiently sensitive and specific to replace the endomyocardial biopsy as a primary diagnostic tool. It does seem, even at this early stage of investigation, that a decline in some antibody titers during treatment may predict a favorable therapeutic response (Müller et al., 2000).

None of these antibodies to cardiac antigens is known to play a direct pathogenetic role in the disease. However, the presence of antibodies to β1-adrenergic receptors in DCM and Chagas' disease is highly suggestive of a direct pathogenetic effect, since the antigen is accessible on the surface of the myocardiocyte. β1-Adrenergic receptor antibodies can induce apoptosis in isolated adult cardiomyocytes (Staudt et al., 2003) and antibodies activating the receptors are associated with reduced cardiac function in chronic heart failure (Jahns et al., 1999). Antibodies to the mitochondrial antigens, ANT and BCKD, may also have adverse consequences on cardiac function. It is not clear, however, whether these antibodies have access to their target antigens *in vivo*. Morad et al. (1988) have provided evidence that ANT antibodies interfere with calcium flux in the isolated

myocardial cell, perhaps because a related antigen is expressed at the cell surface.

While the pathogenetic importance of antibodies is still controversial, the finding that reduction of immunoglobulins in plasma by an immunoadsorption column benefits cardiomyopathy patients provides strong evidence of their involvement (Wallukat et al., 1996; Dorffel et al., 2000; Müller et al., 2000). Although immunoadsorption does not establish the specificity of the autoantibodies that are depleted, reduction of certain immunoglobulin subclasses (IgG3) may be especially beneficial (Staudt et al., 2002).

There is little information about circulating T cells in human disease, although findings from studies in experimental animals indicate that they play a key role in inducing myocarditis (Smith and Allen, 1991).

Immunologic Assessment of Biopsies

In addition to studies of circulating antibody, immunologic methods can contribute to the diagnosis of heart disease by the identification of immunoglobulin and complement in biopsy specimens. Hammond et al. (1988) found that 55% of patients with active myocarditis had deposits of IgG and complement component C3 in their biopsies. Thirty-nine percent of borderline myocarditis (inflammation without myocyte necrosis) cases and 6% of DCM cases were also positive. Patients with other autoimmune diseases, such as systemic lupus erythematosus and scleroderma, sometimes showed deposits of IgG and C3 in their heart tissue, but these usually were coarse, granular deposits in the interstitial spaces of the myocardium or endocardium, probably representing immune complexes.

Immunofluorescence with defined antisera has been used to identify infiltrating cells in cardiac biopsies. Luppi et al. (2003) found that the myocardium of myocarditis and DCM patients was infiltrated by macrophages and $CD4^+$ and $CD8^+$ T lymphocytes. In the majority of patients, the T-cell receptor (TCR) repertoire was restricted with a polyclonal expansion of the Vβ7 gene family. Evidence of Coxsackievirus infection was also found.

The expression of MHC class I and class II antigens in biopsy specimens was evaluated by Herskowitz et al. (1990). In control samples, only low levels of MHC class I molecules were expressed on interstitial cells and vascular epithelium, while MHC class II could not be demonstrated immunohistologically. Increased myocardial expression of MHC class I and *de novo* expression of class II antigens were found in 85% of the myocarditis patients and 33% of the DCM patients.

GENETIC FEATURES

Because of the possible autoimmune origin of myocarditis and DCM in humans, and the well-documented association of experimental myocarditis with the major histocompatibility complex (MHC) in mice (Rose et al., 1988), a number of studies to determine the relationship with the human MHC (HLA) have been carried out. Anderson et al. (1984) reported that DCM patients had an increased frequency of HLA-DR4 and a decreased frequency of HLA-DR6. These findings were corroborated by Limas et al. (1990), who also demonstrated an increased frequency of HLA-DR4 in DCM patients. A genetic predisposition toward cardiac autoimmunity was demonstrated, in that 72% of $HLA\text{-}DR4^+$ patients had anti-β1-adrenergic receptor antibodies compared with 21% of $HLA\text{-}DR4^-$ patients. In the largest study to date, Carlquist et al. (1991) reconfirmed these findings and also found that the DR4-DQw4 haplotype conferred heightened risk of disease. In a meta-analysis of five studies, they confirmed that the DR4 association with myocarditis was sustained among different patient populations. No differences in disease phenotypes have been reported.

A predominance of myocarditis in males has been reported in a number of studies. The proportion of male patients is about 60% (Lieberman et al., 1991; Mason et al., 1995). In this respect, myocarditis differs from most autoimmune diseases, which predominantly affect females.

ENVIRONMENTAL FEATURES

Myocarditis has both infectious and noninfectious causes. As noninfectious agents of myocarditis, a number of drugs have been implicated, acting either directly as toxic agents or as triggers of an allergic response. This section deals with infectious myocarditis; there is little information on cardiac autoimmunity in other forms.

Acute myocarditis is associated with infections of many types, including bacterial, rickettsial, viral, mycotic, protozoan, and helminthic. Several viruses have been implicated in this disease and, in some cases, multiple viruses may be detected in the heart. In Europe and North America, among the most common agents are the enteroviruses and the adenoviruses. Grist and Bell (1974) reported that Coxsackievirus group B infections were associated with at least half of the acute cases of myocarditis. By immunofluorescence, Burch et al. (1968) found Coxsackievirus B antigen in the myocardium of 30.9% of routine autopsy specimens of myocarditis. Serotype B3 is identified most frequently. More recent studies have suggested that adenoviruses are more prevalent in pediatric patients (Bowles et al., 2003).

By using molecular genetic methods, enteroviral and adenoviral genomes were detected in 10–35% of endomyocardial biopsies from patients with myocarditis or DCM (Baboonian and McKenna, 2003; Pauschinger et al., 2004). Among the other viruses found were parvovirus, cytomegalovirus (CMV), influenza A virus, Epstein–Barr virus, and respiratory syncytial virus (Bowles et al., 2003).

Like other enteroviruses, Coxsackieviruses enter the alimentary tract and are acid stable. They multiply in the small intestine. Following replication, viremia develops, seeding the infectious agents in selected tissues. As an obligatory intracellular parasite, the virus must enter a cell through receptor-mediated endocytosis. The virus receptor determines the tropism of the virus. Cardiotropic CB3 employs myocyte surface molecules as receptors, whereas hepatotropic or diabetogenic virus strains utilize receptors on hepatocytes or pancreatic islet cells, respectively. The infection may cause cell death directly, or act indirectly to stimulate an immunopathic host response (Huber, 1997). Coxsackievirus B3 RNA can persist in the myocardium for many days after infectious virus is no longer demonstrable (Klingel et al., 1992) and may, even without the ability to multiply, cause ventricular compromise in the face of a stimulated immune system (Wessely et al., 1998).

The CB3 genome is a single molecule of positive-sense RNA of approximately 7400 nucleotides in length. The genome codes for four capsid proteins as well as for the nonstructural proteins necessary for viral replication. A comparison of cloned cDNA from cardiovirulent and noncardiovirulent strains showed that sites within a nontranslated region and in the capsid protein affect virulence (Tracy et al., 1996).

Nutrition also plays a role in determining susceptibility to viral myocarditis. Beck et al. (1995) demonstrated that mice fed a diet deficient in selenium and infected with a noncardiovirulent strain of CB3 developed severe myocardial damage. Virus isolated from the hearts was fully virulent; six point mutations distinguished the virulent strains. The accumulation of these multiple mutations may be the result of greater viral replication in the hearts of selenium-deficient mice and may be attributable to decreased immune responses compared to selenium-adequate mice (Levander and Beck, 1997). These results may shed light on the etiology of an endemic form of cardiomyopathy known as Keshan disease, seen primarily in selenium-deficient regions of China (Abelmann, 1984).

Chagas' disease is a major cause of heart muscle disease in Latin America, responsible for 50,000 deaths annually (Tanowitz et al., 1992). It is caused by the hemoflagellate, *Trypanosoma cruzi*, which is transmitted to humans via the bite of the reduviid bug, triatomina. Most patients initially have only mild, influenza-like symptoms, but 10–30% of infected individuals develop fulminant myocarditis. Chronic Chagas' disease may present with arrhythmias, thromboembolic events, and congestive heart failure. It represents a particularly lethal form of cardiomyopathy, as survival after presentation is two- to four-fold shorter than that of patients with other forms of DCM (Cunha-Neto et al., 2004).

Antibodies to a number of cardiac antigens, including fibronectin, laminin, and myosin are found in many patients with chronic Chagas' disease, as well as with other forms of DCM (Ballinas-Vedugo et al., 2003). Molecular mimicry between *T. cruzi* and heart disease has been cited as a mechanism to explain the production of autoantibodies during infection (Kalil and Cunha-Neto, 1996). A number of candidate antigens have been described, including a heart-specific epitope of cardiac myosin heavy chain and a 12-amino acid peptide of Fl160, a 160-kDa protein on the surface of *T. cruzi* that mimics a similar protein found on mammalian axonal and myenteric plexus cells (Van Voorhis et al., 1991). The latter antibody is of special interest because of the occurrence of megacolon, megaesophagus, and other neuropathies during chronic Chagas' disease. Among the other antigens described as possible initiators of cross-reactive responses are a peptide of the second extracellular loop of the β1-adrenergic receptor (Ferrari et al., 1995), a *T. cruzi* ribosomal protein R13 (Motran et al., 2000), a ribosomal P protein (Kaplan et al., 1997), and cha. The latter antigen is recognized by T-cells of patients as well as antibodies (Girones et al., 2001).

Kawasaki syndrome is an acute febrile disease of infants and young children that is often associated with myocarditis. Although the etiology is uncertain, available evidence implicates bacterial infection. Cunningham et al. (1999) showed that sera from 5 of 13 patients with Kawasaki syndrome recognized peptides from the light meromysin region of human cardiac myosin and had a different pattern of reactivity from acute rheumatic fever sera.

ANIMAL MODELS AND PATHOGENIC MECHANISMS

Since enteroviruses are most often implicated in human myocarditis and DCM, these agents have been widely used to investigate the pathogenetic mechanisms of these diseases. Although infections by CB3 are relatively common, the development of clinically significant myocardial disease in humans is quite uncommon, suggesting that differences in host response play a critical role in disease susceptibility. These differences are likely to be genetically determined and may relate to the expression of virus-specific receptor on heart tissue or to the immune response of the host. Because it is difficult to examine the role that genetic polymorphisms play in humans, investigators have developed models of Coxsackievirus-induced myocarditis in mice, for which a large number of genetically different, inbred strains are available.

All strains of mice tested developed acute myocarditis, starting 2 or 3 days after CB3 infection. The disease reached its peak on day 7 and gradually resolved, so that by day 21 the heart was histologically normal. In a few strains of mice, however, the myocarditis persisted (Rose et al., 1987). Further studies resolved CB3-induced myocarditis into two distinct phases (Rose et al., 1986). The first phase occurred during the first week after infection and was characterized by focal necrosis of myocytes and an accompanying focal

acute inflammatory response with a mixed-cell infiltrate consisting of polymorphonuclear and mononuclear cells. The second phase became evident about 9 days after infection and was fully manifest 15–21 days after infection. Histologically, the inflammatory process was diffuse rather than focal and consisted mainly of a mononuclear interstitial infiltrate. Little or no myocyte necrosis was evident at that time. Infectious virus could be cultured only during the first phase of disease; no virus was isolated after day 9. Heart-reactive autoantibodies were present in all strains that developed the second phase of myocarditis. Only certain inbred strains of mice developed this secondary autoimmune myocarditis. Susceptibility was determined primarily by non-H2 background genes, although H2-encoded differences influenced the severity of the autoimmune disease (Rose et al., 1988). This finding suggested that the second phase represented an autoimmune response initiated by molecular mimicry between the virus and heart antigens (Cunningham, 2004). Available evidence suggests that the autoimmune response depends upon virus-induced damage to the heart, since Horwitz et al. (2000) found that transgenic mice expressing interferon (IFN)-γ in their pancreatic β cells failed to develop CB3-induced myocarditis, even though the virus proliferated in other sites. This work challenges the concept of molecular mimicry as the mechanism initiating the autoimmune response and suggests that it is due to a "bystander effect" (Rose, 2000). The virus infections may serve as an adjuvant for the cardiac antigens liberated during the viral infection of the heart (Rose, 2000).

The demonstration of autoantibodies in the late phase of CB3-induced myocarditis provided the opportunity to evaluate the pathogenic significance of the autoimmune response. The first step was characterization and isolation of the target antigen. Alvarez et al. (1987) and Neu et al. (1987a) showed that cardiac myosin heavy chain was the major antigen. Using purified mouse cardiac myosin incorporated in complete Freund's adjuvant (CFA), Neu et al. (1987b) were able to produce cardiac lesions that resembled the late phase of CB3-induced myocarditis. Moreover, the inbred strains of mice that were genetically susceptible to the late-phase disease were also susceptible to myosin-induced myocarditis, whereas the strains resistant to the autoimmune phase following viral myocarditis were also resistant to myosin-induced myocarditis. The disease is also induced by short peptides derived from cardiac myosin heavy chain, and by injecting dendritic cells loaded with peptide (Pummerer et al., 1996; Kohno et al., 2000; Eriksson et al., 2003). Taken together, these investigations show that the late phase of CB3-induced myocarditis, which occurs in a few genetically susceptible strains of mice, results from an autoimmune response to cardiac-specific determinants on the myosin heavy chain molecule.

Coincident with the humoral autoimmune response to myosin among both CB3-infected and cardiac myosin-immunized mice, autoreactive T cells are evident. Huber and Job (1983) demonstrated that CB3 infection of BALB/c mice results in the induction of two populations of cytotoxic T cells, one of which is specific for CB3-infected myocytes and one specific for uninfected myocytes. Although the antigen specificity of the latter population has not been determined, Huber et al. (1988) suggested that the reactivity was directed against novel determinants that were expressed on the myocyte surface as a consequence of altered myocyte metabolism. Neumann et al. (1991) reported that spleen cells from CB3-infected mice respond to stimulation *in vitro* with myosin. Also, Smith and Allen (1991) were able to demonstrate, in mice immunized with cardiac myosin, the presence of myosin-specific spleen cells by stimulation *in vitro* of cellular proliferation. Mice depleted of either $CD4^+$ or $CD8^+$ T cells failed to develop myocarditis when immunized with cardiac myosin (Pummerer et al., 1993). Several groups have reported successful transfer of disease from immunized to susceptible mice with T cells (Pummerer et al., 1996; Afanasyeva et al., 2001a).

The pathogenetic role of antibody has also been investigated (see Chapter 7). Neumann et al. (1992) eluted antibody from the hearts of myosin-immunized or CB3-infected mice and showed that it reacted with cardiac myosin. These findings strengthened earlier reports of IgG and complement deposition in the hearts of mice with myocarditis and suggested that antibody as well as T cells participate in the pathologic process. They further suggested that myosin, an intracellular protein, is expressed on the cell surface wholly or in part during the disease. Liao et al. (1995) showed that myosin-specific monoclonal antibody can transfer disease in DBA/2 mice, which express myosin or a myosin-like molecule in the myocardial extracellular matrix. It is possible, therefore, that both humoral and cellular immune processes contribute in differing degrees to the continuing pathology in accordance with the mouse strain investigated. In A/J mice, the disease shows the classic signs of both type 1 helper T-cell (Th1) and Th2 responses (Afanasyeva et al., 2001a; 2001b). Both interleukin (IL)-12 and IL-4 are required for full expression of the typical lesions. Unexpectedly, IFN-γ downregulates the pathogenic immune response to cardiac myosin and reduces the post-viral phase of myocarditis (Afanasyeva et al., 2004; Fairweather et al., 2004a). IFN-γ-deficient mice develop a severe form of heart muscle disease, resembling DCM and often leading to functional evidence of heart failure (Afanasyeva et al., 2005).

Recent investigations direct attention to the critical role of the initial, innate immune response in switching from harmless autoimmunity to a pathogenic autoimmune response (Lane et al., 1991; 1992; 1993; Fairweather et al., 2004a; 2004b). Administration of pro-inflammatory cytokines, IL-1 or TNF-α, to genetically resistant B10.A mice confers susceptibility to myocarditis after challenge

with CB3 or murine myosin in CFA. On the other hand, antibody to TNF-α or IL-1 receptor antagonist prevents or delays the onset of myocarditis in genetically susceptible A/J mice, leading to the conclusion that these early inflammatory mediators are required for autoimmune myocarditis, possibly by inducing nitric oxide and reactive oxygen intermediates (Rose and Hill, 1996).

Myocarditis can be induced in mice by other viruses, including mouse CMV, a herpes virus. Sublethal mouse CMV infection of BALB/c mice induces inflammation of the heart similar to that seen in the CB3-induced disease of mice (Lawson et al., 1992; Lenzo et al., 2003). Infectious virus was not detected in the heart after 10 days, but antibodies to cardiac myosin were evident. As described above, Chagas' disease in humans is caused by *T. cruzi*, and is also associated with autoimmunity to cardiac myosin heavy chain (Cunha-Neto et al., 2004). Thus, immunity to cardiac myosin is a feature common to autoimmune myocarditis induced by diverse infectious agents, making it more likely that endogenous antigen of the host is responsible for the induction of disease.

Myocarditis and autoantibodies to cardiac myosin heavy chain occur spontaneously in mice expressing the human MHC molecule HLA-BQ8 (Taylor et al., 2004), showing that autoimmune disease can occur in the absence of infections.

Okazaki et al. (2003) reported that mice deficient in the programmed cell death-1 (PD-1) immunoinhibitory coreceptor develop DCM accompanied by production of antibodies to cardiac troponin I. Administration of monoclonal antibody to troponin induced cardiac dilatation and dysfunction, indicating that different cardiac-specific antigens may induce a similar autoimmune disease in mice.

TREATMENT AND OUTCOME

Until recently, the only treatment for myocarditis and DCM has been supportive therapies, such as bedrest and treatment of heart failure, arrhythmias, and embolic events if present. In many centers, cardiac transplantation has become the eventual treatment of choice in patients with refractory heart failure. The role of immunosuppressive therapy in myocarditis remains controversial. Numerous reported studies on relatively small numbers of patients have generally found that, while some individuals respond well to immunosuppression (prednisone and cyclosporine), others fail to respond or even have serious adverse reactions that preclude continued treatment. The major problem at present is surely the difficulty in distinguishing immune-mediated cardiac disease from infectious, genetic or toxic forms of the disease. Obviously, until there are reliable biomarkers to distinguish autoimmune myocarditis/DCM, treatment cannot be rational or capable of evaluation.

A placebo-controlled study of the treatment of idiopathic DCM was performed by Parrillo et al. (1989). The study demonstrated that unselected patients with DCM overall did not benefit substantially from immunosuppressive therapy, but a small, if transient, benefit was demonstrated in patients who had histologic evidence of active inflammation by biopsy.

Mason et al. (1995) assigned a series of patients with a histopathologic diagnosis of myocarditis (based on the Dallas criteria) and a low left ventricular ejection fraction to receive conventional therapy, with or without a 24-week course of prednisone plus cyclosporine or azathioprine. The outcome assessed was improvement in the left ventricular ejection fraction at 28 weeks compared with a placebo control group. No significant functional improvement was seen with immunosuppressive therapy. It should be noted, however, that during the establishment of the trial as many as 2233 patients with a clinical and pathologic diagnosis of myocarditis were presented for entry by their cardiologists, but only 111 met the strict Dallas criteria; i.e., some 95% of patients with a preliminary diagnosis of myocarditis failed to fulfill the Dallas criteria. The patients who would be expected to benefit most from immunosuppressive treatment are those with primarily autoimmune rather than viral myocarditis.

This interpretation is favored by the report of Kühl and Schultheiss (1995), who selected 48 patients presenting with mild-to-severe heart failure and immunohistologic evidence of an active immunologic process on biopsy. After a 6-month treatment with 6-methylprednisolone, 23 experienced objective improvement in cardiac function. The suggestion of this study is that in a subgroup of patients with an active immunopathologic process immunosuppressive treatment will confer clinical benefit.

Wojnicz (2001) evaluated 202 patients with idiopathic DCM by endomyocardial biopsy. Eighty-four of these 202 had HLA class II^+ biopsies. Those patients were randomized to immunosuppressive therapy or placebo. While the major outcome of the trial (death, transplantation or hospitalization) was unchanged by treatment, the ejection fraction in the treated population rose from 24% to 36%, while it was virtually unchanged in the placebo group (25% to 27%). In addition, subjective parameters of improvement were noted at 3 months in 72% of the immunosuppressive therapy patients compared with only 21% of the control group. Frustaci et al. (2003) identified 112 of 652 patients with new-onset left ventricular compromise who had a presentation compatible with myocarditis. Forty-one of the 112 had progressive heart failure despite standard heart failure management. This patient population was treated with prednisone and azathiaprine. Twenty of the 41 patients responded while 21 did not. The investigators determined retrospectively that those who responded had antiheart antibodies by immunofluorescence, and those who failed to

respond had persistent virus demonstrated by PCR analysis of the endomyocardial biopsy. Additionally, Jones et al. (1991) demonstrated that in 20 patients with Dallas criteria-positive myocarditis, those with borderline myocarditis had a greater improvement in their ejection fraction by echocardiography and stroke work index from right heart catheterization than those with frank myocarditis. These studies suggest that there soon may be more sensitive and specific biomarkers for immune-mediated heart disease than are currently available by the Dallas criteria.

Different myocarditis populations may respond differently. Patients with fulminant myocarditis usually resolve spontaneously and there is suggestive evidence that immunosuppressive therapy may worsen the outcome. In contrast, the treatment of giant-cell myocarditis with immunosuppression may improve the prognosis by slowing progression of the disease (Cooper, 2002), suggesting that this form of the disease is an autoimmune variant.

Other therapeutic approaches have been based on the possible pathogenic role of humoral antibodies. As described above, several investigators reduced the level of circulating immunoglobulin by means of an immunoabsorption column, and showed improvement in cardiac function. McNamara et al. (2001) demonstrated in a trial of 72 patients with new onset cardiomyopathy that 2 g/kg of intravenous immunoglobulin failed to improve ejection fraction or survival when compared with placebo-treated patients. Importantly, both groups increased their ejection fraction by 14–16% at 6 and 12 months and had transplant-free survivals of 92% at 1 year.

On the premise that many patients with myocarditis or idiopathic DCM have an undisclosed persistent viral infection, Miric et al. (1994) treated a series of 180 patients with IFN-α or thymomodulin. Left ventricular function, exercise tolerance, and survival rate were significantly improved in patients given the immunomodulatory therapy. Kühl et al. (2003) treated 22 patients with proven viral myocarditis with IFN-β. The treatment was well tolerated. All patients cleared the viral genome and showed improved left ventricular function.

The above studies make it obvious that treatment of myocarditis and DCM is still problematic. As more is learned about the pathogenetic mechanisms in these diseases, treatments can be individualized.

PERSONAL THOUGHTS

The diseases described in this chapter exemplify the broad range of cardiovascular disorders with which autoimmune responses have been implicated. Until very recently the role of autoimmunity in cardiovascular diseases had been rather neglected. Yet, before the early 1960s, rheumatic fever was a major topic of investigation. Although the decline of rheumatic fever in the 1960s accounted for a loss of interest in this topic, it must be recognized that the studies of rheumatic fever were the stimulus for many current concepts of autoimmunity and autoimmune disease. The renaissance in cardiovascular immunology followed efforts to define the role of autoimmunity in myocarditis and DCM. There is now a substantial challenge in developing reliable and robust *in vitro* assays that define autoimmune heart disorders with the same sensitivity and specificity now available in autoimmune disorders affecting other major organs. These studies have also served as the impetus to delineate the contribution of autoimmunity to other enigmatic cardiovascular diseases such as atherosclerosis (see Chapter 64). Further, they have served as a general model for studies relating infection to the onset of autoimmune disease.

Acknowledgment

The figures were prepared by Dr Marc Halushka, Department of Pathology, Johns Hopkins School of Medicine.

References

Abelmann, W.H. (1984). Classification and natural history of primary myocardial disease. *Prog. Cardiovasc. Dis. 27*, 73–94.

Afanasyeva, M., Wang, Y., Kaya, Z., Park, S., Zilliox, M.J., Schofield, B.H., Hill, S.L., and Rose, N.R. (2001a). Experimental autoimmune myocarditis in A/J mice is an interleukin-4-dependent disease with a Th2 phenotype. *Am. J. Pathol. 159*, 193–203.

Afanasyeva, M., Wang, Y., Kaya, Z., Stafford, E.A., Dohmen, K.M., Sadighi Akha, A.A., and Rose, N.R. (2001b). Interleukin-12 recepter/STAT4 signaling is required for the development of autoimmune myocarditis in mice by an interferon-gamma-independent pathway. *Circulation 104*, 3145–3151.

Afanasyeva, M., Georgakopoulos, D., Belardi, D.F., Ramsundar, A.C., Barin, J.G., Kass, D.A., and Rose, N.R. (2004). Quantitative analysis of myocardial inflammation by flow cytometry in murine autoimmune myocarditis. *Am. J. Pathol. 164*, 807–815.

Afanasyeva, M., Georgakopoulos, D., Belardi, D.F., Bedja, D., Fairweather, D., Wang, Y., Kaya, Z., Gabrielson, K.L., Rodriguez, E.R., Caturegli, P., Kass, D.A., and Rose, N.R. (2005). Impaired up-regulation of CD25 on $CD4^+$ T cells in IFN-γ knockout mice is associated with progression of myocarditis to heart failure. *Proc. Natl. Acad. Sci. USA 102*, 180–185.

Alvarez, F.L., Neu, N., Rose, N.R., Craig, S.W., and Beisel, K.W. (1987). Heart-specific autoantibodies induced by coxsackievirus B_3: Identification of heart autoantigens. *Clin. Immunol. Immunopathol. 43*, 129–139.

Anderson, J.L., Carlquist, J.F., Lutz, J.R., DeWitt, C.W., and Hammond, E.H. (1984). HLA A, B and DR typing in idiopathic dilated cardiomyopathy: a search for immune response factors. *Am. J. Cardiol. 53*, 473–487.

Ansari, A.A., Herskowitz, A., Danner, D.J., Neckelmann, N., Gershwin, M.E., Gravanis, M.B., and Sell, K.W. (1988). Identification of mitochondrial proteins that serve as targets for autoimmunity in human dilated cardiomyopathy. *Circulation 78*, 457 (abs.).

Aretz, H.T., Billingham, M.E., Edwards, W.D., Factor, S.M., Fallon, J.T., Fenoglio, J.J., Jr., Olsen, E.G.J., and Schoen, F.J. (1986). Myocarditis. A histopathologic definition and classification. *Am. J. Cardiovasc. Pathol. 1*, 3–14.

Arola, A., Jokinen, E., Ruuskanen, O., Saraste, M., Pesonen, E., Kuusela, A.L., Tikanoja, T., Paavilainen, T., and Simell, O. (1997). Epidemiology of idiopathic cardiomyopathies in children and adolescents. A nationwide study in Finland. *Am. J. Epidemiol. 146*, 385–393.

Ayuthya, P.S.N., Jayavasu, J., and Pongpanich, B. (1974). Coxsackie group B virus and primary myocardial disease in infants and children. *Am. Heart J. 88*, 311–314.

Baboonian, C. and McKenna, W. (2003). Eradication of viral myocarditis. Is there hope? *J. Am. Coll. Cardiol. 42*, 473–476.

Ballinas-Vedugo, M.A., Alejandre-Aguilar, R., Aranda-Fraustro, A., Reyes, P., and Monteon, M. (2003). *Int. J. Cardiol. 92*, 101–102.

Beck, M.A., Shi, Q., Morris, V.C., and Levander, O.A. (1995). Rapid genomic evolution of a non-virulent Coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. *Nat. Med. 1*, 433–436.

Bowles, N.E., Richardson, P.J., Olsen, E.G.J., and Archard, L.C. (1986). Detection of Coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. *Lancet i*, 1120–1122.

Bowles, N.E., Ni, J., Kearney, D.L., Pauschinger, M., Schultheiss, H.-P., McCarthy, R., Hare, J., Bricker, J.T., Bowles, K.R. and Towbin, J.A. (2003). Detection of viruses in myocardial tissues by polymerase chain reaction; evidence of adenovirus as a common cause of myocarditis in children and adults. *J. Am. Coll. Cardiol. 42*, 466–472.

Burch, G.E., Sun, S.C., Chu, K.C., Sohal, R.S., and Colcolough, H.L. (1968). Interstitial and Coxsackievirus B myocarditis in infants and children. A comparative histologic and immunofluorescent study of 50 autopsied hearts. *J. Am. Med. Assoc. 203*, 55–62.

Caforio, A.L.P., Grazzini, M., Mann, J.M., Keeling, P.J., Bottazzo, G.F., McKenna, W.J., and Schiaffino, S. (1992). Identification of α- and β-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. *Circulation 85*, 1734–1742.

Caforio, A.L.P., Mahon N.J., Tona, F., and McKenna, W.J. (2002). Circulating cardiac autoantibodies in dilated cardiomyopathy and myocarditis: pathogenetic and clinical significance. *Eur. J. Heart Failure 4*, 411–417.

Carlquist, J.F., Menlove, R.L., Murray, M.B., O'Connell, J.B., and Anderson, J.L. (1991). HLA class II (DR and DQ) antigen associations in idiopathic dilated cardiomyopathy. *Circulation 83*, 515–522.

Cetta, F., and Michels, V.V. (1995). The autoimmune basis of dilated cardiomyopathy. *Ann. Med. 27*, 169–173.

Chow, L.H., Radio, S.J., Sears, T.D., and McManus, G.M. (1989). Insensitivity of right ventricular endomyocardial biopsy in the diagnosis of myocarditis. *J. Am. Coll. Cardiol. 14*, 915–920.

Cooper, L.T. (2002). Idiopathic giant cell myocarditis. In L. T. Cooper (ed.) *Myocarditis from Bench to Bedside* (pp. 405–420). Totowa, NJ: Humana Press.

Cooper, L.T., Berry, G.J., and Shabetai, R. for the Multicenter Giant Cell Myocarditis Study Group Investigators. (1997). Idiopathic giant-cell myocarditis: Natural history and treatment. *N. Engl. J. Med. 336*, 1860–1866.

Corvisart, J.N. (1812). Essai sur les maladies et les lesions organiques du coeur. *Gates J. M.M.M.S.* 1812, 182–189; 299–303. [Cited by Gravanis, M.B., and Sternby, N.H. (1991). *Arch. Pathol. Lab. Med. 115*, 390–392.]

Cunha-Neto, E., Iwai, L.K., Morand, B., Bilate, A., Goncalves-Fonseca, S., and Kalil, J. (2004). In Y. Shoenfeld and N.R. Rose (eds.) *Infection and Autoimmunity* (pp. 449–470). Amsterdam: Elsevier.

Cunningham, M.W. (2004). T cell mimicry in inflammatory heart disease. *Mol. Immunol.* 40, 1121–1127.

Cunningham, M.W., McCormack, J.M., Talaber, L.R., Harley, J.B., Ayoub, E.M., Muneer, R.S., Chun, L.T., and Reddy, D.V. (1988). Human monoclonal antibodies reactive with antigens of the group A streptococcus and human heart. *J. Immunol. 141*, 2760–2706.

Cunningham, M.W., Meissner, H.C. Heuser, J.S., Pietra, B.A., Kurahara, D.K., and Leung, D.Y.M. (1999). Anti-human cardiac myosin autoantibodies in Kawasaki syndrome. *J. Immunol. 163*, 1060–1065.

Dec, G.W., Jr., Palacios, I.F., Fallon, J.T., Aretz, H.T., Mills, J., Lee, D.C., and Johnson, R.A. (1985). Active myocarditis in the spectrum of acute dilated cardiomyopathies. Clinical features, histologic correlates, and clinical outcome. *N. Engl. J. Med. 312*, 885–890.

Dorffel, W.V., Wallukat, G., Baumann, G. and Felix, S.B. (2000). Immunoadsorption in dilated cardiomyopathy. *Ther. Apher. 4*, 235–238.

Eriksson, U., Ricci, R., Hunziker, L., Kurrer, M.O., Oudit, G.Y., Watts, T.H., Sonderegger, I., Bachmaier, K., Kopf, M., and Penninger, J.M. (2003). Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. *Nat. Med. 9*, 1484–1490.

Fairweather, D., Frisancho-Kiss, S., Gatewood, S., Njoku, D., Steele, R., Barrett, M., Rose, N.R. (2004a). Mast cells and innate cytokines are associated with susceptibility to autoimmune heart disease following Coxsackievirus B3 infection. *Autoimmunity 37*, 131–145.

Fairweather, D., Afanasyeva, M., and Rose, N.R. (2004b). Cellular immunity: A role for cytokines. In A. Doria and P. Pauletto (eds), Handbook of Systemic Autoimmune Diseases, Vol 1: The Heart in Systemic Autoimmune Diseases (pp. 3–17). Amsterdam: Elsevier.

Felker, G.M., Thompson, R.E., Hare, J.M., Hurban, R.H., Clemetson, D.E., Howard, D.L., Baughman, K.L., and Kasper, E.K. (2000). Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N. Engl. J. Med. 342*, 1077–1084.

Ferrari, I., Levin, M.J., Wallukat, G., Elies, R., Lebesgue, D., Chiale, P., Elizari, M, Rosenbaum, M. and Hoebeke, J. (1995). Molecular mimicry between the immunodominant ribosomal protein P0 of *Trypanosoma cruzi* and a functional epitope on the human beta$_1$-adrenergic receptor. *J. Exp. Med. 182*, 59–65.

Frustaci, A., Chimenti, C., Calabrese, F, Pieroni, M., Thiene, G., and Maseri, A. (2003). Immunosuppressive therapy for active lymphocytic myocarditis. Virological and immunologic profile of responders versus nonresponders. *Circulation 107*, 857–863.

Girones, N., Rodriguez, C.I., Carrasco-Marin, E., Hernaez, R.F., de Rego, J.L., and Fresno, M. (2001). Dominant T- and B-cell epitopes in an autoantigen linked to Chagas' disease. *J. Clin. Invest. 107*, 985–993.

Gravanis, M.B. and Ansari, A.A. (1987). Idiopathic cardiomyopathies. A review of pathologic studies and mechanisms of pathogenesis. *Arch. Pathol. Lab. Med. 111*, 915–929.

Gravanis, M.B. and Sternby, N.H. (1991). Incidence of myocarditis: A 10-year autopsy study from Malmö, Sweden. *Arch. Pathol. Lab. Med. 115*, 390–392.

Grist, N.R. and Bell, E.J. (1974). A six-year study of coxsackievirus B infections in heart disease. *J. Hygiene 73*, 165–172.

Grogan, M., Redfield, M.M., Bailey, K.R., Reeder, G.S., Gersh, B.J., Edwards, W.D., and Rodeheffer, R.J. (1995). Long-term outcome of patients with biopsy-proved myocarditis: comparison with idiopathic dilated cardiomyopathy. *J. Am. Coll. Cardiol. 26*, 80–84.

Hammond, E.H., Menlove, R.L., and Anderson, J.L. (1988). Immunofluorescence microscopy in the diagnosis and follow-up of myocarditis. A critical review. In H.-P. Schultheiss (ed.), *New Concepts in Viral Heart Disease* (pp. 303–311). Berlin: Springer-Verlag.

Hauck, A.J., Kearney, D.L., and Edwards, W.D. (1989). Evaluation of postmortem endomyocardial biopsy specimens from 38 patients with lymphocytic myocarditis: Implications for role of sampling error. *Mayo Clinic Proc. 64*, 1235–1245.

Herskowitz, A., Ahmed-Ansari, A., Neumann, D.A., Beschorner, W.E., Rose, N.R., Soule, L.M., Burek, C.L., Sell, K.W., and Baughman, K.L. (1990). Induction of major histocompatibility complex antigens within the myocardium of patients with active myocarditis: A nonhistologic marker of myocarditis. *J. Am. Coll. Cardiol. 15*, 624–632.

Horwitz, M. S., La Cava, A., Fine, C., Rodriguez, E., Ilic, A. and Sarvetnick, N. (2000). Pancreatic expression of interferon-gamma

protects mice from lethal coxsackievirus B3 infection and subsequent myocarditis. *Nat. Med. 6*, 693–697.

Huber, S.A. (1997). Animal models of human disease. Autoimmunity in myocarditis: relevance of animal models. *Clin. Immunol. Immunopathol. 83*, 93–102.

Huber, S.A., and Job, L.P. (1983). Differences in cytotoxic T cell response of Balb/c mice infected with myocarditic and non-myocarditic strains of coxsackievirus group B, type 3. *Infect. Immun. 39*, 1419–1427.

Huber, S.A., Heintz, N., and Tracy, R. (1988). Coxsackievirus B-3-induced myocarditis virus and actinomycin D treatment of myocytes induces novel antigens recognized by cytolytic T lymphocytes. *J. Immunol. 141*, 3214–3219.

Jacobson, D.L., Gange, S.J., Rose, N.R., and Graham, N.M.H. (1997). Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol. 84*, 223–243.

Jahns, R., Boivin, V., Siegmund, C., Inselmann, G., Lohse, M.J. and Boege, F. (1999). Autoantibodies activating human B_1-adrenergic receptors are associated with reduced cardiac function in chronic heart failure. *Circulation 99*, 649–654.

Jones, S.R., Herskowitz, H.M., Hutchins, H.M., and Baughman, K.L. (1991). Effects of immunosuppressive therapy in biopsy-proved myocarditis and borderline myocarditis on left ventricular function. *Am. J. Cardiol. 68*, 370–376.

Kalil, J. and Cunha-Neto, E. (1996). Autoimmunity in Chagas' disease cardiomyopathy: fulfilling the criteria at last? *Parasitol. Today 12*, 396–399.

Kandolf, R., Ameis, D., Kirschner, P., Canue, A., and Hofschneider, P.H. (1987). *In situ* detection of enteroviral genomes in myocardital cells by nucleic acid hybridization: An approach to the diagnosis of viral heart disease. *Proc. Natl. Acad. Sci. USA 84*, 6272–6276.

Kaplan, M.H., Meyeserian, M., and Kushner, I. (1961). Immunologic studies of heart tissue. IV. Serologic reactions with normal heart tissue as revealed by immunofluorescent methods: isoimmune, Wassermann and autoimmune reactions. *J. Exp. Med. 113*, 17.

Kaplan, D., Ferrari, I., Bergami, P.L., Mahler, E., Levitus, G. Chiale, P., Hoebeke, J., Van Regenmortel, M.H. and Levin, M.J. (1997). Antibodies to ribosomal P proteins of *Trypanosoma cruzi* in Chagas' disease possess functional autoreactivity with heart tissue and differ from anti-P autoantibodies in lupus. *Proc. Natl. Acad. Sci. USA 94*, 10301–10306.

Kline, I.K. and Saphir, O. (1960). Chronic pernicious myocarditis. *Am. Heart J. 59*, 681–697.

Klingel, K.C., Hohenadl, A., Canu, M., Albrecht, M., Seemann, M., Mall, G., and Kandolf, R. (1992). Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: quantitative analysis of virus replication, tissue damage, and inflammation. *Proc. Natl. Acad. Sci. USA 89*, 314–318.

Kohno, K., Takagaki, Y., Nakajima, Y., and Izumi, T. (2000). Advantage of recombinant technology for the identification of cardiac myosin epitope of severe autoimmune myocarditis in Lewis rats. *Jpn. Heart J. 41*, 67–77.

Kühl, U. and Schultheiss, H.-P. (1995). Treatment of chronic myocarditis with corticosteroids. *Eur. Heart J. 16*, 168–172.

Kühl, U., Pauschinger, M., Schwimmbeck, P.L., Seeberg, B., Lober, C., Noutsias, M., Poller, W., Schultheiss, H.-P. (2003). Interferon-β treatment eliminates cardiotropic viruses and improves left ventricular function in patients with myocardial persistence of viral genomes and left ventricular dysfunction. *Circulation 107*, 2793–2798.

Lane, J.R., Neumann, D.A., Lafond-Walker, A., Herskowitz, A., and Rose, N.R. (1991). LPS promotes CB3-induced myocarditis in resistant B10.A mice. *Cell. Immunol. 136*, 219–233.

Lane, J.R., Neumann, D.A., Lafond-Walker, A., Herskowitz, A., and Rose, N.R. (1992). Interleukin 1 or tumor necrosis factor can promote Coxsackie B3-induced myocarditis in resistant B10.A mice. *J. Exp. Med. 175*, 1123–1129.

Lane, J.R., Neumann, D.A., Lafond-Walker, A., Herskowitz, A., and Rose, N.R. (1993). Role of IL-1 and tumor necrosis factor in Coxsackie virus-induced autoimmune myocarditis. *J. Immunol. 151*, 1682–1690.

Lawson, C.M., O'Donoghue, H.L., and Reed, W.D. (1992). Mouse cytomegalovirus infection induces antibodies which cross-react with virus and cardiac myosin: a model for the study of molecular mimicry in the pathogenesis of viral myocarditis. *Immunology 75*, 513–519.

Lenzo, J.C., Mansfield, J.P., Sivanoorthy, S., Cull, V.C. and James, C.M. (2003). Cytokine expression in murine cytomegalovirus-induced myocarditis: modulation with interferon-α therapy. *Cell. Immunol. 223*, 77–86.

Levander, O.A. and Beck, M.A. (1997). Interacting nutritional and infectious etiologies of Keshan disease. Insights from Coxsackie virus B-induced myocarditis in mice deficient in selenium or vitamin E. *Biol. Trace Element Res. 56*, 1–16.

Liao, L., Sindhwani, R., Rojkind, M., Factor, S., Leinwand, L., and Diamond, B. (1995). Antibody-mediated autoimmune myocarditis depends on genetically determined target organ sensitivity. *J. Exp. Med. 181*, 1123–1131.

Liberthson, R.R. (1996). Sudden death from cardiac causes in children and young adults. *N. Engl. J. Med. 334*, 1039–1044.

Lieberman, E.B., Hutchins, G.M., Herskowitz, A., Rose, N.R., and Baughman, K.L. (1991). Clinicopathologic description of myocarditis. *J. Am. Coll. Cardiol. 18*, 1617–1626.

Lieberman, E.B., Herskowitz, A., Rose, N.R., and Baughman, K.L. (1993). A clinicopathologic description of myocarditis. *Clin. Immunol. Immunopathol. 68*, 191–196.

Limas, C.J., Goldenberg, I.F., and Limas, C. (1989). Autoantibodies against beta-adrenoreceptors in human idiopathic dilated cardiomyopathy. *Circ. Res. 64*, 97–103.

Limas, C.J., Limas, C., Kubo, S.H., and Olivari, M.T. (1990). Anti-beta receptor antibodies in human dilated cardiomyopathy and correlation with HLA-DR antigens. *Am. J. Cardiol. 65*, 483–487.

Liu, P.P. and Opavsky, M.A. (2000). Viral myocarditis: receptors that bridge the cardiovascular with the immune system. *Circ. Res 86*, 253–254.

Luppi, P. Rudert, W., Licata, A., Riboni, S., Betters, D., Cotrufo, M., Frati, G., Condorelli, G. and Trucco, M. (2003). Expansion of specific $\alpha\beta^+$ T-cell subsets in the myocardium of patients with myocarditis and idiopathic dilated cardiomyopathy associated with Coxsackievirus B infection. *Hum. Immunol. 64*, 194–210.

Mahon, N.G., Madden, B.P., Caforio, A.L.P., Elliott, P.M., Haven, A.J., Keogh, B.E., Davies, M.J. and McKenna, W.J. (2002). Immunohistologic evidence of myocardial disease in apparently healthy relatives of patients with dilated cardiomyopathy. *J. Am. Coll. Cardiol. 39*, 455–462.

Maisch, B. (1987). Immune regulation, humoral and cell-mediated immune reactions in myocarditis and dilated cardiomyopathy. In C. Kawai and W.H. Abelmann (eds), *Pathogenesis of Myocarditis and Cardiomyopathy* (pp. 245–267). Tokyo: University of Tokyo Press.

Maisch, B., Berg, P.A., and Kochsiek, K. (1979). Clinical significance of immunopathological findings in patients with post-periocardiotomy syndrome. I. Relevance of antibody pattern. *Clin. Exp. Immunol. 38*, 189–197.

Mason, J.W. and O'Connell, J.B. (1989). Clinical merit of endomyocardial biopsy. *Circulation 79*, 971–979.

Mason, J.W., O'Connell, J.B., Herskowitz, A., Rose, N.R., McManus, B.M., Billingham, M.E., Moon, T.E., and the Myocarditis Treatment Trial Investigators (1995). A clinical trial of immunosuppressive therapy for myocarditis. *N. Engl. J. Med. 333*, 269–275.

McCarthy, R.M. 3rd, Boehmer, J.P., Hruban, R.H., Hutchins, G.M., Kasper, E.K., Hare, J.M., and Baughman, K.L. (2000). Long-term outcome of

fulminant myocarditis as compared with acute (nonfulminant) myocarditis. *N. Engl. J. Med. 342*, 734–735.

McNamara, D.M., Holubko, V.R., Starling, R.C., Dee, G.W., Loh, E., Tirre-Amione, G., Gass, A., Janosko, K., Tokarczyk, T., Kessler, P., Mann, D.L., and Feldman, A.M. (2001). Controlled trial of intravenous immunoglobulin in recent onset dilated cardiomyopathy. *Circulation 103*, 2254–2259.

Miklozek, C.L., Kingsley, E.M., Crumpaker, C.S., Modlin, J.F., Royal, Henry, D., Come, P.C., Mark, R., and Abelmann, W.H. (1986). Serial cardiac function tests in myocarditis. *Postgrad. Med. J. 62*, 577–579.

Miric, M., Miškovic, A., Brkic, S., Vasiljevic, J., Keserovic, N., and Pešic, M. (1994). Long-term follow-up of patients with myocarditis and idiopathic dilated cardiomyopathy after immunomodulatory therapy. *FEMS Immunol. Med. Microbiol. 10*, 65–74.

Morad, M., Davies, N.W., Ulrich, G., and Schultheiss, H.P. (1988). Antibodies against ADP-ATP carrier enhance Ca^{2+} current in isolated cardiac myocytes. Am. J. Physiol. 255, H960–H964.

Motran, C.C., Fretes, R.E., Cerban, F.M., Rivarola, H.W. and Bottero de Cima, E. (2000). Immunization with the C-terminal region of *Trypanosoma cruzi* ribosomal P1 and P2 proteins induces long-term duration cross-reactive antibodies with heart functional and structural alterations in young and aged mice. *Clin. Immunol. 97*, 89–94.

Müller, J., Wallukat, G., Dandel, M., Bieda, H., Brandes, K., Spiegelsberger, S., Nissen, E., Kunze, R. and Hetzer, R. (2000). Immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy. *Circulation 101*, 385–401.

Neckelmann, N., Li, K., Wade, R.P., Shuster, R., and Wallace, D.C. (1987). cDNA sequence of a human skeletal muscle ADP/ATP translocator, lack of a leader peptide, divergence from a fibroblast translocator cDNA, and coevolution with mitochondrial DNA genes. *Proc. Natl. Acad. Sci. USA 84*, 7580–7584.

Neu, N., Beisel, K.W., Traystman, M.D., Rose, N.R., and Craig, S.W. (1987a). Autoantibodies specific for the cardiac myosin isoform are found in mice susceptible to Coxsackievirus B_3-induced myocarditis. *J. Immunol. 138*, 2488–2492.

Neu, N., Rose, N.R., Beisel, K.W., Herskowitz, A., Gurri-Glass, G., and Craig, S.W. (1987b). Cardiac myosin induces myocarditis in genetically predisposed mice. *J. Immunol. 139*, 3630–3636.

Neumann, D.A., Burek, C.L., Baughman, K.L., Rose, N.R., and Herskowitz, A. (1990). Circulating heart-reactive antibodies in patients with myocarditis or cardiomyopathy. *J. Am. Coll. Cardiol. 16*, 839–846.

Neumann, D.A., Lane, J.R., LaFond-Walker, A., Allen, G.S., Frondoza, C., Herskowitz, A., and Rose, N.R. (1991). Elution of autoantibodies from the hearts of Coxsackie-virus-infected mice. *Eur. Heart J. 12*(Suppl D), 113–116.

Neumann, D.A., Lane, J.R., Wulff, S.M., Allen, G.S., LaFond-Walker, A., Herskowitz, A., and Rose, N.R. (1992). *In vivo* deposition of myosin-specific autoantibodies in the hearts of mice with experimental autoimmune myocarditis. *J. Immunol. 148*, 3806–3813.

Noren, G.R., Staley, N.A., Bandt, C.M., and Kaplan, E.L. (1976). Occurrence of myocarditis in sudden death in children. *J. Forensic Sci. 22*, 188–196.

Okazaki, T., Tanaka, Y., Nishio, R., Mitsuiye, T., Mizoguchi, A., Wang, J., Ishida, M., Hiai, H., Matsumori, A., Minato, N., and Honjo, T. (2003). Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med. 9*, 1477–1483.

Parrillo, J.E., Cunnion, R.E., Epstein, S.E., Parker, M.M., Suffredini, A.F., Brenner, M., Schaer, G.L., Palmeri, S.T., Cannon, R.O., 3rd, Alling, D., Wittes, J., Rerrane, V.J., Rodrigues, E.R., and Fauci, A.J. (1989). A prospective, randomized, controlled trial of prednisone for dilated cardiomyopathy. *N. Engl. J. Med. 321*, 1061–1068.

Pauschinger, M., Chandrasekharan, K., Noutsias, M., Kühl, U., Schwimmbeck, L.P., and Schultheiss, H.-P. (2004). Viral heart disease: molecular diagnosis, clinical prognosis, and treatment strategies. *Med. Microbiol. Immunol. (Berl.) 193*, 65–69.

Pummerer, C.L., Grässl, G., Sailer, M., Bachmaier, K.W., Penninger, J.M., and Neu, N. (1993). Cardiac myosin-induced myocarditis. Target recognition by autoreactive T cells requires prior activation of cardiac interstitial cells. *Lab. Invest. 74*, 845–852.

Pummerer, C.L., Luze, K., Grässl, G., Bachmaier, K., Offner, F., Burrell, S.K., Lenz, D.M., Zamborelli, T.J., Penninger, J.M., and Neu, N. (1996). Identification of cardiac myosin peptides capable of inducing autoimmune myocarditis in Balb/c mice. *J. Clin. Invest. 97*, 2057–2062.

Pummerer, C.L., Luze, K., Grassl, G., Bachmaier, K., Offner, F., Burrell, S.K., Lenz, D.M., Zamborelli, T.J. Penninger, J.M., and Neu, N. (1996). Identification of cardiac myosin peptides capable of inducing autoimmune myocarditis in BALB/c mice. *J. Clin. Invest 97*, 2057–2062.

Rose, N.R. (2000). Viral damage or "molecular mimicry": placing the blame in myocarditis. *Nat. Med. 6*, 5–6.

Rose, N.R. and Hill, S.L. (1996). The pathogenesis of postinfectious myocarditis. *Clin. Immunol. Immunopathol. 80*, S92–S99.

Rose, N.R. and Chihakova, D. (2004). Cardiomyopathies. *Autoimmunity 37*, 347–350.

Rose, N.R., Wolfgram, L.J., Herskowitz, A., and Beisel, K.W. (1986). Postinfectious autoimmunity: two distinct phases of Coxsackievirus B3-induced myocarditis. *Ann. N. Y. Acad. Sci. 475*, 146–156.

Rose, N.R., Beisel, K.W., Herskowitz, A., Neu, N., Wolfgram, L.J., Alvarez, F.L., Traystman, M.D., and Craig, S.W. (1987). Cardiac myosin and autoimmune myocarditis. In D. Evered and J. Whelan (eds) *Ciba Symposium 129* (pp. 3–24). Chichester: John Wiley & Sons.

Rose, N.R., Neumann, D.A., Herskowitz, A., Traystman, M., Beisel, K.W. (1988). Genetics of susceptibility to viral myocarditis in mice. *Pathol. Immunopathol. Res. 7*, 266–278.

Schultheiss, H.-P., Schulze, K., Kühl, U., Ulrich, G., and Klingenberg, M. (1986). The ADP/ATP carrier as a mitochondrial auto-antigen C facts and perspectives. *Ann. N. Y. Acad. Sci. 488*, 44–64.

Schultheiss, H.-P., Kühl, U., Schauer, R., Schulze, K., Kemkes, B., and Becker, B.F. (1988). Antibodies against the ADP/ATP carrier alter myocardial function by disturbing cellular energy metabolism. In H.-P. Schultheiss (ed.), *New Concepts in Viral Heart Disease* (pp. 243–258). Berlin: Springer-Verlag.

Smith, S.C. and Allen, P.M. (1991). Myosin-induced acute myocarditis is a T cell-mediated disease. *J. Immunol. 147*, 2141–2147.

Staudt, A., Böhm, M., Knebel, F., Grosse, Y., Bischoff, C., Hummel, A., Dahm, J.B., Borges, A., Jochmann, N., Wernecke, K.D., Wallukat, G., Baumann, G. and Felix, S.B. (2002). Potential role of autoantibodies belonging to the immunoglobulin G-3 subclass in cardiac dysfunction among patients with dilated cardiomyopathy. *Circulation 106*, 2448–2453.

Staudt, Y., Mobini, R., Fu, M., Felix, S.B., Kuhn, J.P., and Staudt, A. (2003). Beta1-adrenoceptor antibodies induce apoptosis in adult isolated cardiomyocytes. *Eur. J. Pharmacol. 466*, 1–6.

Tanowitz, H.B., Kirchhoff, L.V., Simon, D., Morris, S.A., Weiss, L.M., and Wittner, M. (1992). Chagas' disease. *Clin. Microbiol. Rev. 5*, 400–419.

Taylor, J.A., Havari, E., McInerney, M.F., Bronson, R., Wucherpfennig, K.W., and Lipes M.A. (2004). A spontaneous model for autoimmune myocarditis using the human MHC molecule HLA-DQ8. *J. Immunol. 172*, 2651–2658.

Torp, A. (1981). Incidence of congestive cardiomyopathy. In J.F. Goodwin, A. Hjalmarson, and E.G.J. Olsen (eds), *Congestive Cardiomyopathy* (pp. 18–22). Molndal: A. B. Hässle.

Towbin, J.A., Li, H., Taggart, R.T., Lehman, M.H., Schwartz, P.J., Satler, C.A., Ayyagari, R., Robinson, J.L., Moss, A. and Hejtmancik, J.F. (1994). Molecular and cellular cardiovascular medicine: evidence of genetic heterogeneity in Romano-Ward long QT syndrome: analysis of 23 families. *Circulation 90*, 2635–2644.

Tracy, S., Chapman, N.M., Romero, J., and Ramsingh, A.I. (1996). Genetics of coxsackievirus B cardiovirulence and inflammatory heart muscle disease. *Trends Microbiol. 4*, 175–179.

Van Voorhis, W.C., Schlekewy, L. And Trong, H.L. (1991). Molecular mimicry by *Tryponosoma cruzi*: the Fl-160 epitope that mimics mammalian nerve can be mapped to a 12-amino acid peptide. *Proc. Natl. Acad. Sci. USA 88*, 5993–5997.

Wallukat, G., Reinke, P., Dörffel, W.V., Luther, H.P., Bestvater, K., Felix, S.B. and Baumann, G. (1996). Removal of autoantibodies in dilated cardiomyopathy by immunoadsorption. *Int. J. Cardiol. 54*, 191–195.

Wessely, R., Henke, A., Zell, R., Kandolf, R., and Knowlton, K.U. (1998). Low-level expression of a mutant coxsackieviral cDNA induces a myocytopathic effect in culture: An approach to the study of enteroviral persistence in cardiac myocytes. *Circulation 98*, 450–457.

Williams, D.G. and Olsen, E.G.L. (1985). Prevalence of overt dilated cardiomyopathy in two regions of England. *Br. Heart J. 54*, 153–155.

Wojnicz, R., Nawalany-Kozielska, E., Wojciechowska, C., *et al*. (2001). Randomized, placebo-controlled study for immunosuppressive treatment of inflammatory dilated cardiomyopathy: two-year follow-up results. *Circulation 104*, 39–45.

Wolff, P., Kühl, U., and Schultheiss, H.-P. (1989). Laminin distribution and autoantibodies in idiopathic dilated cardiomyopathy and myocarditis. *Am. Heart J. 117*, 1303–1309.

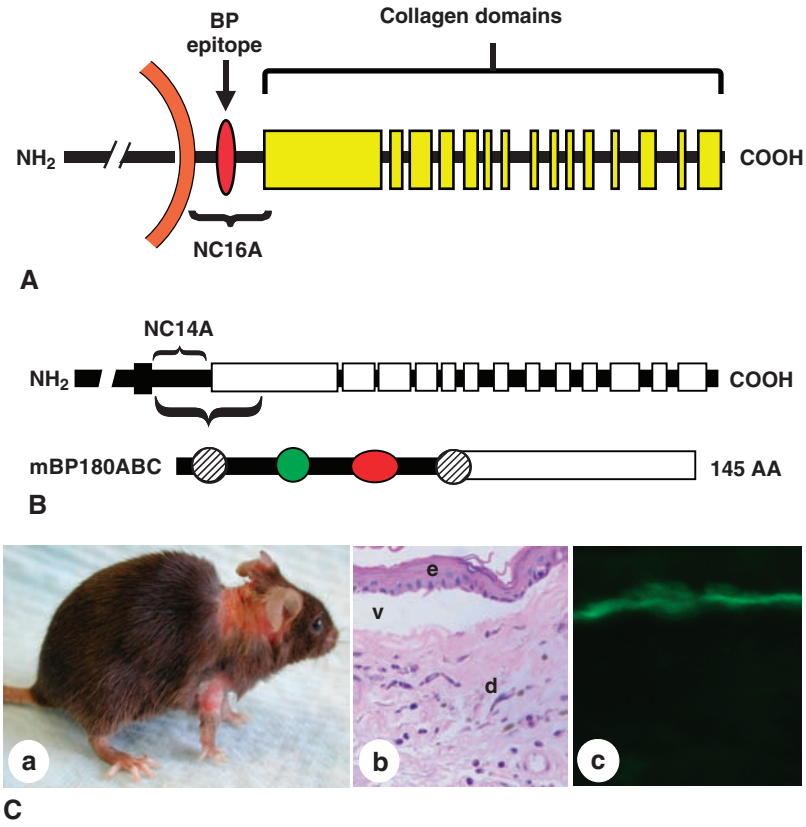


FIGURE 57.8 Active model of bullous pemphigoid (BP). *A*, Schematic diagram of the human BP180 antigen. The C-terminal extracellular region is made up of a series of collagen-like domains (yellow rectangles) interrupted by non-collagen domains (black bars). The major epitope (NC16A) recognized by BP autoantibodies is depicted by the red oval. *B*, Mouse BP180 antigen used to immunize mice. This antigen (referred to as mBP180ABC) contains the largest non-collagen domain (NC14A) and part of the largest collagen-like domain. mBP180ABC harbors four epitopes, one of which (red) is pathogenic. *C*, 8–10-week-old C57BL/6J mice immunized with mBP180ABC. Some of the mice developed BP-like skin lesions, starting the third week after the boosting immunization (a). Histologic examination shows subepidermal blistering (b). Direct immunofluorescence shows in-vivo deposition of autoantibodies at the basement membrane zone (c). d, dermis; e, epidermis; v, vesicle.

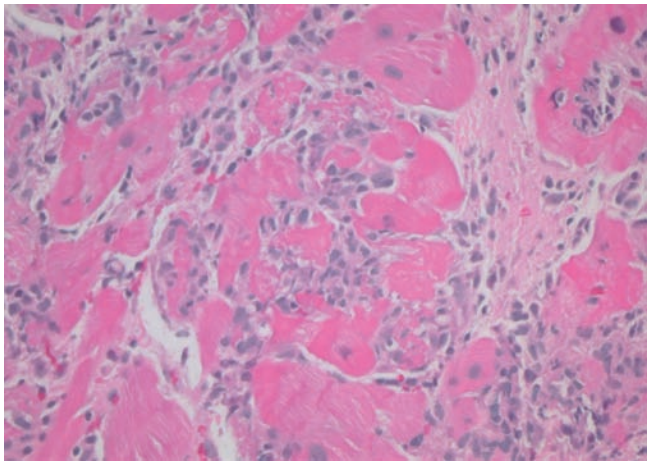


FIGURE 63.1 Lymphocytic myocarditis. There is a heavy infiltrate of large activated lymphocytes throughout the myocardium. Myocyte necrosis is noted in the middle of this image. Fibrosis is present on the right side of the image. (H&E 400×).

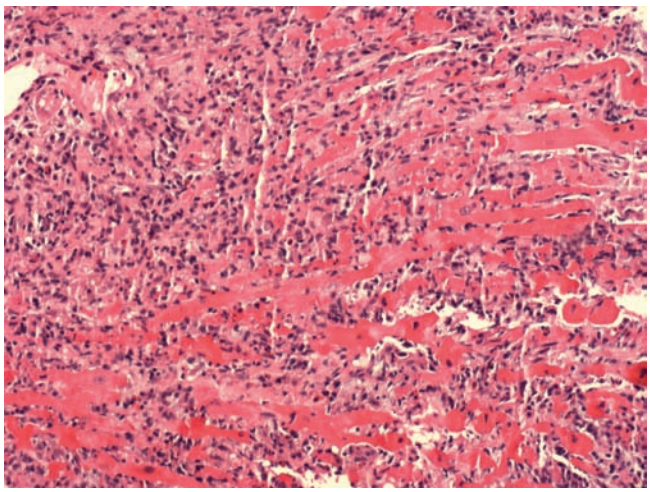


FIGURE 63.3 Fulminant myocarditis. The myocardium is replaced by a marked polymorphous inflammatory infiltrate composed predominantly of lymphocytes and macrophages with rarer eosinophils and neutrophils. Global myocyte injury and loss is noted. No giant cells are present. (H&E 100×).

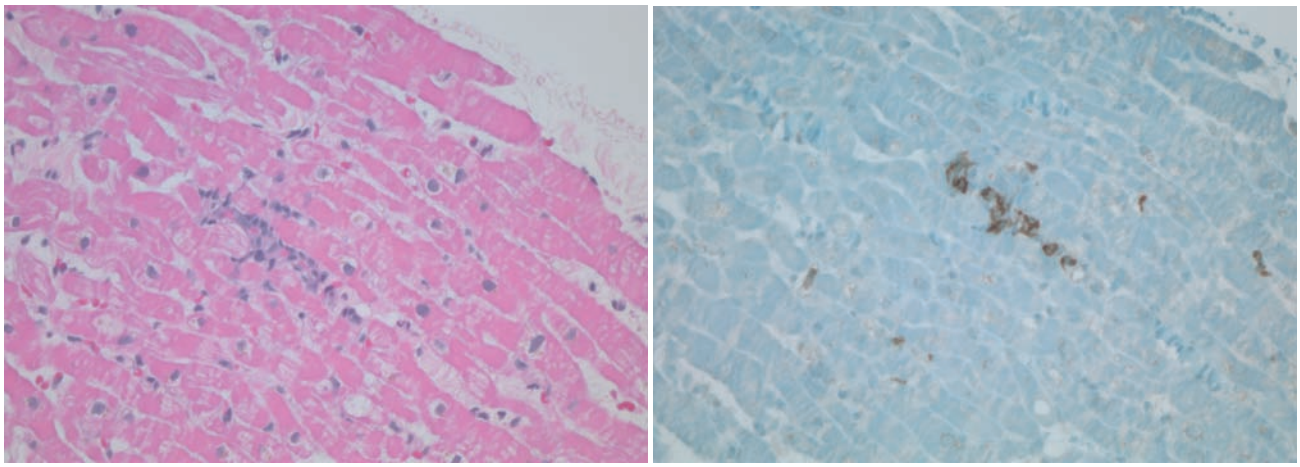


FIGURE 63.2 Borderline myocarditis. *A*, A single cluster of perivascular lymphocytes is present. No myocardial damage is identified. (H&E 400×). *B*, A CD8 immunohistochemical stain highlights the infiltrating T lymphocytes. (H&E 400×). See color plate section.

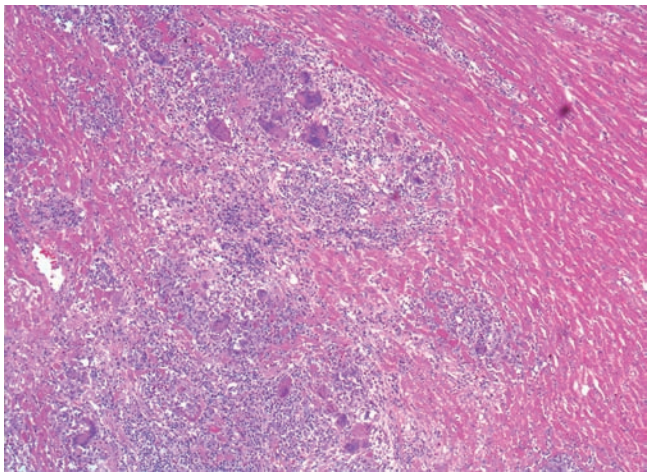


FIGURE 63.4 Giant cell myocarditis. The myocardium is infiltrated by a patchy and diffuse inflammatory infiltrate composed primarily of lymphocytes and macrophages. Multiple collections of giant cells are seen within the infiltrate along with eosinophils. There is significant injury and loss of myocytes in areas of inflammation, while adjacent myocardium is relatively uninvolved. (H&E 50×). See color plate section.

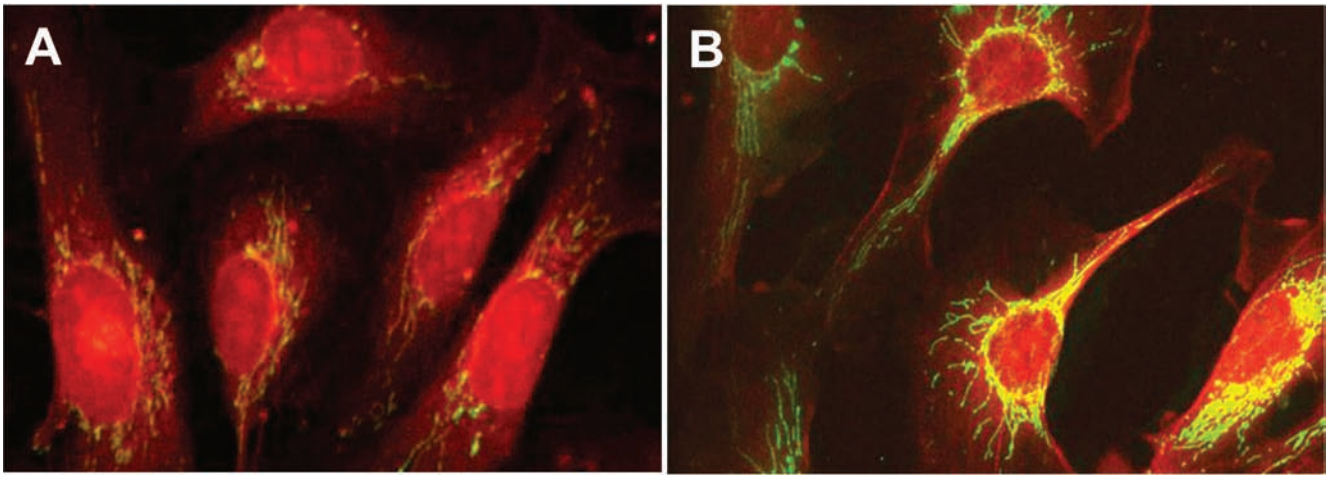


FIGURE 64.1 Acetone-fixed human umbilical vein endothelial cells stained in indirect immunofluorescence for the presence of HSP60 (FITC, green) and counterstained with TRITC-labelled phalloidin (red). A, unstressed cells, and B, heat stressed cells (42 °C for 30 min). Note the weak mitochondrial staining in A, most probably reflecting mild stress from the culture conditions, and the significant upregulation of HSP60 expression in the mitochondria and cytoplasm in B. Laser confocal scanning microscope, original magnification ×1000.

CHAPTER

64

Atherosclerosis: Autoimmunity to Heat-Shock Proteins

GEORGE WICK,* BLAIR HENDERSON,* MICHAEL KNOFLACH,† AGNES MAYR,‡ AND DAVID BERNHARD*

*Division of Experimental Pathophysiology and Immunology, Biocenter, Innsbruck Medical University, †University Hospital for Neurology, Innsbruck, Austria, and ‡Hospital Bruneck, Bruneck, Italy

The pathogenesis of autoimmune diseases is multifactorial. A literature search shows that most groups worldwide working on autoimmune disease continue to concentrate their efforts on the elucidation of an abnormally functioning immune system as the primary cause of clinically overt manifestations of autoimmunity. Our concept for the pathogenesis of autoimmune diseases, including early atherosclerosis, differs markedly from the general dogma, since it assigns a major importance to the *susceptibility of the target structure* to attack by preexisting humoral and cellular autoreactive immune effector mechanisms (Wick et al., 1987). This concept has been developed from studies in the obese strain (OS) of chickens, an animal model with an hereditary, spontaneously occurring, autoimmune disease that resembles human Hashimoto's thyroiditis (Wick et al., 1989). Soon after hatching, OS chicks present with symptoms of hypothyroidism that are due to a severe mononuclear cell (MNC) infiltration of the thyroid glands, leading to their complete destruction within 3–4 weeks. In the periphery, this event is accompanied by humoral and cellular autoimmunity against thyroglobulin. When OS chickens are crossed with birds from a normal healthy inbred strain, the F1 chicks still display the hallmarks of severe peripheral autoimmune reactivity, albeit without thyroid infiltration (Neu et al., 1985) (Table 64.1). Further analyses of F2 and back-cross generations have provided a rather unique picture, since in this model only a relatively small number of genes seem to be involved (Kroemer et al., 1989).

The development of spontaneous autoimmune thyroiditis in OS chickens is based on the presence of two sets of essential genes, one responsible for the autoreactivity of the immune system and the other for target organ susceptibility. The former comprises three to five dominant genes, while organ susceptibility is encoded by a single recessive gene (Hala, 1988). One of the dominant autoimmunity-associated genes is responsible for interleukin (IL)-2 hyperproduction, another for increased expression of the IL-2 receptor (IL-2R) (Kroemer and Wick, 1989). The role of the other two genes has not been identified, but they include neither a certain major histocompatibility complex (MHC) haplotype (*B* locus in chickens) nor a restricted, albeit preferential, use of a given T-cell receptor (TCR) V gene (Cihak et al., 1995). The recessive gene responsible for target organ susceptibility has not been identified, but preliminary evidence points to the possibility that it may be associated with a susceptibility of thyroid epithelial cells to viral infection (Kuhr et al., 1994; Hofmann et al., 2003).

This concept, which attaches a major importance to the role of the target structure in the development of organ-specific as well as systemic autoimmune diseases, has formed the basis of all subsequent work by our group in this field and has gained special momentum in the context of the development of our "autoimmune" hypothesis of atherogenesis (Wick et al., 2004). As described below, the original version of this hypothesis was based on the observation of a preexisting immunity in nearly all humans against a phylogenetically highly conserved group of antigens belonging to the stress proteins, also called heat-shock

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 64.1 Target organ susceptibility in autoimmune disease

	Disease phenotype	
Chicken strain*	**Spontaneous autoimmune thyroiditis**	**Antithyroglobulin autoimmunity**
Obese strain (OS)	++++	++++
Normal white leghorn (NWL)	—	—
(OS × NWL)F1	—	++++

proteins (HSPs) (Young and Elliott, 1989). Specifically, we have shown humoral and cellular immunity against HSPs belonging to the 60-kDa family (HSP60), which show high bacterial–human homology (Karlin and Brocchieri, 2000). This anti-HSP60 immunity can be induced by infection or vaccination, as well as *bona-fide* autoimmunity against altered autologous HSP60. This protective, beneficial anti-HSP60 immunity only becomes detrimental, leading to the development of the first inflammatory stages of atherosclerosis, when the target cells, in this case arterial endothelial cells (ECs), express the stress protein HSP60 as a response to their confrontation with classical risk factors for atherosclerosis, such as hypertension, diabetes, biochemically altered, e.g., oxidized, low-density lipoproteins (oxLDLs), and toxins, e.g., cigarette smoke. In other words, risk factors for atherosclerosis make the target cells susceptible to attack by a pre-existing immune reaction, and atherosclerosis is therefore the price we pay for maltreating our vascular system (Wick et al., 1995). The fact that atherosclerosis can afflict nearly everybody is based on two prerequisites: 1) the presence of adaptive as well as innate immunity against HSP60 in all healthy humans, as mentioned above; and 2) the subjection of mechanically (by arterial blood pressure) pre-stressed arterial ECs to unnatural additional stress conditions in the form of modern risk factors for atherosclerosis not "foreseen" by natural selection during phylogeny and human development. Clinically manifest atherosclerosis is classified as an age-associated disease, since early, still symptomless, lesions do not impair the reproductive capacity that in the past has driven the shaping of the human genome.

It should be emphasized, however, that our interest, as well as the validity of this concept, concerns only the initial stages of atherogenesis rather than the complicated late lesions. Furthermore, we exclusively focus on "conventional" atherosclerosis, which can afflict nearly everyone, rather than special forms, such as the genetically-determined familial hypercholesterolemia in LDL-receptor-deficient patients. This is also the reason why we only rarely resort to animal models of latter forms of atherosclerosis, e.g., LDL-receptor or ApoE knock-out mice.

DEFINITIONS AND EXPERIMENTAL BACKGROUND

The arterial wall is composed of four layers: 1) the innermost monolayer lining of ECs is separated by a basement membrane from 2) the so-called *intima*, which consists of connective tissue and a few smooth muscle cells (SMCs); 3) the *media* consists of SMCs, and the outer layer, 4) the *adventitia*, which connects the artery with its surrounding tissue, also harbors nerves and small vessels (*vasa vasorum*) that penetrate the wall of larger arteries, providing blood supply to areas that are too distant from the lumen to be maintained by simple diffusion. Atherosclerosis starts in the intima and until the early 1990s it was general dogma that so-called fatty streaks, cushion-like cholesterol-rich microscopically visible lesions, represented the first stage of the disease, later followed by more severe changes, so-called atherosclerotic plaques (Stary et al., 1994; 1995). The latter have a tendency to exulcerate and even calcify, leading to the formation of thromboses and hence sequelae such as myocardial infarction, stroke, and occlusion of peripheral arteries, e.g., in the legs.

Pathologists originally distinguished *arteriosclerosis* from *atherosclerosis*. The former, as the name implies, is characterized by thickening and hardening (sclerosis) of the arterial wall due to infiltration by MNCs, proliferation of pre-existing fibroblasts with increased formation of extracellular matrix (ECM) proteins, notably various types of collagen, and the immigration and subsequent proliferation of SMCs from the media into the intima. Atherosclerosis was considered a special form of arteriosclerosis, the hallmark of which is foam cells, i.e., macrophages and SMCs that have taken up biochemically modified LDL, e.g., oxLDL, via scavenger receptors. In recent years, this distinction has been abandoned by most authors and atherosclerosis frequently serves as a common term for the different forms of the disease, and is so used in this chapter.

In the past 20 years, reports pointing to inflammatory processes that take place within atherosclerotic lesions have appeared in the scientific literature with increasing frequency (Ridker, 1999; Ross, 1999, Hansson, 2001; Libby et al., 2002). However, these papers mainly concentrate on the histologic, electron microscopic, and immunohistologic analyses of advanced lesions where, in addition to the above-mentioned macrophages derived from blood-borne monocytes, T cells can also be demonstrated in high frequency (Hansson et al., 1989). Thus, histologic investigations of specimens from young adults secured within the framework of the PDAY (Pathobiological Determinants of Atherosclerosis in Youth) study originally did not mention

pathologic inflammatory processes in general, or identify the phenotype of lesion-infiltrating MNCs in particular (McGill et al., 2000).

Early in our work, we conducted an elaborate immunohistologic study of arterial specimens with early and mild lesions derived from young donors (younger than 35 years) and more severe lesions from old (older than 65 years) donors, and found clear evidence that the first cells to arise in the intima were not macrophages and SMCs but rather $CD3^+$ T-cells (Xu et al., 1990). The majority of these T cells infiltrating the intima were $CD4^+$ and carried the TCR$\alpha\beta$. They showed the characteristics of activation, i.e., expression of CD25 and HLA-DR. Interestingly, an unexpectedly high percentage of T cells in these early lesions were TCR$\gamma\delta^+$ (10–15% as compared to 1–2% in the peripheral blood). These cells mainly expressed the TCR V δ^1 chain, similar to $\gamma\delta^+$ T cells in the mucosa-associated lymphoid tissue (MALT), while the TCR V γ^9/δ^{2+} was present in the same frequency as in peripheral blood (Kleindienst et al., 1993). Here it is important to mention that $\gamma\delta^+$ T cells at different sites of the MALT show a preferential, non-MHC-restricted reactivity with HSP60, i.e., a bacterial antigen that apparently assumed an important antigenic role during evolution (Born, 1991).

In the course of our original immunohistologic studies, we also had the opportunity to analyze the carotid artery of a 1-year-old baby. Unexpectedly, we found considerable accumulations of MNCs in the intima at the arterial branching points, which are subjected to increased (turbulent) hemodynamic stress and are known to be predilection sites for the possible later development of atherosclerosis. As described below, we then performed an extensive series of experiments that led to the concept of the existence of a vascular-associated lymphoid tissue (VALT) as a so far unknown site of an (inner) surface line of defense (Wick et al., 1997; Waltner-Romen et al., 1998; Millonig et al., 2001). In a similar investigation on specimens from the PDAY study, we confirmed the presence of early inflammatory T-cell-dominated intimal infiltrations, a finding supplemented by the demonstration of dendritic cells (DCs) and mast cells. B cells were scarce and $CD56^+$ K/NK cells were not detected (Millonig et al., 2002).

In our attempts to elucidate the antigenic specificity of the intralesional lymphocytes, we resorted to the classical technique of inducing autoimmune diseases in normal animals. We immunized normocholesterolemic rabbits with human plaque proteins as well as plaque proteins derived from Watanabe rabbits, which develop hypercholesterolemia due to the genetically determined absence of the LDL receptor. Delipidated total plaque proteins were emulsified in complete Freund adjuvant (CFA) and used for the immunization. Ovalbumin (OVA) served as a negative control. Our original intention was to identify the culprit protein moiety among the plaque proteins in case atherosclerotic lesions could be induced by this approach (Xu et al., 1992). To our surprise, all three groups, including the OVA-immunized animals, developed atherosclerotic lesions that were histologically characterized by abundant intimal infiltration with MNCs, mainly lymphocytes. Since CFA was the only common denominator in these experiments, we then immunized rabbits with CFA alone, which again resulted in the development of atherosclerotic lesions. Since heat-killed mycobacteria are the main active component in CFA, we then focused on this constituent. It is known from other experiments that HSP60 of mycobacteria (mHSP65, a 65-kDa protein) is a qualitatively and quantitatively important component of these microorganisms (van der Zee, 1998). Immunization with recombinant mHSP65 had the same atherosclerosis-inducing effect as CFA alone. Immunization with mHSP65 plus feeding a cholesterol-rich diet significantly aggravated the development of these lesions. Furthermore, while the early inflammatory lesions in normocholesterolemic rabbits were still reversible, this was not the case for the severe lesions in immunized plus cholesterol-fed rabbits (Xu et al., 1996). We also included controls that were not immunized but only received a cholesterol-rich diet. While it was not unexpected that *in vitro* cultured T-cell lines derived from lesions of mHSP65-immunized rabbits showed a significant reactivity against this antigen, it was surprising that T cells derived from lesions of unimmunized hypercholesterolemic animals also displayed significantly higher stimulation indices in response to mHSP65, as compared to peripheral blood T-cells of the same animals (Xu et al., 1993a).

Similar experiments were then conducted in apoprotein E knock-out and wild-type C57/BL6 mice. In addition, immunization with oxLDL and a non-HSP60-containing adjuvant was performed in these animals. The results showed that the atherosclerosis that develops spontaneously in hypercholesterolemic mice is aggravated by immunization with mHSP65 (George et al., 1999), while induction of immunity to oxLDL had a protective effect (George et al., 1998). So, in addition to confirming the pro-atherogenic effect of HSP60, these studies also solved another controversy, i.e., they disproved a similar effect of immunity to oxLDL. Also, in the murine system, passive transfer of atherosclerosis was achieved by T cells from mHSP65 C57/BL6 immunized donors to histocompatible recipients (George et al., 2001). Furthermore, peroral induction of immune tolerance against mHSP60 made such animals resistant to the subsequent induction of atherosclerosis by appropriate immunization (Harats et al., 2002). As expected, mHSP65 immunized rabbits and mice also developed humoral antibodies against this antigen, but passive transfer via this mechanism has not yet been attempted.

Reactivity of human T-cells derived from advanced lesions against HSP60 has been described (Mosorin et al., 2000), but so far it has not been possible to perform similar

experiments with T cells harvested from early lesions that are clinically not yet apparent.

HEAT-SHOCK PROTEINS

HSPs are proteins that are expressed by prokaryotic and eukaryotic cells as a response to various stress factors, e.g., heat (hence the name), mechanical stress, toxins, and oxygen radicals (Young, 1992). HSPs are classified according to their molecular weights, which range from very large (90–100 kDa) to small (<10 kDa) (Craig et al., 1993). HSP60 is a special case since, as mentioned above, it is not only an important and highly antigenic component of bacteria and parasites, eliciting immune responses in all animals and humans, but also plays a major role in several autoimmune diseases. HSP60 is phylogenetically highly conserved. Thus, it shows over 95% homology between different bacterial species, and an overall 55% homology with eukaryotic, e.g., human, HSP60 (Jones et al., 1993). HSP60 executes an important physiologic task during the assembly and transport of intracellular proteins. In addition, it acts as a chaperone, i.e., it associates with cellular proteins under mildly stressful conditions, protecting them from denaturation (Benjamin and McMillan, 1998; Craig et al., 1993).

HSP60 is a mitochondrial protein (Figure 64.1). However, it is transported from that site into the cytoplasm and even to the cellular surface (Soltys and Gupta, 1997; Xu et al., 1994) where it seems to act as a danger signal for the innate and adaptive immune system. HSP60 is, of course, expressed by many cells of the body, but ECs are a special case because they are the first to be encountered by circulating antibodies and cells of the adaptive and innate immune system, respectively.

It has recently been shown that extracellular HSP60 can also bind passively to highly conserved pattern-recognition receptors (PRRs), i.e., Toll-like receptors (TLRs) and CD14 (Bulut et al., 2002; Habich et al., 2002). HSP60 belongs to the pathogen-associated molecular patterns (PAMPs), which are present on the surface of pathogens and also in solution. Binding of such ligands to TLRs leads to internalization of the ligand–receptor complex and initiation of signal transduction via the mitogen-activated protein kinase (MAPK) pathway (Akira et al., 2001). This activation is effected via various transcription factors, but mainly NF-κB. HSP60 acts as a ligand for TLR-4 and TLR-6, the former in association with CD14. The potential role of the TLR in the development of atherosclerosis has been reviewed recently by Michelsen et al. (2004); the focus is the triggering of signaling via TLR-4 by foreign pathogens and endogenously generated ligands. A recent report based on data from the BRUNECK study (Kiechl and Willeit, 1999) involving a very large number of volunteers suggests a significant role for TLR-4 polymorphism in atherogenesis (Kiechl et al., 2002). It remains, however, to be clarified if HSP60 binding to different TLR isotypes also involves alteration of signal transduction.

With respect to the adaptive immune response against antigenic determinants that are accessible on the surface of target cells, various scenarios are conceivable. Thus, peptides derived from autochronous HSP60 will be presented in the context of MHC class I molecules on the surface of ECs, where they can be recognized by HSP60-reactive $CD8^+$ cells. In addition, conformational HSP60

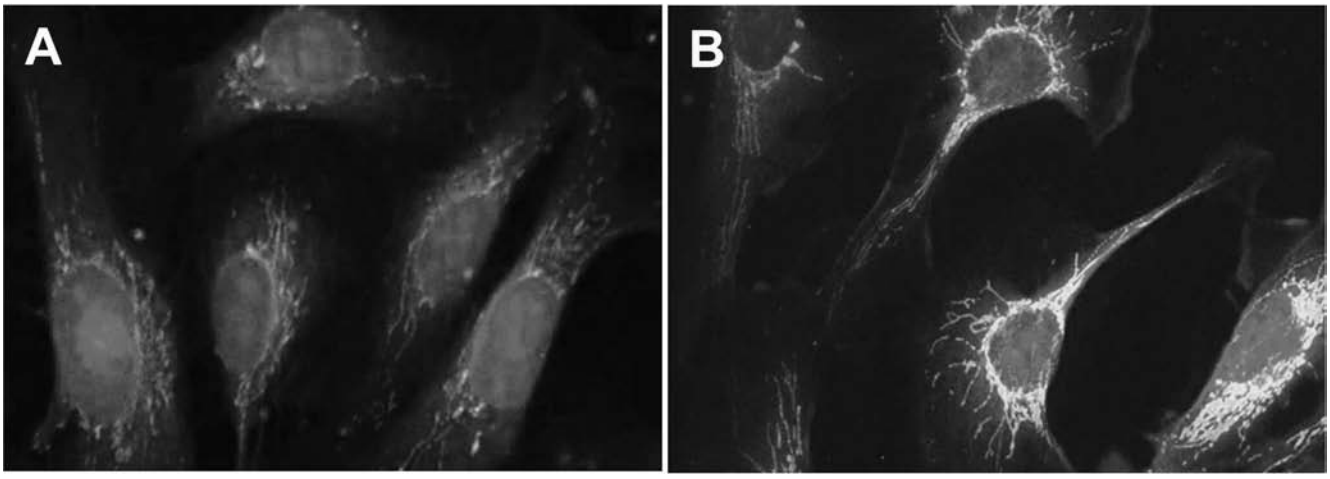


FIGURE 64.1 Acetone-fixed human umbilical vein endothelial cells stained in indirect immunofluorescence for the presence of HSP60 (FITC, green) and counterstained with TRITC-labelled phalloidin (red). *A*, unstressed cells, and *B*, heat stressed cells (42 °C for 30 min). Note the weak mitochondrial staining in *A*, most probably reflecting mild stress from the culture conditions, and the significant upregulation of HSP60 expression in the mitochondria and cytoplasm in *B*. Laser confocal scanning microscope, original magnification ×1000. See color plate section.

epitopes are also exposed on the surface of stressed target cells, where they can be recognized by specific antibodies that will lyse stressed, but not unstressed, cells via complement-mediated cytotoxicity or antibody-dependent cellular cytotoxicity (ADCC) (Schett et al., 1995). Furthermore, soluble HSP60 (sHSP60) that is released by stressed endothelial target cells themselves (Xu et al., 2000) or, to a greater extent, by necrotic cells into the circulation, can bind to the surface of ECs via TLRs and CD14, as described above, or be processed via the MHC class II antigen presentation pathway. In the latter case, they will be recognized by $CD4^+$ T cells or again by specific humoral antibodies. Finally, as mentioned above, γ/δ^+ T cells recognize HSP60 epitopes in a non-MHC-restricted fashion. sHSP60 has proven to be an inflammatory marker that is independent of other classical markers, such as C-reative protein (CRP) (Xu et al., 2000).

CLINICAL STUDIES

Having completed the studies in experimental animals that led to the formulation of our autoimmune hypothesis for atherogenesis (see below), we embarked on studies in a clinical setting within the framework of the BRUNECK study. This is a large population-based atherosclerosis prevention program that has been ongoing since 1990 and originally involved a cohort of nearly 1000 people of both sexes, aged 40–80 years (Willeit and Kiechl, 1993). This cohort is extensively assessed every 5 years with a great number of personal, physical, clinical and laboratory values recorded (Kiechl and Willeit, 1999). Among these, parameters reflecting inflammation in general and immune reactivity in particular are of special interest. The outcome of the three observation points to date, i.e., 1990, 1995 and 2000, have been published and extensively reviewed. In brief, it was shown that although the sera of all volunteers contain antibodies to mHSP65, antibody titers are significantly increased in those who display sonographically demonstrable atherosclerotic lesions (Xu et al., 1993b). Furthermore, such antibodies not only showed the expected high degree of cross-reactivity with HSP60 of other bacterial species (chlamydial HSP60–cHSP60 and GroEL, the HSP60 of *Escherichia coli*), but also with human HSP60 (hHSP60) (Metzler et al., 1997; Mayr et al, 1999). They are cytotoxic for stressed but not unstressed ECs and other target cells, e.g., macrophages (Schett, 1997b). We consider the proatherogenic role of chlamydial infections as compared to other infections a quantitative rather than qualitative phenomenon: chlamydiae are very rich in HSP60, which is highly immunogenic; and chlamydiae have a tropism for ECs and represent an excessive stress factor for these cells, leading to massive eukaryotic HSP60 expression (unpublished observation).

If our concept of the pathogenetic relevance of microbial–human HSP60 cross-reactivity is also of clinical relevance, then the lifelong infectious load should also be associated with an increased odds ratio to develop atherosclerosis. Again, this has been proven within the framework of the BRUNECK study (Mayr et al., 2000; Kiechl et al., 2001). With respect to *Helicobacter pylori* infections, we have shown that an increased odds ratio for the development of atherosclerosis only surfaces when pathogenicity factors are taken into account (Mayr et al., 2003).

The association of anti-HSP60 antibodies with atherosclerosis has subsequently been confirmed by many laboratories [reviewed by Wick et al. (2004)].

We have also identified linear (Perschinka, 2003) and conformational bacterial (H. Perschinka, personal communication) human cross-reactive epitopes that are recognized by human antibodies purified by affinity chromatography over hHSP60 columns.

Numerous reports in the literature concern an association between periodontal disease and atherosclerosis (Kinane and Lowe, 2000). We have, therefore, studied the question whether salivary IgA antibodies to mHSP65 may reflect such an association, and have shown that this is indeed the case. We were also successful in identifying some of the epitopes of HSP60 expressed by the oral bacterial flora (Schett et al., 1997a).

With respect to a possible pathogenetic role of HSP60-reactive T-cells, our data from humans are less clear. We investigated this issue in the peripheral blood of volunteers from the BRUNECK study in 2000, i.e., when the youngest participants were 50 years old. No difference in stimulation indices of peripheral blood T cells derived from volunteers with and without sonographically demonstrable atherosclerotic lesions at various sites of the arterial tree could be demonstrated (B. Mayrl, unpublished observation), although there was still a statistically significant correlation with anti-HSP60 antibodies. Since, as stated above, our concept postulates a pathogenetic role of anti-HSP60 immunity mainly in the early stages of atherosclerosis, we concluded that the first BRUNECK cohort may have been too old for this type of investigation. Therefore, we performed similar studies in a smaller cohort of 17–18-year-old healthy male volunteers within the framework of the ARMY (Atherosclerosis Risk Factors in Male Youth) study (Knoflach et al., 2003). To our surprise, 28% of these young men already showed an increased intima media thickness (IMT) in at least one of eight measured sites. An increased IMT was associated with an increased rate of proliferation of peripheral blood T cells upon incubation with hHSP60 and, less pronounced, with mHSP65, as well as with anti-HSP60/65 antibodies. The association of anti-hHSP60 T-cell reactivity with an increased IMT was only surpassed by an increased odds ratio for atherosclerosis in smokers, but unexpectedly not by an elevated diastolic blood pressure.

This effect of cigarette smoking recently received even greater interest in view of the above-mentioned evidence for binding of HSP60 to cell-surface receptors and the statistically significant association of the concentration of sHSP60 with sonographically diagnosed atherosclerosis. Furthermore, since we have also obtained evidence for biochemical alterations of circulating sHSP60, we consider this fact to be possible basis for the development of *bona-fide* autoimmunity in addition to the already described mechanism of microbial–human HSP60 cross-reactivity. From these data we tentatively conclude that T cells exert a major role in the earliest stages of the disease and humoral antibodies play an initiating and accelerating pathogenetic role. In this context it is of interest that preliminary data show that the injection of a monoclonal anti-HSP60 antibody into mice leads to the development of atherosclerosis (Foteinos et al., 2005).

RISK FACTORS FOR THE SUSCEPTIBILITY OF ENDOTHELIAL TARGET CELLS TO ANTI-HSP60 IMMUNITY

The classical theories for atherogenesis are the "response to injury" hypothesis (Ross and Glomset, 1976a; 1976b) and the "oxLDL" hypothesis (Steinberg et al., 1989). In the former, damage to arterial endothelium is considered the initiating event in the disease, followed by thrombus formation, and eventual occlusion of the afflicted vessel. In the latter, a transgression of oxLDL via the endothelium into the intima has been postulated as the first event leading to foam-cell formation and extracellular cholesterol deposition at that site. More recently, however, evidence has been provided that the LDL oxidation process occurs in the intima itself rather than in the peripheral blood, and that this biochemically altered LDL acts as a chemoattractant for blood-borne monocytes and SMCs from the media, as well as leading to the formation of foam cells. This variant of the oxLDL hypothesis was called the "oxLDL retention" hypothesis (Nishi et al., 2002). We are convinced that the autoimmune hypothesis of atherogenesis comprises and expands these former concepts by explaining the development of the earliest stages of this disease. This view is based on the fact that we do not, of course, deny the well-proven role of atherosclerosis risk factors, but that we assign a different primary importance to them, i.e., as stress factors for ECs. The data that support this notion with respect to *endothelial target susceptibility* to both innate and adaptive defense mechanisms are briefly reviewed below.

In rats, we have shown that injection of bacterial endotoxin [lipopolysaccharide (LPS)] *in vivo* leads to the simultaneous expression by aortic ECs of HSP60 and the adhesion molecule ICAM-1 (Seitz et al., 1996). The latter is reflected by the increased adhesion of leukocytes with a predilection for arterial branching points.

In the rat system we also have demonstrated that increasing the blood flow in one common carotid artery by occlusion of the contralateral vessel leads to an increased expression of HSP60, both at the RNA and protein level (Hochleitner et al., 2000).

Similarly, other stress factors, such as proinflammatory cytokines (TNF-α) and oxygen radicals (H_2O_2), have a HSP60-inducing effect on human ECs *in vitro*. Perhaps most importantly, oxLDL has been shown to be a potent EC stressor (Amberger et al., 1997). All these *in vitro* effects were more pronounced in arterial than venous ECs. We have interpreted these results as showing that arterial ECs have a lower threshold for the effects of various stressors because they are subjected to a lifelong mechanical pre-stress from the higher arterial blood pressure compared with venous blood pressure.

A special case that supports the role of mechanical stress in atherogenesis is arteriovenous bypass restenosis. Since the early stages of atherosclerotic changes in bypass conduits are obviously not available to examination, except in cases of postoperative surgical complications, we addressed this issue in a unique murine arteriovenous bypass model (Zou et al., 1998). In this experimental situation, HSP60 expression by ECs also proved to be the first histochemically sizeable event, followed by mononuclear, i.e., T cell and macrophage, infiltration of the intima, which later even extended into the media and adventitia, and was finally followed by the thickening of the intima and media with complete occlusion as the final result.

Two interesting sidelines of our research are worth mentioning. First, the addition of cyclosporine A to *in vitro* cultures of human ECs in concentrations equal to those found in patients results in a massive expression of both HSP60 and adhesion molecules (Amberger et al., 1999). In contrast, pharmacologic doses of aspirin have no influence on HSP60 expression but downregulate the expression of adhesion molecules. The former observation may in part explain the increased frequency of atherosclerosis in patients treated with cyclosporine A; the latter adds an additional facet to the atherosclerosis preventive properties of aspirin.

Second, we have used our experimental repertoire to answer the often-raised question, do electromagnetic inductions (so-called "electrosmog") represent an atherosclerosis risk factor? We subjected human umbilical vein ECs (HUVECs) *in vitro* (Henderson et al., 2003a) or murine arteriovenous bypass graft-carrying mice *in vivo* (Henderson et al., 2003b) to electrosmog conditions (50 Hz, 700 μT), which can be reached in certain workplace settings. In neither case were we able to demonstrate induction of HSP60 or HSP70, thus making an atherosclerosis-inducing effect of such low-frequency electromagnetic inductions very improbable.

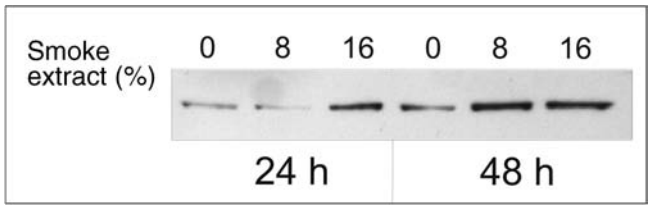


FIGURE 64.2 HSP60 release into supernatant of smoke-extract treated endothelial cells (ECs). Human umbilical vein ECs incubated with cigarette smoke extract, adjusted to *in vivo* compound concentrations, for the times indicated. Supernatants were transferred into 50-ml tubes and centrifuged to remove suspended cells and cell fragments. Supernatants were then concentrated with centrifugal filter devices. Electrophoresis buffer was added to the concentrated supernatants and the samples were subsequently subjected to SDS–polyacrylamide gel electrophoresis. Finally, supernatant proteins were transferred onto nitrocellulose membranes and HSP60 was detected via an antibody-mediated detection system.

In the ARMY study, smoking was identified, as expected, as the most important atherosclerosis risk factor in young male subjects, with an odds ratio of 3.5. Although the many health-deteriorating effects of cigarette smoke have been known for a long time and are highlighted on cigarette packs, we were surprised to learn that very little is known, or published, on the specific effects of smoke components on endothelial function. We have, therefore, approached this issue by devising an experimental method that allows the treatment of HUVECs with water-soluble components of cigarette smoke, again in concentrations that are seen in real life (Bernhard et al., 2004). With respect to this discussion, it is of interest that such treatment leads to EC necrosis rather than apoptosis (Bernhard et al., 2003). Before succumbing to necrosis, the cells undergo a stage of HSP60 expression, which shows that constituents of cigarette smoke act as potent cellular stressors. Subsequently, necrotic cells release abundant amounts of HSP60, which may contribute significantly to the serum concentrations of sHSP60 under *in vivo* conditions (Figure 64.2).

In summary, all risk factors for atherosclerosis that we have analyzed showed HSP60 and simultaneous adhesion molecule induction on endothelial target cells as their first effect. If corroborated by investigations in other laboratories, this crucial observation will further support our autoimmune hypothesis of atherogenesis.

CONCLUSION: THE AUTOIMMUNE HYPOTHESIS OF ATHEROGENESIS

The autoimmune hypothesis of atherogenesis postulates that pre-existing adaptive and innate immunity to HSP60 exposed on the surface of arterial ECs leads to the development of an initial inflammatory stage of this disease. Immunity against HSP60 seems to be an important facet of the defense system, e.g., as reflected by the fact that a considerable proportion of lymphocytes within the VALT show reactivity against HSP60. Also, every healthy animal and human possesses humoral antibodies against HSP60. Cells of the innate immune system recognize HSP60 as a danger signal, be it of microbial or autologous origin. The development of atherosclerosis therefore depends on the expression and accessibility of atherogenic HSP60 epitopes on the surface of ECs. This disease is obviously a price that we pay for maltreating our arterial vascular system with classical risk factors that have as their common denominator the ability to induce the expression of HSP60. Since everybody apparently has either microbial–human cross-reactive immunity or bona-fide autoimmunity, as well as innate immunity against HSP60, whether or not atherosclerosis develops depends exclusively on the state of the endothelial target cell.

Acknowlegments

We would like to thank all present and past members of our group whose work has contributed to our understanding of the immunology of atherosclerosis. We especially thank Dr Gerald Pfister for preparation of *Figure 64.1*, Michaela Kind and Christina Mayerl for expert technical help, Barbara Gschirr for preparation of the manuscript, and Ilona Atzinger for artwork. This work has been supported by the Austrian Research Fund (FWF, Project P14741), the Austrian Ministry of Defense (BMLV) and the Austrian Association of Electricity Companies (VEÖ).

References

Akira, S., Takeda, K., and Kaisho, T. (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat. Immunol. 2*, 675–680.

Amberger, A., Maczek, C., Jurgens, G., Michaeles, D., Schett, G., Trieb, K., Eberl, T., Jindal, S., Xu, Q., and Wick, G. (1997). Co-expression of ICAM-1, VCAM-1, ELAM-1 and Hsp60 in human arterial and venous endothelial cells in response to cytokines and oxidized low-density lipoproteins. *Cell Stress Chaperones 2*, 94–103.

Amberger, A., Hala, M., Saurwein-Teissl, M., Metzler, B., Grubeck-Loebenstein, B., Xu, O., and Wick, G. (1999). Suppressive effects of anti-inflammatory agents on human endothelial cell activation and induction of heat shock proteins. *Mol. Med. 5*, 117–128.

Benjamin, I.J. and McMillan, D.R. (1998). Stress (heat shock) proteins: molecular chaperones in cardiovascular biology and disease. *Circ. Res. 83*, 117–132.

Bernhard D., Pfister G., Huck C.W., Kind, M., Salvenmoser, W., Bonn, G.K., and Wick, G. (2003). Disruption of vascular endothelial homeostasis by tobacco smoke: impact on atherosclerosis. *FASEB J. 17*, 2302–2304.

Bernhard, D., Huck, C.W., Jakschitz, T., Pfister, G., Henderson, B., Bonn, G.K., and Wick, G. (2004). Development and evaluation of an *in vitro* model for the analysis of cigarette smoke effects on cultured cells and tissues. *J. Pharmacol. Toxicol. Methods 50*, 45–51.

Born, W., Harbeck, R., and O'Brien, R.L. (1991). Possible links between immune system and stress response: the role of gamma delta T lymphocytes. *Semin. Immunol. 3*, 43–48.

Bulut, Y., Faure, E., Thomas, L., Karahashi, H., Michelsen, K.S., Equils, O., Morrison, S.G., Morrison, R.P., and Arditi, M. (2002). Chlamydial

heat shock protein 60 activates macrophages and endothelial cells through Toll-like receptor 4 and MD2 in a MyD88-dependent pathway. *J. Immunol. 168*, 1435–1440.

Cihak, J., Hoffmann-Fezer, G., Koller, A., Kaspers, B., Merkle, H., Hala, K., Wick, G., and Losch, U. (1995). Preferential TCR V beta 1 gene usage by autoreactive T cells in spontaneous autoimmune thyroiditis of the obese strain of chickens. *J. Autoimmun. 8*, 507–520.

Craig, E.A., Gambill, B.D., and Nelson R.J. (1993). Heat shock proteins: molecular chaperones of protein biogenesis. Microbiol. Rev. 57, 402–441.

Foteinos, G., Afzal, A. R., Mandal, K., Jahangiri, M., and Xu, Q. (2005). Anti-HSP60 autoantibodies induce atherosclerosis in ApoE-deficient mice via endothelial damage. *Circulation 112*, 1206–1213.

George, J., Afek, A., Gilburd, B., Levkovitz, H., Shalish, A., Goldberg, I., Kapolovic, Y., Wick, G., Schoenfeld, Y., and Harats, D. (1998). Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis 138*, 147–152.

George, J., Shoenfeld, Y., Afek, A., Gilburd, B., Keren, P., Shaish, A., Kapolovic, J., Wick, G., and Harats, D. (1999). Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein–65. *Arterioscler. Thromb. Vasc. Biol. 19*, 505–510.

George, J., Afek, A., Gilburd, B., Schoenfeld, Y., and Harats, D. (2001). Cellular and humoral immune responses to heat shock protein 65 are both involved in promoting fatty-streak formation in LDL-receptor deficient mice. *J. Am. Coll. Cardiol. 38*, 900–905.

Habich, C., Baumgart, K., Kolb, H., and Burkart, V. (2002). The receptor for heat shock protein 60 on macrophages is saturable, specific, and distinct from receptors for other heat shock proteins. *J. Immunol. 168*, 569–576.

Hala, K. (1988). Hypothesis: immunogenetic analysis of spontaneous autoimmune thyroiditis in obese strain (OS) chickens: a two-gene family model. *Immunobiology 176*, 354–373.

Hansson, G.K., Holm, J., and Jonasson, L. (1989). Detection of activated T lymphocytes in the human atherosclerotic plaque. *Am. J. Pathol. 135*, 169–175.

Hansson, G.K. (2001). Immune mechanisms in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol. 21*, 1876–1890.

Harats, D., Yacov, N., Gilburd, B., Schoenfeld, Y., and George, J. (2002). Oral tolerance with heat shock protein 65 attenuates *Mycobacterium tuberculosis*-induced and high-fat-diet-driven atherosclerotic lesions. *J. Am. Coll. Cardiol. 40*, 1333–1338.

Henderson, B., Tagwerker, A., Mayrl, C., Pfister, G., Boeck, G., Ulmer, H., Dietrich, H, and Wick, G. (2003a). Progression of arteriovenous bypass restenosis in mice exposed to a 50 Hz magnetic field. *Cell Stress Chaperones 8*, 373–380.

Henderson, B.R., Pfister, G., Boeck, G., Kind, M., and Wick, G. (2003b). Expression levels of heat shock protein 60 in human endothelial cells *in vitro* are unaffected by exposure to 50 Hz magnetic fields. *Cell Stress Chaperones 8*, 172–182.

Hochleitner, B.W., Hochleitner, E.O., Obrist, P., Eberl, T., Amberger, A., Xu, Q., Margreiter, R., and Wick, G. (2000). Fluid shear stress induces heat shock protein 60 expression in endothelial cells *in vitro* and *in vivo*. *Arterioscler. Thromb. Vasc. Biol. 20*, 617–623.

Hofmann, A., Plachy, J., Hunt, L., Kaufman, J., and Hala, K. (2003). v-scr oncogene-specific carboxy-terminal peptide is immunoprotective against Rous sarcoma growth in chickens with MHC class I allele B-F12. Vaccine 21, 4694–4699.

Jones D.B., Coulson A.F., and Duff G.W. (1993). Sequence homologies between hsp60 and autoantigens. *Immunol. Today 14*, 115–118.

Karlin, S. and Brocchieri, L. (2000). Heat shock protein 60 sequence comparisons: duplications, lateral transfer, and mitochondrial evolution. *Proc. Natl. Acad. Sci. USA 97*, 11348–11353.

Kiechl, S. and Willeit, J. (1999). The natural course of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol. 19*, 1484–1498.

Kiechl, S., Egger, G., Mayr, M., Wiedermann, C.J., Bonora, E., Oberhollenzer, F., Muggeo, M., Xu, Q., Wick, G., Poewe, W., and Willeit, J. (2001). Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. *Circulation 103*, 1064–1070.

Kiechl, S., Lorenz, E., Reindl, M., Wiedermann, C.J., Oberhollenzer, F., Bonora, E., Willeit, J., and Schwartz, D.A. (2002). Toll-like receptor 4 polymorphisms and atherogenesis. *N. Engl. J. Med. 347*, 185–192.

Kinane, D.F. and Lowe, G.D. (2000). How periodontal disease may contribute to cardiovascular disease. *Periodontology 23*, 121–126.

Kleindienst R., Xu Q., Willeit J., Waldenberger, F.R., Weimann, S., and Wick, G. (1993). Immunology of atherosclerosis. Demonstration of heat shock protein 60 expression and T lymphocytes bearing alpha/beta or gamma/delta receptor in human atherosclerotic lesions. *Am. J. Pathol. 142*, 1927–1937.

Knoflach, M., Kiechl, S., Kind, M., Said, M., Sief, R., Gisinger, M., van der Zee, R., Gaston, H., Jarosch, E., Willeit, J., and Wick, G. (2003). Cardiovascular risk factors and atherosclerosis in young males: ARMY study (Atherosclerosis Risk-Factors in Male Youngsters). *Circulation 108*, 1064–1069.

Kroemer, G., Neu, N., Kuehr, T., Dietrich, H., Fassler, R., Hala, K., and Wick, G. (1989). Immunogenetic analysis of spontaneous autoimmune thyroiditis of obese strain chickens. *Clin. Immunol. Immunopathol. 52*, 202–213.

Kroemer, G. and Wick, G. (1989). The role of interleukin 2 in autoimmunity. *Immunol. Today 10*, 246–251.

Kuhr, T., Hala, K., Dietrich, H., Herold, M., and Wick, G. (1994). Genetically determined target organ susceptibility in the pathogenesis of spontaneous autoimmune thyroiditis: aberrant expression of MHC-class II antigens and the possible role of virus. *J. Autoimmun. 7*, 13–25.

Libby, P., Ridker, P.M., and Maseri, A. (2002). Inflammation and atherosclerosis. *Circulation 105*, 1135–1143.

Mayr, M., Metzler, B., Kiechl, S., Williet, J., Schett, G., Xu, Q., and Wick, G. (1999). Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of *Escherichia coli* and *Chlamydia pneumoniae*: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. *Circulation 99*, 1560–1566.

Mayr, M., Kiechl, S., Willeit, J., Wick, G., and Xu, Q. (2000). Infections, immunity, and atherosclerosis: associations of antibodies to *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis. *Circulation 102*, 833–839.

Mayr, M., Kiechl, S., Mendall, M.A., Willeit, J., Wick, G., and Xu, Q. (2003). Increased risk of atherosclerosis is confined to CagA-positive *Helicobacter pylori* strains: prospective results from the Bruneck study. *Stroke 34*, 610–615.

McGill, H.C., Jr., McMahan, C.A., Zieske, A.W., Sloop, G.D., Walcott, J.V., Troxclair, D.A., Malcolm, G.T., Tracy, R.E., Oalmann, M.C., and Strong, J.P. (2000). Associations of coronary heart disease risk factors with the intermediate lesion of atherosclerosis in youth. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Arterioscler. Thromb. Vasc. Biol. 20*, 1998–2004.

Metzler, B., Schett, G., Kleindienst, R., van der Zee, R., Ottenhoff, T., Hajeer, A., Bernstein, R., Xu, Q., and Wick, G. (1997). Epitope specificity of anti-heat shock protein 65/60 serum antibodies in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol. 17*, 536–541.

Michelsen, K.S., Doherty, T.M., Shah, P.K., and Arditi, M. (2004). TLR signaling: an emerging bridge from innate immunity to atherogenesis. *J. Immunol. 173*, 5901–5907.

Millonig, G., Schwentner, C., Mueller, P., Mayerl, C., and Wick, G. (2001). The vascular-associated lymphoid tissue: a new site of local immunity. *Curr. Opin. Lipidol. 12*, 547–553.

Millonig, G., Malcom, G.T., and Wick, G. (2002). Early inflammatory-immunological lesions in juvenile atherosclerosis from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. *Atherosclerosis 160*, 441–448.

Mosorin, M., Surcel, H.-M., Laurila, A., Lehtinen, M., Karttunen, R., Juvonen, J., Paavonen, J., Morrison, R.P., Saikku, P., and Juvonen, T. (2000). Detection of *Chlamydia pneumoniae*-reactive T lymphocytes in human atherosclerotic plaques of carotid artery. *Arterioscler. Thromb. Vasc. Biol. 20*, 1061–1067.

Neu, N., Hala, K., Dietrich H., and Wick, G. (1985). Spontaneous autoimmune thyroiditis in obese strain chickens: a genetic analysis of target organ abnormalities. *Clin. Immunol. Immunopathol. 37*, 397–405.

Nishi, K., Itabe, H., Uno, M., Kitazato, K.T., Heriguchi, H., Shinno, K., and Nagahiro, S. (2002). Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler. Thromb. Vasc. Biol. 22*, 1649–1654.

Perschinka, H., Mayr, M., Millonig, G., Mayerl, C., van der Zee, R., Morrison, S. G., Morrison, R.P., Xu, Q., and Wick, G. (2003). Cross-reactive B-cell epitopes of microbial and human heat shock protein 60/65 in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol. 23*, 1060–1065.

Ridker, P.M. (1999). Inflammation, atherosclerosis, and cardiovascular risk: an epidemiologic view. *Blood Coagul. Fibrinolysis 10*(Suppl 1), S9–12.

Ross, R. (1999). Atherosclerosis—an inflammatory disease. *N. Engl. J. Med. 340*, 115–126.

Ross, R. and Glomset, J.A. (1976a). The pathogenesis of atherosclerosis (first of two parts). *N. Engl. J. Med. 295*, 369–377.

Ross, R. and Glomset, J.A. (1976b). The pathogenesis of atherosclerosis (second of two parts). *N. Engl. J. Med. 295*, 420–425.

Schett, G., Metzler, B., Kleindienst, R., Moschen, I., Hattmannsdorfer, R., Wolf, H., Ottenhoff, T., Xu, Q., and Wick, G. (1997a). Salivary anti-hsp65 antibodies as a diagnostic marker for gingivitis and a possible link to atherosclerosis. *Int. Arch. Allergy Immunol. 114*, 246–250.

Schett, G., Metzler, B., Mayr, M., Amberger, A., Niederwiesser, D., Gupta, R.S., Mizzen, L., Xu, Q., and Wick, G. (1997b). Macrophage-lysis mediated by autoantibodies to heat shock protein 65/60. *Atherosclerosis 128*, 27–38.

Schett, G., Xu, Q., Amberger, A., van der Zee, R., Recheis, H., Willeit, J., and Wick, G. (1995). Autoantibodies against heat shock protein 60 mediate endothelial cytotoxicity. *J. Clin. Invest 96*, 2569–2577.

Seitz, C.S., Kleindienst, R., Xu, Q., and Wick, G. (1996). Coexpression of heat-shock protein 60 and intercellular-adhesion molecule-1 is related to increased adhesion of monocytes and T cells to aortic endothelium of rats in response to endotoxin. Lab. Invest. 74, 241–252.

Soltys, B.J. and Gupta, R.S. (1997). Cell surface localization of the 60 kDa heat shock chaperonin protein (hsp60) in mammalian cells. *Cell Biol. Int. 21*, 315–320.

Stary, H.C., Chandler, A.B., Glagov, S., Guyton, J.R., Insull, W., Jr, Rosenfeld, M.E., Schaffer, S.A., Schwartz, C.J., Wagner, W.D., and Wissler, R.W. (1994). A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation 89*, 2462–2478.

Stary, H.C., Chandler, A.B., Dinsmore, R.E., Fuster, V., Glagov, S., Insull, W., Jr, Rosenfeld, M.E., Schwartz, C.J., Wagner, W.D., and Wissler, R.W. (1995). A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler. Thromb. Vasc. Biol. 15*, 1512–1531.

Steinberg, D., Parthasarathy, S., Carew, T.E., Khoo, J.C., and Witztum, J.L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med. 320*, 915–924.

van der Zee, R., Anderton, S.M., Prakken, A.B., Lisbeth Paul, A.G., and van Eden, W. (1998). T cell responses to conserved bacterial heat-shock-protein epitopes induce resistance in experimental autoimmunity. *Semin. Immunol. 10*, 35–41.

Waltner-Romen, M., Falkensammer, G., Rabl, W., and Wick, G. (1998). A previously unrecognized site of local accumulation of mononuclear cells. The vascular-associated lymphoid tissue. *J. Histochem. Cytochem. 46*, 1347–1350.

Wick, G., Kromer, G., Neu, N., Fassler, R., Ziemiecka, A., Muller, R.G., Ginzel, M., Beladi, I, Kuhr, T., and Hala, K. (1987). The multifactorial pathogenesis of autoimmune disease. *Immunol. Lett. 16*, 249–257.

Wick, G., Brezinschek, H.P., Hala, K., Dietrich, H., Wolf, H., and Kroemer, G. (1989). The obese strain of chickens: an animal model with spontaneous autoimmune thyroiditis. *Adv. Immunol. 47*, 433–500.

Wick, G., Schett, G., Amberger, A., Kleindienst, R., and Xu, Q. (1995). Is atherosclerosis an immunologically mediated disease? *Immunol. Today 16*, 27–33.

Wick, G., Romen, M., Amberger, A., Metzler, B., Mayr, M., Falkensammer, G., and Xu, Q. (1997). Atherosclerosis, autoimmunity, and vascular-associated lymphoid tissue. *FASEB J. 11*, 1199–1207.

Wick, G., Knoflach, M., and Xu, Q. (2004). Autoimmune and inflammatory mechanisms in atherosclerosis. *Annu. Rev. Immunol. 22*, 361–403.

Willeit, J. and Kiechl, S. (1993). Prevalence and risk factors of asymptomatic extracranial carotid artery atherosclerosis. A population-based study. *Arterioscler. Thromb. 13*, 661–668.

Xu, Q.B., Oberhuber, G., Gruschwitz, M., and Wick, G. (1990). Immunology of atherosclerosis: cellular composition and major histocompatibility complex class II antigen expression in aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens. *Clin. Immunol. Immunopathol. 56*, 344–359.

Xu, Q., Dietrich, H., Steiner, H.J., Gown, A.M., Schoel, B., Miku, L.G., Kaufmann, S.H., and Wick, G. (1992). Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. *Arterioscler. Thromb. 12*, 789–799.

Xu, Q., Kleindienst, R., Waitz, W., Dietrich, H., and Wick, G. (1993a). Increased expression of heat shock protein 65 coincides with a population of infiltrating T lymphocytes in atherosclerotic lesions of rabbits specifically responding to heat shock protein 65. *J. Clin. Invest 91*, 2693–2702.

Xu, Q., Willeit, J., Marosi, M., Kleindienst, R., Oberhollenzer, F., Kiechl, S., Luef, G., and Wick, G. (1993b). Association of serum antibodies to heat-shock protein 65 with carotid atherosclerosis. *Lancet 341*, 255–259.

Xu, Q., Schett, G., Seitz, C. S., Hu, Y., Gupta, R. S., Wick, G. (1994). Surface staining and cytotoxic activity of heat-shock protein 60 antibody in stressed aortic endothelial cells. *Circ. Res. 75*, 1078–1085.

Xu, Q., Kleindienst, R., Schett, G., Waitz, W. Jindal, S., Gupta, R.S., Dietrich, H., and Wick, G. (1996). Regression of arteriosclerotic lesions induced by immunization with heat shock protein 65-containing material in normocholesterolemic, but not hypercholesterolemic, rabbits. *Atherosclerosis 123*, 145–155.

Xu, Q., Schett, G., Perschinka, H., Mayr, M., Egger, G., Oberhollenzer, F., Williet, J., Kiechl, S, and Wick, G. (2000). Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation 102*, 14–20.

Young, D.B. (1992). Heat-shock proteins: immunity and autoimmunity. *Curr. Opin. Immunol. 4*, 396–400.

Young, R.A. and Elliott, T.J. (1989). Stress proteins, infection, and immune surveillance. *Cell 59*, 5–8.

Zou, Y., Dietrich, H., Hu, Y., Metzler, B., Wick, G., and Xu, Q. (1998). Mouse model of venous bypass graft arteriosclerosis. *Am. J. Pathol. 153*, 1301–1310.

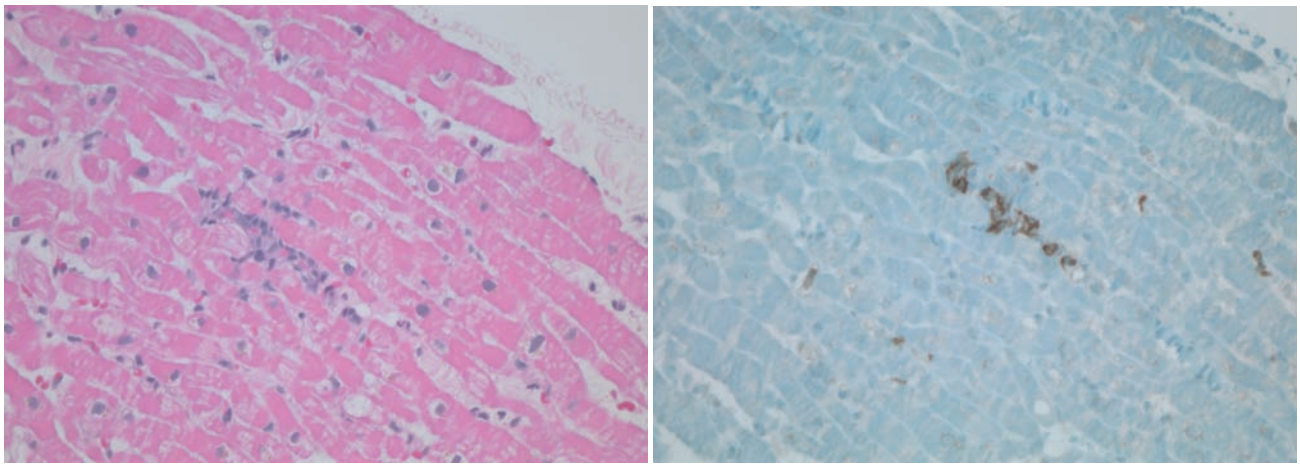


FIGURE 63.2 Borderline myocarditis. *A*, A single cluster of perivascular lymphocytes is present. No myocardial damage is identified. (H&E 400×). *B*, A CD8 immunohistochemical stain highlights the infiltrating T lymphocytes. (H&E 400×). See color plate section.

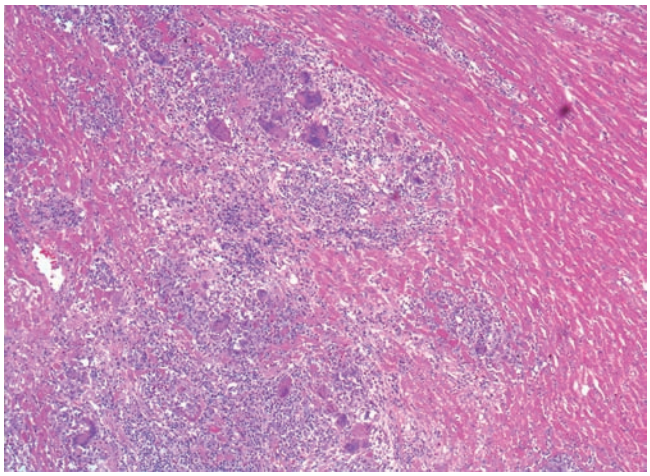


FIGURE 63.4 Giant cell myocarditis. The myocardium is infiltrated by a patchy and diffuse inflammatory infiltrate composed primarily of lymphocytes and macrophages. Multiple collections of giant cells are seen within the infiltrate along with eosinophils. There is significant injury and loss of myocytes in areas of inflammation, while adjacent myocardium is relatively uninvolved. (H&E 50×). See color plate section.

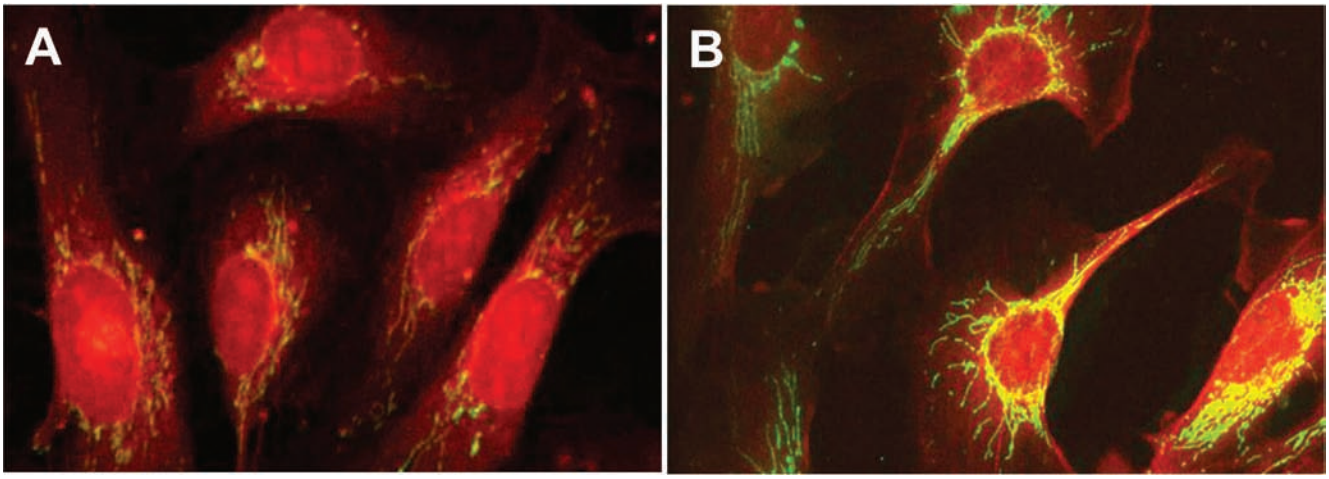


FIGURE 64.1 Acetone-fixed human umbilical vein endothelial cells stained in indirect immunofluorescence for the presence of HSP60 (FITC, green) and counterstained with TRITC-labelled phalloidin (red). A, unstressed cells, and B, heat stressed cells (42 °C for 30 min). Note the weak mitochondrial staining in A, most probably reflecting mild stress from the culture conditions, and the significant upregulation of HSP60 expression in the mitochondria and cytoplasm in B. Laser confocal scanning microscope, original magnification ×1000.

CHAPTER

65

Necrotizing Arteritis and Small Vessel Vasculitis

J. CHARLES JENNETTE*
*Department of Pathology and Laboratory Medicine

RONALD J. FALK†
†Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, USA

The three major categories of systemic vasculitis are large vessel vasculitis (chronic granulomatous arteritis), medium-sized vessel vasculitis (necrotizing arteritis), and small vessel vasculitis (necrotizing polyangiitis) (Figure 65.1; Table 65.1). This chapter will focus on medium-sized

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

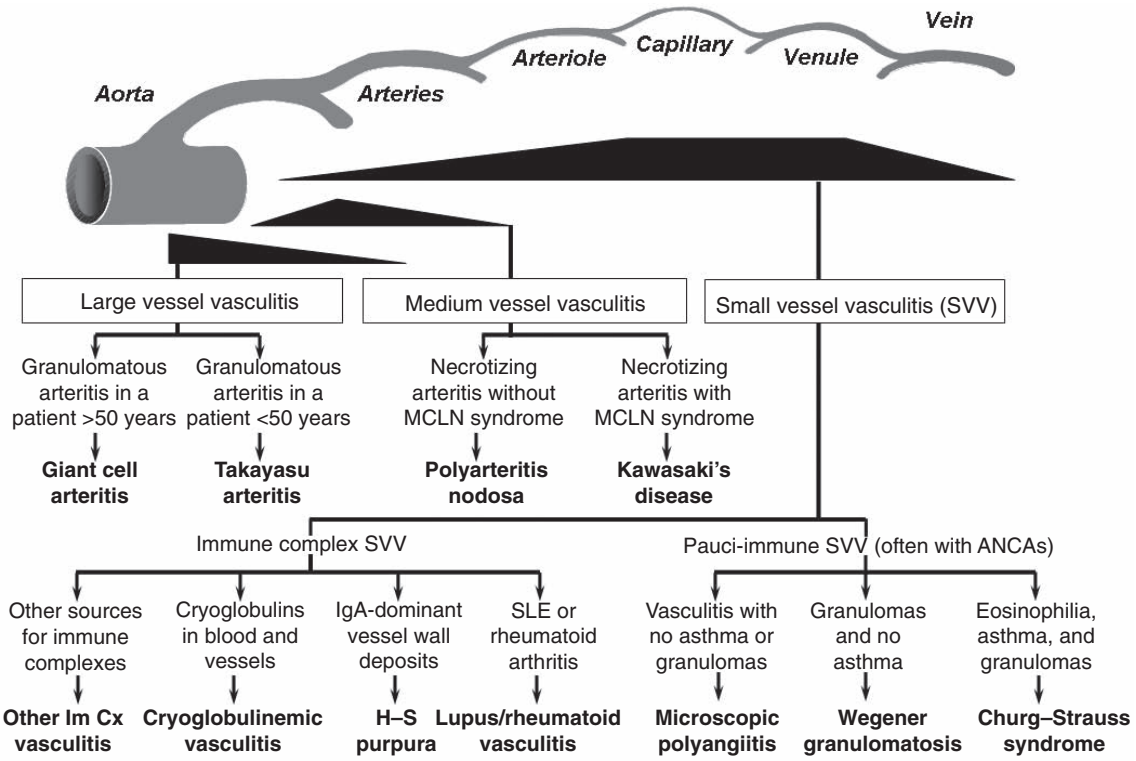


FIGURE 65.1 Overlapping vascular distributions (black triangles and trapezoid) of large vessel vasculitis, medium-sized vessel vasculitis, and small vessel vasculitis, as well as some of the features that differentiate between different clinicopathologic categories of vasculitis. ANCAs, antineutrophil cytoplasmic autoantibodies; H–S, Henoch–Schönlein; Im Cx, immune complex; MCLN, mucocutaneous lymph node system; SLE, systemic lupus erythematosus.

vessel vasculitis (necrotizing arteritis) and small vessel vasculitis. Chapter 66 deals with large vessel vasculitis.

HISTORIC BACKGROUND

Necrotizing arteritis was first recognized because of the grossly discernible segmental inflammatory nodular lesions that occur along major arteries (Kussmaul and Maier, 1866). Small vessel vasculitis (SVV) was first recognized because of the palpable purpura that is caused by inflammation of dermal venules (Willan, 1808).

Necrotizing Arteritis

Kussmaul and Maier (1866) gave the first detailed pathologic and clinical description of systemic necrotizing arteritis. They reported a patient with fever, anorexia, muscle weakness, paresthesias, myalgias, abdominal pain, and oliguria, who was found to have inflammatory nodules scattered over medium-sized and small arteries in many organs. They coined the term "periarteritis nodosa" for this process, which, over the following decades, evolved into "polyarteritis nodosa" as it became clear that the inflammation arose in the walls of arteries rather than in the perivascular tissue.

Soon after the recognition of polyarteritis nodosa (PAN), a number of investigators noted that some patients with necrotizing arteritis also had vasculitis affecting arteries that could be seen only by microscopy, and that some patients also had vasculitis affecting glomerular capillaries (glomerulonephritis) and pulmonary capillaries (alveolar capillaritis) (Arkin, 1930; Davson et al., 1948; Zeek et al., 1948; Zeek, 1952; Godman and Churg, 1954). Davson, and Godman and Churg called this the "microscopic form of periarteritis", and Zeek called it "hypersensitivity angiitis." Today, this form of vasculitis usually is designated microscopic polyangiitis (Jennette et al., 1994).

By the 1950s, Wegener's granulomatosis and Churg–Strauss syndrome had been recognized as variants of vasculitis that could have necrotizing arteritis combined with granulomatous inflammation and vasculitis in small vessels (Klinger, 1931; Wegener, 1939; Churg and Strauss, 1951; Godman and Churg, 1954). In their landmark publication in 1954, Godman and Churg concluded that the "microscopic form or periarteritis," Wegener granulomatosis, and Churg–Strauss syndrome are pathologically and clinically distinct from PAN, and probably have a related etiology and

TABLE 65.1 Names and definitions of vasculitis adopted by the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis [Modified from Jennette et al. (1994)]

Systemic vasculitis	Definition
Large vessel vasculitis*	
Giant cell (temporal) arteritis	Granulomatous arteritis of the aorta and its major branches, with a predilection for the extracranial branches of the carotid artery. *Often involves the temporal artery. Usually occurs in patients older than 40 years and often is associated with polymyalgia rheumatica*
Takayasu arteritis	Granulomatous inflammation of the aorta and its major branches. *Usually occurs in patients younger than 40 years*
Medium-sized vessel vasculitis*	
Polyarteritis nodosa (classic polyarteritis nodosa)	Necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries or venules
Kawasaki's disease	Arteritis involving large, medium-sized and small arteries, and associated with mucocutaneous lymph node syndrome. *Coronary arteries are often involved. Aorta and veins may be involved. Usually occurs in children*
Small vessel vasculitis*	
Wegener granulomatosis	Granulomatous inflammation involving the respiratory tract, and necrotizing vasculitis affecting small to medium-sized vessels, e.g., capillaries, venules, arterioles, and arteries. *Necrotizing glomerulonephritis is common*
Churg–Strauss syndrome	Eosinophil-rich and granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small to medium-sized vessels, and associated with asthma and blood eosinophilia
Microscopic polyangiitis (microscopic polyarteritis)	Necrotizing vasculitis with few or no immune deposits affecting small vessels, i.e., capillaries, venules, or arterioles. *Necrotizing arteritis involving small and medium-sized arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs*
Henoch–Schönlein purpura	Vasculitis with IgA-dominant immune deposits affecting small vessels, i.e., capillaries, venules, or arterioles. *Typically involves skin, gut, and glomeruli, and is associated with arthralgias or arthritis*
Essential cryoglobulinemic vasculitis	Vasculitis with cryoglobulin immune deposits affecting small vessels, i.e., capillaries, venules, or arterioles, and associated with cryoglobulins in serum. *Skin and glomeruli are often involved*
Cutaneous leukocytoclastic angiitis	Isolated cutaneous leukocytoclastic angiitis without systemic vasculitis or glomerulonephritis

*Large artery refers to the aorta and the largest branches directed toward major body regions (e.g., to the extremities, and the head and neck); medium-sized artery refers to the main visceral arteries (e.g., renal, hepatic, coronary, and mesenteric arteries), and small artery refers to the distal arterial radicals that connect with arterioles. Note that some small and large vessel vasculitides may involve medium-sized arteries, but large and medium-sized vessel vasculitides do not involve vessels smaller than arteries.

pathogenesis. This concept has been born out by the discovery that microscopic polyangiitis, Wegener granulomatosis, and Churg–Strauss syndrome are associated with, and are probably caused by, antineutrophil cytoplasmic autoantibodies (ANCAs), whereas PAN is not (Jennette and Falk, 1997).

An additional form of necrotizing arteritis was discovered that is associated with the mucocutaneous lymph node syndrome (Kawasaki, 1967; Tanaka et al., 1971). This disease has been called "infantile polyarteritis nodosa" because it almost always occurs in young children (Magilavy et al., 1977); however, the clinical, pathologic, and pathogenetic features of Kawasaki's disease are clearly distinct from those of PAN (Jennette et al., 1994).

Purpura and Small Vessel Vasculitis

The evaluation of patients with cutaneous palpable purpura revealed vasculitis involving predominantly small vessels. Although purpura had been described in medical writings at least since the time of Hippocates, Willan (1808) was one of the first to separate purpura caused by febrile infectious disease from noninfectious purpura. The occurrence of purpura in children with arthralgias and arthritis was reported by Schönlein in 1837. Later, Henoch (1868, 1882) described the association of purpura with abdominal pain, nephritis, small visceral hemorrhages, and pathologic changes in small vessels in the skin and internal organs. These pediatric patients probably had what today would be called Henoch–Schönlein purpura.

At the turn of the century, William Osler (1895; 1914) reported numerous adult patients with a more aggressive from of SVV most consistent with what today would be called microscopic polyangiitis. These patients had a broad spectrum of SVV manifestations, including purpura, arthritis, peripheral neuropathy, abdominal pain, pulmonary hemorrhage, epistaxis, iritis, and nephritis, including rapidly progressive renal disease with death from uremia in several months and the autopsy finding of glomeruli with "every tuft compressed by a crescentic mass."

The pathologic findings in SVV of extensive acute inflammation with numerous neutrophils and conspicuous

leukocytoclasia that resembled the Arthus reaction suggested a possible "hypersensitivity" pathogenesis (Winkleman, 1958). A hypersensitivity or allergic cause also was supported by the association of SVV with exposure to certain drugs and with serum sickness (Winkleman, 1958; Clark and Kaplan, 1937; Alarcon Segovia and Brown, 1964; Zeek et al., 1948). In the 1960s, the widespread application of immunofluorescence microscopy revealed that certain forms of SVV had substantial vascular localization of immunoglobulins and complement, suggesting an immune complex pathogenesis. Some patients with pulmonary–renal syndrome had linear deposits of immunoglobulin along glomerular and pulmonary capillary basement membranes (Sturgill and Westervelt, 1965), which were shown to be pathogenic autoantibodies directed against basement membrane collagen (Lerner et al., 1967). Patients with circulating cryoglobulins associated with signs and symptoms of SVV, such as purpura and glomerulonephritis, were found to have granular deposits of IgM, IgG, and complement in vessels walls (Meltzer et al., 1966), indicating an immune complex pathogenesis. This concept of immune complex-mediated SVV was further supported by the detection of hepatitis B antigens and antibodies in the walls of vessels in patients with SVV associated with hepatitis B virus (HBV) infection (Gower et al., 1978). Children with Henoch–Schönlein purpura were found to have deposits of IgA and C3 in dermal venules and glomerular capillaries supporting a distinct type of immune complex pathogenesis (Baart de la Faille-Kuyber et al., 1973). Neutrophilic SVV with extensive leukocytoclastic angiitis also was identified in the acute phase of Behçet's disease (Jorizzo et al., 1985).

By the end of the 1970s, there was a widespread belief that most if not all SVV was mediated by immune complexes (Fauci et al., 1978). However, not all immunohistologic studies of vasculitis showed evidence for substantial vessel wall deposition of immunoglobulins or complement. This was especially true in some of the most common forms of SVV in adults, including Wegener's granulomatosis (Ronco et al., 1983; Weiss and Crissman, 1984). In 1982, Davies et al. reported a new type of autoantibody that reacted with neutrophil cytoplasm in patients with SVV who did not have deposits of immunoglobulin or complement in inflamed glomeruli. Three years later, van der Woude et al. (1985) identified these autoantibodies in patients with Wegener' granulomatosis. Numerous subsequent studies have documented the association of these ANCAs with several clinicopathologic expressions of SVV (Wegener' granulomatosis, microscopic polyangiitis, Churg–Strauss syndrome) that are characterized by the absence or paucity of immunoglobulin and complement deposits in the vessel walls, which suggests a pathogenesis that does not involve extensive vascular deposition of immune complexes (Jennette and Falk, 1997). In fact, there is strong evidence that ANCA-associated pauci-immune SVV is caused by direct activation of neutrophils by autoantibodies directed against granule proteins (Xiao et al., 2002).

POLYARTERITIS NODOSA

Clinical, Pathologic, and Epidemiologic Features

The hallmark of PAN is necrotizing inflammation of medium-sized or small arteries. The prevalence is somewhere between 10 and 30 cases per million population (Watts and Scott, 2004; Mahr et al., 2004). The vascular inflammation initially contains predominantly neutrophils, but within a few days the infiltrates contain predominantly mononuclear leukocytes. Segmental inflammation and necrosis may produce pseudoaneurysms by eroding through the vessel wall into the surrounding tissue. Thrombosis can cause acute ischemia, including infarction. Rupture of pseudoaneurysms results in hemorrhage, which may be severe and life-threatening.

PAN is an acute disease that typically has one major episode with only rare recurrences if remission is attained (Guillevin, 1999). Clinical manifestations include cutaneous erythematous nodules and ulcers caused by dermal and subcutaneous arteritis; peripheral neuropathy (e.g., mononeuritis multiplex) caused by arteritis in epineural arteries; myalgias and elevated circulating muscle enzymes caused by arteritis in skeletal muscle; and pain and dysfunction in virtually any viscera caused by arteritis in the parenchyma. Involvement of the skin and gut is common, whereas involvement of the lungs is rare (Agard et al., 2003). Imaging studies may reveal arterial aneurysms (pseudoaneurysms), visceral infarcts or gut perforation. Diagnosis of PAN in a patient with necrotizing arteritis requires the exclusion of other diseases that also cause arteritis, including Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, and Churg–Strauss syndrome (Jennette et al., 1994; Agard et al., 2003; Mahr et al., 2004). Glomerulonephritis or alveolar capillaritis rule out a diagnosis of PAN and indicate some form of SVV, such as microscopic polyangiitis (Jennette et al., 1994; Agard et al., 2003). Renal involvement can result in renal insufficiency, hematuria, and low-level proteinuria, but this is the result of infarction and hemorrhage rather than glomerulonephritis. Myocardial infarction caused by coronary artery involvement is a rare complication.

Autoimmune Features

Most patients with PAN do not have recognized evidence for an autoimmune pathogenesis. Exceptions are the few patients with systemic lupus erythematosus who have

a vasculitis that is pathologically similar to idiopathic PAN (Korbet et al., 1984). PAN is not associated with ANCAs. As noted later, the serologic detection of ANCAs in a patient who is suspected of having PAN should raise the possibility of pauci-immune SVV instead (Guillevin et al., 1995).

Genetic Features

There is no evidence that genetic factors play a substantial role in the development of PAN. Familial occurrences of PAN are rare and may be related to hepatitis B virus (HBV) infection (Reveille et al., 1989; Mason et al., 1994). No genetic features of HBV have been identified that correlate with the induction of PAN (Janssen et al., 2004).

Environmental Influences

The most commonly recognized environmental factor is infection by HBV (Guillevin, 1999; Janssen et al., 2004). There are anecdotal reports of PAN associated with other infections, such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), and parvovirus B19, but a statistically significant relationship has been established only for HBV infection.

Animal Models

Rich (1942) proposed that PAN was mediated by immunologic mechanisms because he observed arteritis in patients who had serum sickness or had been treated with antibiotics. He acquired support for this hypothesis by inducing necrotizing arteritis in rabbits after injection of horse serum (Rich and Gregory, 1943). However, serum sickness in animals actually is a better model for SVV than for PAN because glomerulonephritis is a very frequent component (Germuth, 1953; Dixon et al., 1958). Interestingly, Pearl Zeek, who was one of the pioneers in delineating the clinical and pathologic features of PAN, studied an animal model of systemic arteritis that resembled PAN and was induced by implanting pieces of silk in the perirenal tissue of rats (Zeek et al., 1948).

Pathogenic Mechanisms

As noted above, at least some examples of PAN are thought to be mediated by immune complex deposition in vessel walls (Figure 65.2). HBV infection may be the source of pathogenic antigens in patients with PAN (Han, 2004; Janssen et al., 2004); however, the vasculitis that is associated with HBV infection often has more features of SVV than necrotizing arteritis alone.

When arterial wall immune complexes are present, they cause inflammation by activating the many interconnected humoral and cellular inflammatory mediator systems; e.g., the complement, kinin, plasmin, and coagulation humoral systems, and the neutrophil, mononuclear phagocyte, lymphocyte, and platelet cellular systems. Endothelial cells and mural smooth muscle cells are capable of modulating vascular inflammation, e.g., by producing lipid metabolites with cytokine activity, and, in the case of endothelial cells, by secreting cytokines, upregulating leukocyte adhesion molecules, and altering surface thrombogenicity (Pober, 1988). This complex interplay of humoral and cellular events results in the influx of inflammatory cells (especially neutrophils), necrosis, and sometimes thrombosis.

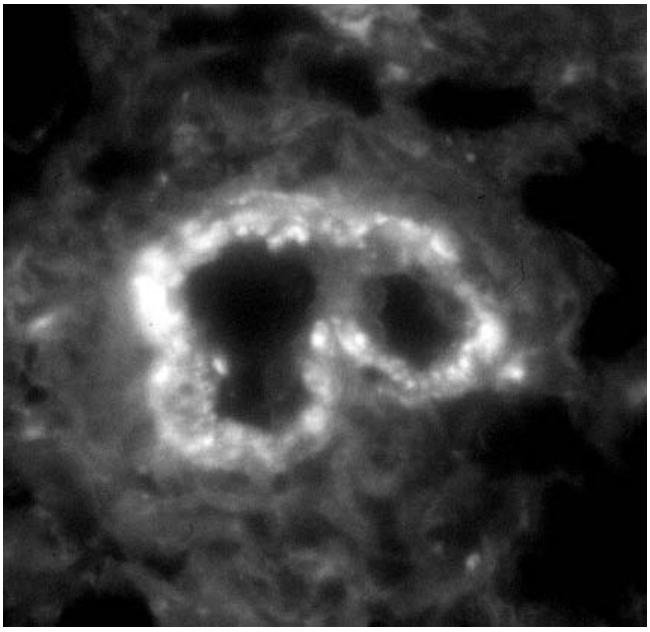


FIGURE 65.2 Direct immunofluorescence photomicrograph demonstrating granular IgG deposits in an artery from the subcutaneous tissue of a patient with hepatitis B-associated polyarteritis nodosa, showing granular vessel wall staining for C3.

Immunologic Markers in Diagnosis

Serologic testing for ANCA helps distinguish between PAN and pauci-immune SVV in a patient with arteritis because <5% of patients with PAN have a positive ANCA (Agard et al., 2003) compared to >80% of patients with pauci-immune SVV. For example, if a patient with mononeuritis multiplex and a peripheral nerve biopsy showing arteritis is ANCA-positive, pauci-immune SVV (e.g., microscopic polyangiitis) is a more likely possibility than PAN. Serologic identification of HBV infection by detection of antigens or antibodies supports a diagnosis of PAN because of the strong association between PAN and hepatitis B (Agard et al., 2003; Janssen et al., 2004) and the

rare occurrence of hepatitis B in other forms of vasculitis (Guillevin, 1999).

Treatment and Outcome

Corticosteroids and cytotoxic drugs have been the most frequently used therapy for PAN and many other form of noninfectious vasculitis. Guillevin (1999) advocates the use of corticosteroids alone for patients with PAN who lack all of the following five factors that indicate a worse prognosis: renal insufficiency, proteinuria >1 g/day, cardiac involvement, central nervous system (CNS) involvement, or gastrointestinal involvement. If 1 or more of these five factors are present, treatment should include combined steroids and cyclophosphamide. If one of these five factors is identified, the 5-year mortality is 25%, if two are identified it is 46%, and if none is identified it is 12%.

The optimal treatment of HBV-associated PAN includes a combination of antiviral and immunosuppressive therapies (Guillevin, 1999; Han, 2004; Janssen et al., 2004). In fact, control of HBV replication may be the most important factor in the resolution of HBV-associated PAN (Janssen et al., 2004). Guillevin (1999) advocates an initial short regimen of corticosteroids to subdue the arteritis, which is most intense during the first weeks of disease. Steroids are then stopped. Continued treatment of the putative immune complex mechanism can be pursued with plasmapheresis if manifestations of arteritis persist. Antiviral therapy also should be instituted, e.g., with interferon (IFN)-α or lamivudine.

KAWASAKI'S DISEASE

Clinical, Pathologic, and Epidemiologic Features

Kawasaki's disease is a self-limited febrile illness with systemic necrotizing arteritis that affects predominantly children, usually under 2 years of age. It was first described in Japan (Kawasaki, 1967), but occurs worldwide. The incidence of Kawasaki's disease in children under 5 years of age ranges from roughly 100 in 100,000 in Japan to 15 in 100,000 in the US to 10 in 100,000 in Europe (Watts and Scott, 2004).

The clinical hallmark of Kawasaki's disease is the mucocutaneous lymph node syndrome, which is characterized by oral mucosal changes, e.g., erythema of the oropharynx; "strawberry tongue," and dryness, redness or fissuring of the lips; skin lesions, e.g., erythema, indurative edema, or desquamation of the distal extremities, or polymorphous macular exanthema on the trunk; bilateral conjunctivitis, and lymphadenopathy (Kawasaki, 1967; Rauch and Hurwitz, 1985; Newburger and Fulton, 2004). The vasculitis of Kawasaki's disease involves medium-sized and small arteries, most notably the coronary arteries. The lesions are characterized histologically by segmental mural necrosis with infiltration by predominantly mononuclear leukocytes with less conspicuous neutrophils (Jennette, 2002). Necrosis occurs but typically has less fibrinoid material, more edema, and more macrophages than seen with PAN. Mural thickening, especially form intimal inflammation, may result in narrowing of the lumen and ischemia. Pseudoaneurysms are most common in the proximal coronary arteries and may be occluded by thrombus, resulting in myocardial infarction. Kawasaki's disease is the major cause of myocardial infarction in young children. Kawasaki's disease has surpassed acute rheumatic fever as the leading cause of acquired heart disease in children in the USA (Newburger and Fulton, 2004).

Autoimmune Features

Patients with Kawasaki's disease have circulating autoantibodies that react with activated endothelial cells (Leung et al., 1986a; 1986b; 1989; Grunebaum et al., 2002; Kaneko et al., 2004). Kaneko et al. (2004) identified a number of candidate target antigens using a cDNA expression library derived from tumor necrosis factor (TNF)-α-stimulated human umbilical vein endothelial cells. Tropomyosin was the most likely autoantigen recognized by anti-endothelial cell autoantibodies. Mor et al. (2002) have identified autoantibodies to tropomyosin in another form of vasculitis, Behçet's disease.

Cunningham et al. (1999) have reported IgM autoantibodies to cardiac myosin in Kawasaki's disease. These autoantibodies differ from the antimyosin antibodies of patients with acute rheumatic fever.

Genetic Features

A role for genetic factors in the pathogenesis of Kawasaki's disease is supported by the observation that children of parents who had Kawasaki's disease in childhood are at greater risk for developing the disease (Uehara et al., 2003). In addition, a child is at 10-fold greater risk of developing the disease within 1 year of onset of the disease in a sibling (Fujita et al., 1989). However, the genetic basis for this familial susceptibility has not been defined. No definite HLA genotype associations have been detected.

Environmental Influences

The clinical and epidemiologic features of Kawasaki's disease, especially the temporal clustering and seasonality, suggest an infectious or environmental etiology. However, in spite of extensive efforts over many decades, no specific infectious agent or environmental factor has been identified (Newburger and Fulton, 2004; Burns et al., 2005).

Animal Models

Takahashi et al. (2004) have developed an animal model of vasculitis that has a remarkable pathologic similarity to the arteritis of Kawasaki's disease. They injected a *Candida albicans* extract intraperitoneally for 5 consecutive days into a variety of mouse strains. Arteritis developed in 66% of CD-1 mice and most often affected the coronary arteries and aortic root close to the orifice of coronary arteries. The gross distribution and histologic pattern of injury closely mimics coronary arteritis in patients with Kawasaki's disease. Not all strains of mice developed disease, indicating a genetic susceptibility in certain strains.

Duong et al. (2003) have described a similar model of coronary arteritis induced by injection of *Lactobacillus casei* cell wall extract into mice. They hypothesized that the pathogenicity of *L. casei* cell wall extract may derive from its ability to function as a superantigen.

Pathogenic Mechanisms

Anti-endothelial cell antibodies (AECAs) have been incriminated in the pathogenesis of vascular injury in patients with Kawasaki's disease by Leung et al. (1986; 1980). They observed that patients with Kawasaki's disease have IgG and IgM AECAs that cause complement-mediated lysis of human umbilical and saphenous vein endothelial cells that had been pretreated with IFN-γ, interleukin (IL)-1, or TNF. From these observations, they hypothesized that the pathogenesis of vasculitis in Kawasaki's disease entails two events: production of AECAs (possibly related to the polyclonal B-cell activation that occurs in Kawasaki's disease) and increased cytokine production (possibly related to the increased activity of CD4 T-lymphocytes and monocytes that also occurs in Kawasaki's disease). The AECAs would bind to upregulated endothelial antigens, and cause endothelial death and vascular inflammation.

A role for bacterial superantigen has been postulated because of selective expansion of T-cell receptor (TCR) V β families in some patients with Kawasaki's disease (Newburger and Fulton, 2004). As noted earlier, observations in animal models also support this possibility.

Immunologic Markers in Diagnosis

No immunologic markers are used in the routine diagnosis of Kawasaki's disease (Newburger and Fulton, 2004). Erythrocyte sedimentation rate and C-reactive protein are consistently elevated but are completely nonspecific.

Treatment and Outcome

The conventional therapy for Kawasaki's disease is aspirin and intravenous gamma-globulin (Furusho et al., 1984; Nagashima, et al., 1987; Rowley, et al., 1988; Newburger and Fulton, 2004). If the interaction of AECAs with cytokine-activated endothelial cells is the major pathogenic mechanism in Kawasaki's disease, intravenous gamma-globulin could be acting by reducing, e.g., through negative feedback control, or neutralizing, e.g., by anti-idiotypic binding, AECAs, or preventing cytokine stimulation of endothelial cells. Leung et al. (1989) have studied these possibilities, and have concluded that the beneficial effects of intravenous gamma-globulin result from reduced circulating cytokines and reduced endothelial cell activation, and not from reduced AECA activity. Interestingly, intravenous gamma-globulin therapy but not aspirin is effective in reducing the severity and complications of the coronary arteritis (Newburger and Fulton, 2004), which indirectly supports the role of antibodies in the pathogenesis of the arteritis.

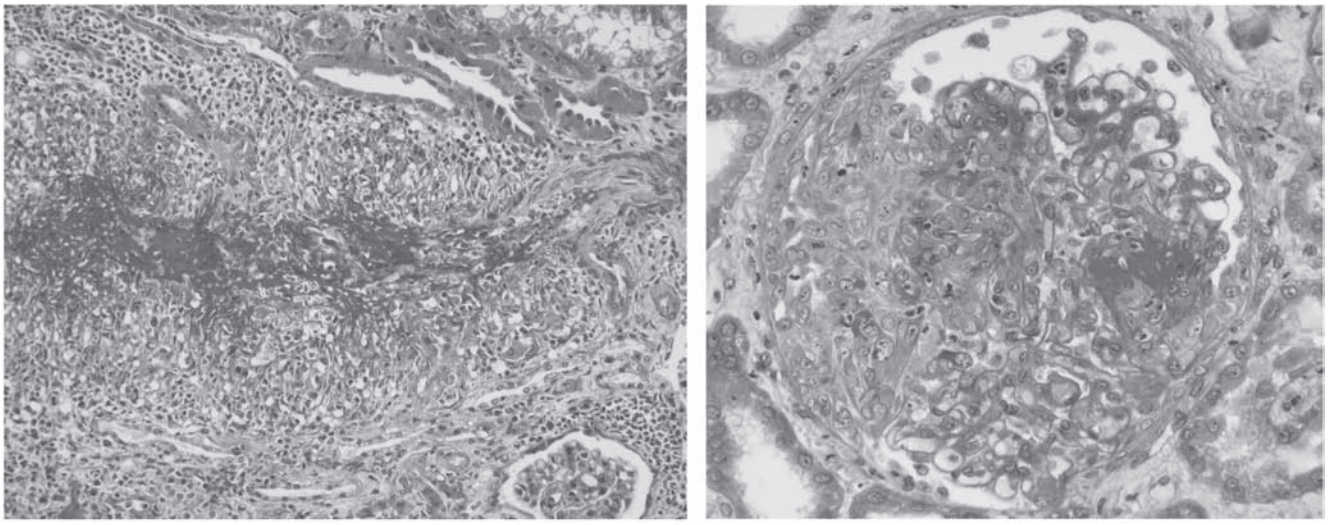
PAUCI-IMMUNE SMALL VESSEL VASCULITIS: WEGENER'S GRANULOMATOSIS, MICROSCOPIC POLYANGIITIS, AND CHURG–STRAUSS SYNDROME

Clinical, Pathologic, and Epidemiologic Features

Pauci-immune SVV has three major clinicopathologic expressions: Wegener's granulomatosis, Churg–Strauss syndrome, and microscopic polyangiitis (Jennette et al., 1994; Jennette and Falk, 1997) (see Table 65.1). All three are characterized by a pauci-immune SVV. Wegener's granulomatosis has necrotizing granulomatous inflammation superimposed on the vasculitis. Churg–Strauss syndrome has asthma, eosinophilia, and granulomatous inflammation in addition to the vasculitis. Microscopic polyangiitis has only the vasculitis, without granulomatous inflammation, asthma, or eosinophilia. Pauci-immune SVV is associated with circulating ANCAs in 80–90% of patients and thus also is called ANCA-associated SVV.

Patients with all clinicopathologic expressions of pauci-immune SVV share a common pathologic manifestation of small vessel inflammation (Jennette, 1991; Jennette and Falk, 1995; Jennette, 2002). This lesion is characterized by mural fibrinoid necrosis with karyorrhexis and infiltrating leukocytes (Figure 65.3). Neutrophils predominate in early lesions but are replaced by mononuclear leukocytes as soon as 48 h after the onset of acute lesions.

The clinical manifestations are protean and can affect many different organs individually or in combination. For example, vasculitis affecting dermal venules causes palpable purpura in skin; inflammation of glomerular capillaries causes glomerulonephritis (see Figure 65.3); pulmonary capillaritis causes pulmonary hemorrhage; vasculitis of



A B

FIGURE 65.3 *A*, Necrotizing arteritis in a small artery and *B*, necrotizing glomerulonephritis in a patient with microscopic polyangiitis. The artery and glomerulus have bright red staining for fibrinoid necrosis in this Masson trichrome stain. The artery and adjacent tissue are infiltrated by neutrophils and mononuclear leukocytes. The glomerulus has a cellular crescent. See color plate section.

TABLE 65.2 Approximate frequency of organ system manifestations in several forms of small vessel vasculitis (Jennette and Falk, 1997)

Organ system	Microscopic polyangiitis (%)	Wegener granulomatosis (%)	Churg–Strauss syndrome (%)	Henoch–Schönlein purpura (%)	Cryoglobulinemic vasculitis (%)
Renal	90	80	45	50	55
Cutaneous	40	40	60	90	90
Pulmonary	50	90	70	<5	<5
Ear, nose, and throat	35	90	50	<5	<5
Musculoskeletal	60	60	50	75	70
Neurologic	30	50	70	10	40
Gastrointestinal	50	50	50	60	30

small vessels in the upper respiratory tract mucosa causes sinusitis and otitis; vasculitis of the small vessels in the uvea (vascular tunic) of the eye causes uveitis; vasculitis affecting small epineural arteries and arterioles causes peripheral neuropathy (usually mononeuritis multiplex); vasculitis in small vessels in the gastrointestinal mucosa and submucosa causes abdominal pain and blood in the stool; and vasculitis affecting small visceral arteries, e.g., in the liver and pancreas, causes pain, dysfunction, and release of intracellular enzymes into the blood. The different clinicopathologic categories of pauci-immune SVV have overlapping clinical features with each other and with other forms of SVV (Table 65.2). Patients with Wegener's granulomatosis may have clinical manifestations of the necrotizing granulomatous inflammation, such as pulmonary nodules and cavities, perforation of the nasal septum, or collapse of the nasal septum causing saddle nose deformity.

In a review of multiple studies, Watts and Scott (2004) reported that the prevalence of Wegener's granulomatosis ranged from 24 to 157 in 1,000,000, of microscopic polyangiitis from 9 to 66, and of Churg–Strauss syndrome from 2 to 38. In accord with this, Mahr et al. (2004) determined that the prevalence of pauci-immune SVV in 1,000,000 adults in France was 25 for microscopic polyangiitis, 24 for Wegener's granulomatosis, and 11 for Churg–Strauss syndrome. The incidence in 1,000,000 adults ranged from 5 to 11 for Wegener's granulomatosis, from 3 to 12 for microscopic polyangiitis, and from 1 to 3 for Churg–Strauss syndrome (Watts and Scott, 2004).

Autoimmune Features

Over 80% of patient with acute untreated pauci-immune SVV have circulating ANCAs (Jennette and Falk, 1995;

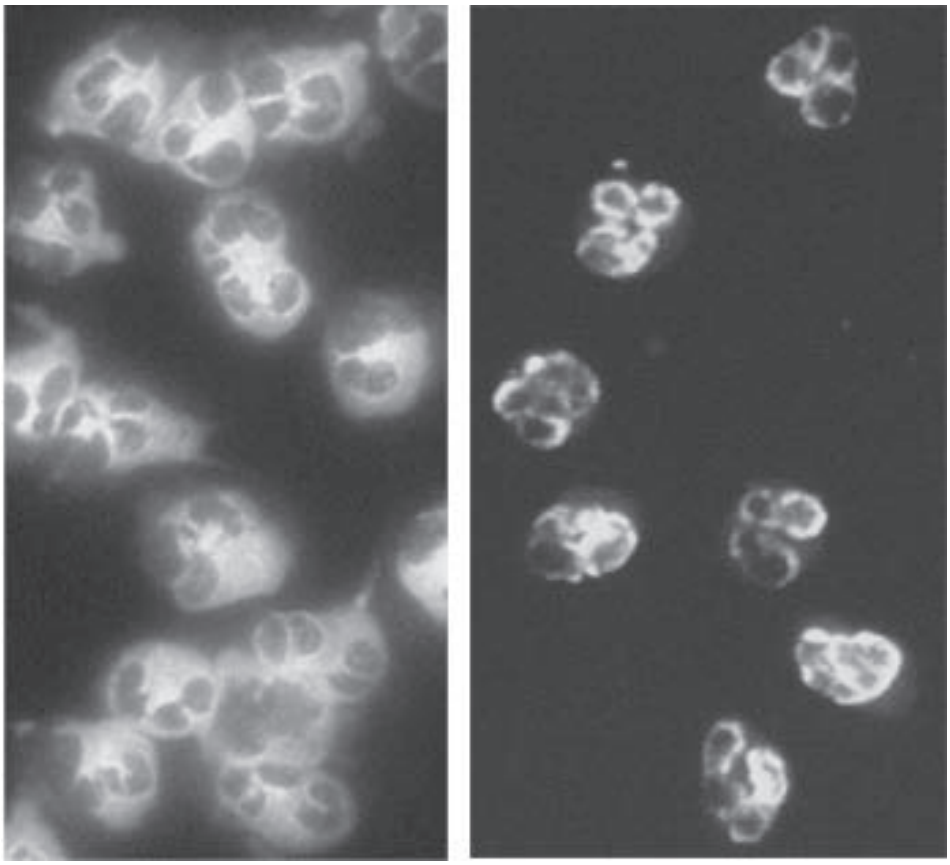


FIGURE 65.4 Indirect immunofluorescence microscopy photomicrograph of *A*, C-ANCA- and *B*, P-ANCA-staining patterns on alcohol-fixed human neutrophils.

1997). ANCAs are specific for proteins in the cytoplasm of neutrophils and monocytes. When detected by indirect immunofluorescence microscopy using alcohol-fixed neutrophils as substrate, the two major antigen specificities cause two different staining patterns: cytoplasmic (C-ANCA) and perinuclear (P-ANCA) (Figure 65.4). The perinuclear pattern is an artifact of substrate preparation caused by diffusion of antigens from the cytoplasm to the nucleus (Charles et al., 1989). When analyzed by specific immunoassays, the most frequent C-ANCA antigen specificity is for proteinase 3 (PR3-ANCA) (Goldschmeding et al., 1989; Niles et al., 1989; Jennette et al., 1990; Ludemann et al., 1990) and the most frequent P-ANCA specificity is for myeloperoxidase (MPO-ANCA) (Falk and Jennette, 1988).

T-cells with specificity for ANCA antigens are present in patients with ANCA vasculitis (Griffith et al., 1996; Clayton and Savage, 2000). T-cells are probably involved in the immunogenesis of the autoimmune response, but their role in the pathogenesis of injury is unknown.

Some patients with Wegener granulomatosis and microscopic polyangiitis have circulating AECAs, often concurrent with ANCA (Chan et al., 1993; Del Papa et al., 1996; Holmen et al., 2004). There is controversy over the diagnostic utility and pathogenic significance of AECAs in pauci-immune SVV.

Genetic Features

The predilection for the disease in white persons and the low prevalence in African-Americans suggests that a genetic background contributes to disease induction. An analysis of 94 patients with Wegener granulomatosis, microscopic polyangiitis or renal-limited vasculitis found no significant association between these vasculitides and any MHC class II allele (Zhang et al., 1995). However, there are several reports of relatively weak associations with a number of HLA-DR alleles (Jagiello et al., 2004; Williams et al., 2005).

Esnault et al. (1993) and Elzouki et al. (1994) reported the association of α-1 antitrypsin-deficiency phenotypes with PR3-ANCA–positive SVV. The fact that α-1 antitrypsin is the major inhibitor in the blood of PR3 is intriguing, but the relevance of this to the relationship between α-1 antitrypsin deficiency and PR3-ANCA is unknown.

Gencik et al. (2000) identified an association between Wegener granulomatosis and a polymorphism in the PR3 promotor affecting a possible transcription factor-binding site. In addition, Borgmann and Haubitz (2004) concluded that the neutrophil expression of PR3 is genetically regulated and correlates with onset and relapse of PR3-ANCA-associated SVV.

Environmental Influences

Drug exposure can induce ANCA formation and SVV. For example, a minority of patients who receive propylthiouracil develop ANCAs concurrent with the onset of pauci-immune SVV and glomerulonephritis (Dolman et al., 1993; Vogt et al., 1994; Tanemoto et al., 1995). Minocycline and hydralazine also have been associated with the development of ANCA disease (Elkayam et al., 1996; Choi et al., 2000).

Silica exposure and farming are risk factors for the development of ANCA SVV (Gregorini et al., 1993; Hogan et al., 2001; Lane et al., 2003). For example, Hogan et al. noted that silica dust exposure was reported by 46% of patients with ANCA SVV, compared with 20% of control subjects ($P = 0.001$). The odds ratio of exposure to silica dust was 4.4 times greater for patients with ANCA SVV compared with control subjects ($P = 0.013$).

Approximately two-thirds of patients with Wegener granulomatosis are chronic nasal carriers of *Staphylococcus aureus*. This is a risk factor for relapse, and prophylactic treatment with antibiotics that eliminate staphylococcal nasal carriage reduces relapse frequency (Stegman et al., 1996). The mechanistic relationship between staphylococcal infection and ANCA disease onset or exacerbation is not clear. However, recent observations by Pendergraft et al. (2004; 2005) raise the possibility that exposure to certain pathogens can result in the induction of the autoimmune ANCA response (see Chapter 59). They propose that pathogens can initiate an autoantibody response through induction of an appropriate antibody response to microbial proteins that have an amino acid sequence that mimics the antisense sequence (complementary sequence) of the autoantigen. These antibodies to the complementary peptide in turn induce anti-idiotypic antibodies that cross-react with the autoantigen (i.e., are autoantibodies). In support of this theory, patients with PR3-ANCA disease have circulating antibodies that react with peptides that have an amino acid sequence that is complementary to PR3, and these antibodies react with anti-PR3 antibodies as an anti-idiotypic pair. Further, immunization of mice with a complementary PR3 peptide induces not only an antibody response to the complementary PR3 peptide but also to native PR3. Interestingly, *Staph. aureus*, which is associated with PR3-ANCA disease, contains a protein that has a sequence that mimics the antisense sequence of PR3. According to the theory of autoantigen complementarity, an antibody response to the *Staph. aureus* peptide that mimics a complementary PR3 peptide results in an anti-idiotypic immune response that targets PR3, resulting in PR3-ANCA production. Also in support of this concept is the association between PR3-ANCA and infection with Ross River virus and *Entamoeba histolytica*, both of which contain proteins with amino acid sequences that mimic complementary peptides of PR3 (Pendergraft et al., 2004).

Animal Models

The most convincing model of ANCA-induced SVV has been described by Xiao et al. (2002). MPO knock-out mice were immunized with murine MPO and produced anti-MPO antibodies. Splenocytes (including T and B cells) or isolated antibodies were then transferred from $MPO^{-/-}$ mice that had been immunized with MPO to $Rag2^{-/-}$mice (lacking functional T- and B-cells), resulting in the development of necrotizing and crescentic glomerulonephritis in all mice and varying degrees of extrarenal SVV in many mice, including pulmonary capillaritis or necrotizing granulomatous inflammation, leukocytoclastic angiitis in the skin, and necrotizing arteritis in multiple viscera (Figure 65.5). The disease in the mice that received splenocytes is complicated by a background of immune complex localization in glomeruli; however, the $Rag2^{-/-}$ mice or wild-type mice that receive anti-MPO IgG alone develop a pauci-immune glomerulonephritis and vasculitis that is pathologically identical to human ANCA-associated pauci-immune disease. Because

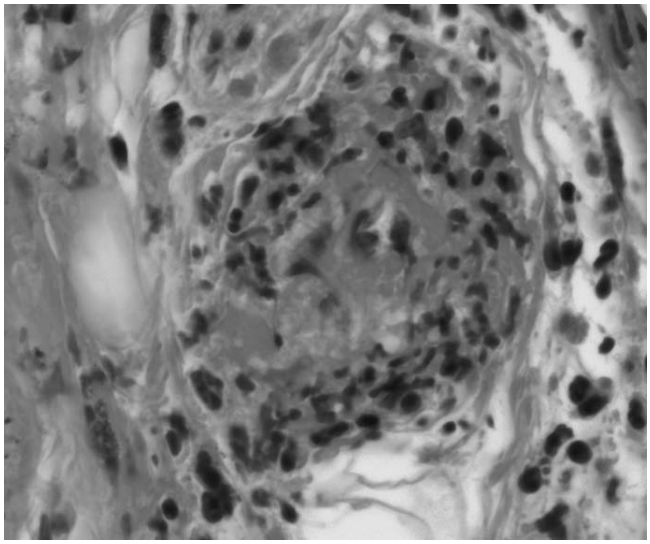


FIGURE 65.5 Necrotizing arteritis in a small artery in the dermis of a mouse 6 days after intravenous injection of mouse antimyeloperoxidase IgG. There is a central area of deeply eosinophilic fibrinoid necrosis surrounded by leukocytes with leukocytoclasia (H&E stain).

this disease can be induced in mice with no functional T cells, T cells are not required for pathogenesis. However, neutrophils are required because elimination of circulating neutrophils with a cytotoxic rat antibody completely abrogates disease induction by anti-MPO IgG (Xiao et al., 2005).

Pathogenic Mechanisms

By definition, pauci-immune SVV has little or no localization of immunoglobulin in vessel walls and thus appears to have a pathogenic mechanism that differs from classical immune complex-mediated vasculitis. The presence of ANCA in the circulation and the correlation of ANCA titers with disease activity raise the possibility that ANCAs have a pathogenic role (Jennette and Falk, 1994; Kallenberg et al., 1994; Han et al., 2003). The induction of ANCA and pauci-immune SVV by drug exposure, discussed above, is strong clinical evidence for the pathogenetic importance of ANCA. The most compelling clinical evidence for the pathogenicity of ANCA is the report of a neonate who developed pulmonary hemorrhage and nephritis following transplacental transfer of maternal MPO-ANCA IgG (Bansal and Tobin, 2004).

In addition to the animal models described earlier, there are numerous *in vitro* observations that support the pathogenic potential of ANCA IgG (Jennette and Falk, 1994; Rarok et al., 2003; Williams et al., 2005). Incubation of ANCA IgG with primed neutrophils induces the release of toxic reactive oxygen species and lytic granule enzymes (Falk et al., 1990a). Neutrophil priming, as occurs with exposure to certain cytokines, results in the expression of small amounts of ANCA antigens at the surface of neutrophils where they can interact with ANCA. The resultant activation of neutrophils is mediated by a combination of Fc receptor-dependent events (Porges et al., 1994; Mulder et al., 1994), as well as Fab′2-mediated events (Kettritz et al., 1997; Williams and Savage, 2005).

In vitro, ANCA-activated neutrophils kill primed endothelial cells (Ewert et al., 1992a; Savage et al., 1992). This process requires adhesion of the neutrophils to endothelial cells via β2 integrins (Ewert et al., 1992b). Further, Radford et al. (2000) have shown that exposure to ANCA IgG causes rolling neutrophils to adhere to endothelial cells in culture through integrin-mediated adhesion.

Instead of, or in addition to, reacting with antigens expressed on the surface of neutrophils and monocytes, ANCAs might react with antigens (e.g., MPO and PR3) that have become planted in vessel walls. Vargunam et al. (1992) have shown that MPO binds to cultured endothelial cells by a charge-dependent mechanism and can subsequently react with ANCA to form immune complexes *in situ*. If this occurs *in vivo*, the magnitude of vessel wall immune complex formation must be substantially less than in conventional immune complex disease because of the absence or paucity of staining for immunoglobulin in vessel walls in ANCA SVV.

Inflammatory process, such as a viral respiratory tract infection, may cause increased levels of circulating cytokines, which in turn prime neutrophils to interact with circulating ANCAs to induce vasculitis (Jennette and Falk, 1994). With respect to this hypothesis, it is interesting to note that approximately 90% of patients report a "flu-like illness" shortly before the onset of the signs and symptoms of ANCA vasculitis (Falk et al., 1990b). Experimental support for this hypothesis is provided by the observation that injection of bacterial lipopolysaccharide into mice prior to induction of glomerulonephritis with anti-MPO IgG causes more severe injury (Huugen et al., 2005).

In summary, the *in vitro* observations support the hypothesis that antigens (PR3 and MPO) that are expressed at the surface of cytokine-primed neutrophils react with ANCA, causing neutrophil activation through both Fc receptor engagement and Fab′2 attachment to antigens, resulting in the attachment to and injury of vascular endothelial cells (Figure 65.6).

AECAs have been detected in some patients with pauci-immune SVV. Del Papa et al. (1996) observed that AECA from patients with Wegener granulomatosis are not toxic to endothelial cells but cause these cells to upregulate adhesion molecules and to release pro-inflammatory cytokines. Holmen et al. (2004) reported that patients with Wegener granulomatosis have noncytotoxic AECAs that selectively bind surface antigens on unstimulated nasal, kidney, and lung endothelial cells, and that serum from patients with Wegener granulomatosis caused agglutination of cytokine-stimulated nasal endothelial cells. AECA could synergize with ANCA to cause disease; however, the pathogenic importance of AECA in pauci-immune SVV has not been clearly delineated.

Immunologic Markers in Diagnosis

In a patient with signs and symptoms of SVV, serology and/or immunohistology may demonstrate diagnostic immunologic markers that distinguish between vasculitides that have very different prognoses and treatments (Table 65.3). For example, in a patient with purpura, arthralgias, hematuria, and proteinuria, IgA-dominant immune deposits in dermal vessels support a diagnosis of Henoch–Schönlein purpura; serum cryoglobulins and antihepatitis C antibodies support cryoglobulinemic vasculitis; serum anti-DNA and hypocomplementemia support lupus vasculitis; and serum ANCAs support one of the categories of pauci-immune SVV. Similarly, in a patient with hemoptysis and rapidly progressive glomerulonephritis (i.e., pulmonary–renal vasculitic syndrome), serum antiglomerular basement membrane (GBM) antibodies indicate Good-

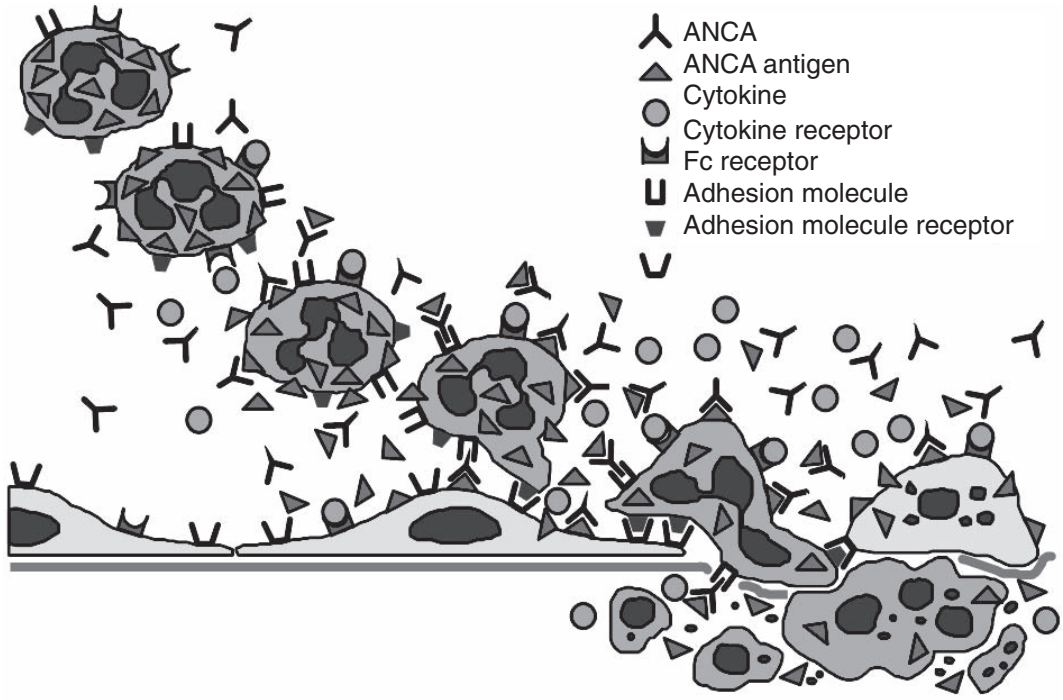


FIGURE 65.6 Putative pathogenic mechanism for antineutrophil cytoplasmic autoantibody (ANCA)-induced vasculitis. ANCA antigens (granule proteins) that are normally within the cytoplasm of neutrophils are transferred to the surface by cytokine priming, where they can interact with ANCAs. Some antigens also are released and can bind to endothelial cells. These free and bound antigens can also react with ANCAs. The interaction of neutrophils with ANCAs induces full activation with respiratory burst and degranulation, which causes vascular injury (vasculitis). See color plate section.

Reproduced with permission from Jennette and Falk (1998).

TABLE 65.3 Differential diagnostic features of several forms of small vessel vasculitis (SVV) (Jennette and Falk, 1997)

Diagnostic feature	Microscopic polyangiitis	Wegener granulomatosis	Churg–Strauss syndrome	Henoch–Schönlein purpura	Cryoglobulinemic vasculitis
SVV signs and symptoms*	+	+	+	+	+
IgA-dominant immune deposits	–	–	–	+	–
Cryoglobulins in blood and vessels	–	–	–	–	+
ANCAs in blood	+	+	+	–	–
Necrotizing granulomas	–	+	+	–	–
Asthma and eosinophilia	–	–	+	–	–

ANCAs, antineutrophil cytoplasmic autoantibodies.

*All of these SVVs can manifest any or all of the shared features of SVV, such as purpura, nephritis, abdominal pain, peripheral neuropathy, myalgias, and arthralgias. Each is distinguished by the presence and just as importantly the absence of certain specific features.

pasture syndrome, whereas ANCAs indicate one of the ANCA vasculitides.

ANCA testing should include a specific immunochemical method [e.g., enzyme-linked immunosorbent assay (ELISA)] that will detect MPO-ANCA and PR3-ANCA, not merely an indirect immunofluorescence assay for C-ANCA and P-ANCA (Lim et al., 1999; Savige et al., 1999). This improves diagnostic specificity without a significant drop in sensitivity.

An added complexity of serologic testing for ANCA disease and anti-GBM disease is the increased frequency of anti-GBM antibodies in patients with ANCA and *vice versa* (Saxena et al., 1991; Jennette, 2003; Levy et al., 2004). Patients with both autoantibodies have a worse prognosis than patients with ANCA alone. When both autoantibodies are present, a patient is at risk for manifesting vasculitic features of ANCA-associated disease that do not occur with anti-GBM disease alone, such as cutaneous, skeletal muscle

TABLE 65.4 Approximate frequency of PR3-ANCA and MPO-ANCA in patients with pauci-immune small vessel vasculitis.

	Wegener granulomatosis	Microscopic polyangiitis	Churg–Strauss syndrome	Renal-limited glomerulonephritis
PR3-ANCA	75	40	10	20
MPO-ANCA	20	50	60	70
Negative	5	10	30	10

or gut vasculitis. In some patients, the anti-GBM component of their autoimmune disease resolves, leaving more persistent ANCA disease.

Serology alone can not make the distinction between microscopic polyangiitis, Wegener granulomatosis, Churg–Strauss syndrome or isolated pauci-immune crescentic glomerulonephritis because each syndrome can be associated with either C-ANCA (PR3-ANCA) or P-ANCA (MPO-ANCA). However, the relative frequencies of ANCA specificities vary among the disease variants. For example, most patients with Wegener granulomatosis have C-ANCA (PR3-ANCA) and most patients with renal-limited disease have P-ANCA (MPO-ANCA) (Jennette et al., 1994) (Table 65.4).

Diseases other than pauci-immune SVV can be associated with ANCAs, especially with ANCAs that are not specific for MPO or PR3. P-ANCAs that do not have specificity for MPO are associated with a number of nonvasculitic inflammatory diseases, such as ulcerative colitis, sclerosing cholangiitis, autoimmune hepatitis, and Felty syndrome (Jennette and Falk, 1993; Bartunkova et al., 2002). C-ANCAs that are not associated with SVV occur in patients with cystic fibrosis (Bartunkova et al., 2002).

Detection of circulating endothelial cells may be of value in diagnosing or monitoring ANCA SVV (Haubitz and Woywodt, 2004), but this has not yet been adequately validated for clinical use.

Treatment and Outcome

ANCA vasculitis is usually a very aggressive disease that often will be complicated by endstage renal disease or life-threatening pulmonary hemorrhage if not treated with high-dose corticosteroids and cytotoxic drugs, especially cyclophosphamide (Nachman et al., 1996; Jayne, 2003; Little and Pusey, 2004). Although there is general agreement about the use of corticosteroid and cytotoxic therapy for the induction of remission in patients with pauci-immune SVV, there is controversy over the dose, method of administration, and duration of treatment. Approximately 80% of ANCA SVV patients will enter remission with aggressive immunosuppressive therapy. However, up to 40% will have a relapse within 2 years. There is controversy over how best to treat relapses. A repeat course of corticosteroids and cyclophosphamide often is used for relapses, but less toxic alternatives are azathioprine or mycophenolate mofetil (Little and Pusey, 2004).

Intravenous gamma-globulin and plasma exchange may have a beneficial effect on ANCA SVV, especially in patients with aggressive or persistent disease that does not respond well to conventional therapy (Jordan, 1995; Jayne et al., 2000; Little and Pusey, 2004). Anti-idiotypic antibodies that inhibit ANCA *in vitro* are present in pooled human gamma-globulin preparations, but whether or not an anti-idiotypic effect is the basis for the therapeutic benefits has not been determined. Plasma exchange reduces circulating ANCA levels, which is potentially beneficial if these autoantibodies are in fact pathogenic.

CRYOGLOBULINEMIC VASCULITIS

Clinical, Pathologic, and Epidemiologic Features

Cryoglobulinemic vasculitis is characterized pathologically by inflammation in small vessels (Figure 65.7A) that is associated with deposits of cryoglobulins and complement in vessel lumens and walls. Skin, joints, gut, and glomeruli often are involved (see Table 65.2). Cryoglobulinemic vasculitis affects vessels of many types, including postcapillary venules (e.g., in the dermis), capillaries (e.g., glomerular and rarely pulmonary alveolar capillaries), arterioles, and rarely small arteries (Gorevic et al., 1980; Ferri et al., 2004). Immunofluorescence microscopy reveals granular deposits of immunoglobulins and complement in vessel walls, and sometimes lumenal aggregates of cryoglobulins and complement. The clinical and pathologic manifestations of cryoglobulinemic vasculitis overlap with those of other systemic vasculitides, including purpura with leukocytoclastic angiitis, proliferative and membranoproliferative glomerulonephritis, and, in rare severe cases, pulmonary hemorrhage with alveolar capillaritis. As with many systemic vasculitides, arthralgias and arthritis caused by synovitis are a common feature.

The prevalence of cryoglobulinemic vasculitis is not well defined, but it is more common in southern Europe than in northern Europe or North America (Ferri et al., 2004).

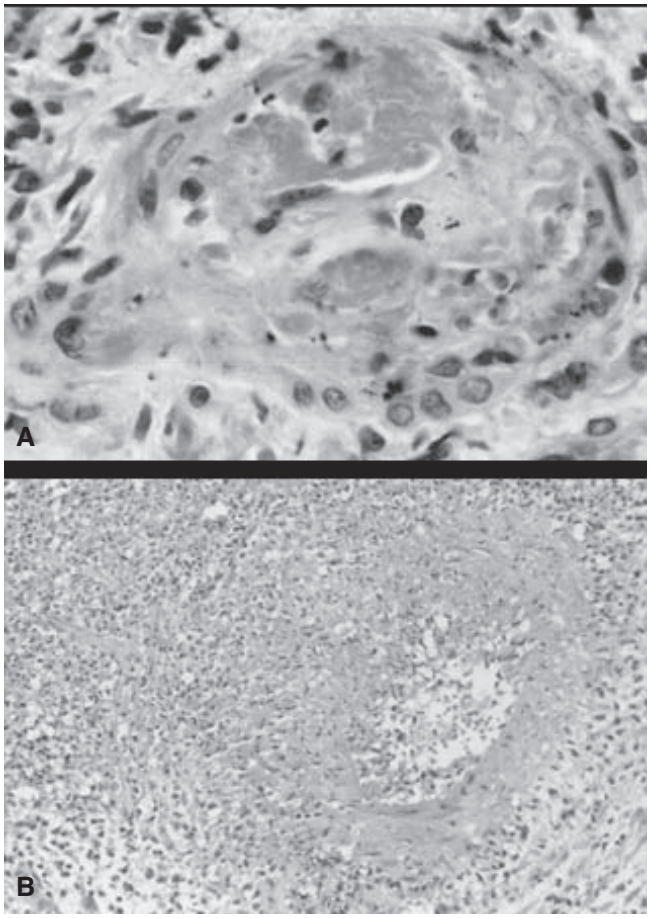


FIGURE 65.7 *A*, Cryoglobulinemic vasculitis affecting a small artery in the kidney. The deeply acidophilic material in the vessel probably is a mixture of thrombus, fibrinoid necrosis, and aggregated cryoglobulins. The vessel wall and adjacent tissue are infiltrated by leukocytes with leukocytoclasia. *B*, Intense neutrophilic inflammation and necrosis in a small dermal artery from a patient with Behçet's disease. The neutrophilic infiltrate also extends into the adjacent dermis, especially on the left.

There is a close association between HCV infection and cryoglobulinemia (D'Amico and Fornasieri, 1995; Ferri et al., 2004).

Autoimmune Features

Most cryoglobulins that cause vasculitis contain autoantibodies directed against immunoglobulins, i.e., rheumatoid factors (Grey and Kohler, 1973; Ferri et al., 2004). Cryoglobulins are divided into three major types: monoclonal (type I), mixed monoclonal–polyclonal (type II), and polyclonal (type III). Mixed cryoglobulins are more pathogenic than monoclonal or polyclonal cryoglobulins. Monoclonal cryoglobulins usually occur in patients with plasma cell dyscrasias or B-cell lymphomas, and cause morbidity primarily by precipitating within vessels and producing occlusion. Monoclonal cryoglobulins are not effective activators of inflammatory mediator systems, and therefore rarely cause overt vasculitis. Mixed cryoglobulins, however, are immune complexes that are capable of activating inflammatory mediators, including the complement system, and therefore characteristically cause systemic vasculitis.

Genetic Features

Not all patients with HCV infection develop cryoglobulinemia. The likelihood of developing cryoglobulinemia is not determined by serum HCV RNA titer or HCV genotype; thus, host factors, including genetic susceptibility, are incriminated as risk factors for this complication. In studies of relatively small numbers of patients, Amoroso et al. (1998) reported an increased frequency of the DRB1*11 (DR5) (36% vs 20%, $P = 0.0035$), Congia et al. (1996) an increased frequency of DRB1*1101–4 (DR5), DQB1*0301, DRB1*0301 (DR3), and DQB1*0201, and Lenzi et al. (1998) an increased frequency of HLA-B8 and HLA-DR3 in patients with cryoglobulinemia compared to healthy controls or patients with hepatitis C but no cryoglobulinemia.

Environmental Influences

There is a strong association between cryoglobulinemic vasculitis and HCV infection (D'Amico et al., 1995; Ferri et al., 2004). HCV antibodies and RNA are detected in the serum of 75–95% of patients with vasculitis and glomerulonephritis caused by mixed cryoglobulins. The HCV is both hepatotropic and lymphotropic. One hypothesis proposes that infection of B cells by HCV triggers the production of polyclonal and monoclonal rheumatoid factors that participate in cryoglobulin formation (D'Amico et al., 1995). Another proposes that HCV lipoprotein induces an IgM response that is initially reactive with a virus–self complex but subsequently mutates to have rheumatoid factor activity (Agnello, 1995).

Animal Models

Gyotoku et al. (1987) have described an experimental model of cryoglobulinemic vasculitis. They established monoclonal IgG rheumatoid factor-secreting hybridomas from MRL/lpr mice. These monoclonal rheumatoid factor antibodies were capable of forming cryoglobulins, and, when injected into normal mice, caused peripheral vasculitis and glomerulonephritis resembling that seen in patients with mixed cryoglobulinemia. Kikuchi et al. (2002) implanted mice with a hybridoma that secreted an IgG3 anti-IgG2a rheumatoid factor. The mice developed circulating cryoglobulins, acute glomerulonephritis, and cutaneous leukocytoclastic vasculitis.

Pathogenic Mechanisms

Cryoglobulinemic vasculitis is mediated by the deposition of mixed cryoglobulins in small vessels. These immune complexes composed of mixed cryoglobulins activate complement and initiate acute inflammation through mechanisms that were discussed above in the section on PAN.

Immunologic Markers in Diagnosis

The laboratory hallmark of cryoglobulinemic vasculitis is the detection of cryoglobulins in the circulation (see Table 65.3). Testing for cryoglobulins requires that the blood be kept warm prior to testing. In a proper clinical setting, hypocomplementemia, positive rheumatoid factor assay, and positive serology for HCV infection support a diagnosis of cryoglobulinemia. The hypocomplementemia characteristically has low C4 but normal or near normal C3.

Treatment and Outcome

If there are circulating pathogenic autoantibodies or immune complexes in patients with vasculitis, theoretically, plasmapheresis combined with immunosuppression would be beneficial. Plasmapheresis has been used in severe cryoglobulinemia (D'Amico et al., 1995). Plasmapheresis is combined with corticosteroid and cytotoxic therapy, which makes it difficult to determine what effect plasmapheresis alone is having on the disease. Therapy with IFN-α, in addition to the immunosuppressive drugs, has also been advocated in patients with cryoglobulinemic vasculitis secondary to HCV infection (D'Amico et al., 1995).

HENOCH–SCHÖNLEIN PURPURA

Clinical, Pathologic, and Epidemiologic Features

Henoch–Schönlein purpura is the most common form of vasculitis in children with an incidence of approximately 20 in 100,000 children, with the highest frequency between 4 and 6 years old (Gardner-Medwin et al., 2002), although it can occur at any age. Henoch–Schönlein purpura is SVV with IgA-dominant immune deposits affecting small vessels, i.e., capillaries, venules, or arterioles (Jennette et al., 1994). It typically involves skin, gut, and glomeruli, and is associated with arthralgias or arthritis (Ting and Hashkes, 2004). The purpura typically affects the buttocks and lower extremities. Table 65.2 provides estimates of involvement of different organs, although this differs among cohorts. Immunofluorescence microscopy of dermal venules and renal glomeruli reveals granular vessel wall deposits of predominantly IgA and C3, supporting a pathogenic mechanism that involves IgA-dominant immune complexes (Baart de la Fille-Kuyber et al., 1973; Namba et al., 1977).

Autoimmune Features

The role of autoimmunity in Henoch–Schönlein purpura is unclear. There have been conflicting reports about the presence of IgA-ANCA in patients with Henoch–Schönlein purpura and IgA nephropathy that is usually detected only by ELISA. This most likely is not an antigen–antibody reaction but rather binding of IgA mediated by the abnormal composition of IgA carbohydrate side chains in patients with Henoch–Schönlein purpura and IgA nephropathy (Coppo et al., 1997).

Davin et al. (1987) detected IgA anti-α-galactosyl antibodies in the serum of children with active Henoch–Schönlein purpura nephritis and IgA nephropathy. This finding is intriguing because of the known association between Henoch–Schönlein purpura and infection with pathogens that have α-galactosyl residues on their surfaces, but the relevance of these antibodies to the pathogenesis of Henoch–Schönlein purpura is uncertain.

Genetic Features

No HLA-A, HLA-B or HLA-C associations were observed in 26 patients with Henoch–Schönlein purpura (Ostergaard et al., 1990). Amoli et al. (2002a; 2002b) reported an increased prevalence of HLA-DRB101 and a decreased prevalence of HLA-DRB107 among Henoch–Schönlein purpura patients. They also observed an increased prevalence of HLA-B35 and IL-1 allele polymorphism in Henoch–Schönlein purpura patients with glomerulonephritis (Amoli et al., 2004). The severity of nephritis and renal outcome were influenced by the IL-1 gene polymorphism.

Environmental Influences

Approximately 40% of children have an upper respiratory tract infection near the time of onset of Henoch–Schönlein purpura, most commonly with group A Streptococcus (Masuda et al., 2003; Gonzalez-Gay et al., 2004; Ting and Hashkes, 2004). The pathogenic relationship between the infection and the onset of disease is unclear. Theoretically, this association could be because of a specific acquired immune response that is initiated by the infection, or because of a nonspecific amplification of immune activation that augments an underlying pathogenic mechanism, such as production of aberrantly glycosylated IgA (Allen et al., 1998).

Animal Models

There is no good animal model for Henoch–Schönlein purpura. There are a few animal models of IgA nephropathy, which is the glomerular lesion of Henoch–Schönlein purpura. Many of these involve oral or nasal immunization with dietary or infectious antigens (Amore et al., 2004). Uteroglobin gene knock-out and uteroglobin antisense transgenic mice develop pathologic features of human IgA nephropathy (Zheng et al., 1999), but the relevance of this to human disease is unclear because reduced uteroglobin has not been observed in patients with IgA nephropathy (Coppo et al., 2002).

Pathogenic Mechanisms

A leading hypothesis is that Henoch–Schönlein purpura is an immune complex vasculitis resulting from a dysregulated mucosal immune response to environmental or infectious antigens in a genetically predisposed individual. IgA-dominant immune complex deposits in vessel walls are the putative mediators of the inflammation. The predominant subclass is IgA1. The major antigen in the complexes is unknown. Because of the association with infections, an antigen derived from a pathogen has been sought. Masuda et al. (2003) reported the discovery of streptococcal antigens in the glomeruli of patients with Henoch–Schönlein purpura glomerulonephritis, but this has not been independently confirmed.

Human IgA1 has an O-glycosylated hinge region that is not found in other immunoglobulin classes. There is a reduction of galactosyl residues in this hinge region in IgA1 from patients with IgA nephropathy and Henoch–Schönlein purpura (Allen et al., 1998). This reduced galactosylation may be due to a functional defect in plasma cell β-1,3-galactosyltransferase. The abnormal hinge region glycosylation alters IgA1 structure, resulting in IgA1 homo aggregation, greater affinity for matrix proteins in vessel walls (including glomerular mesangium), and greater complement activation, which could result in localization of IgA-dominant deposits in vessel walls with resultant complement activation and vasculitis.

Immunologic Markers in Diagnosis

There are no specific immunologic markers for Henoch–Schönlein purpura other than the immunohistologic identification of IgA-dominant immune deposits in vessels. Increased levels of serum IgA–fibronectin aggregates have been observed in patients with IgA nephropathy and Henoch–Schönlein purpura (Jennette et al., 1991), but this finding is not specific or sensitive enough for diagnostic use.

Treatment and Outcome

Henoch–Schönlein purpura usually is a mild, self-limited, vasculitis that does not warrant corticosteroid or cytotoxic therapy (Robson and Leung, 1994). Less than 5% of patients have serious complications, usually progressive renal failure. Patients with rapidly progressive glomerulonephritis or severe CNS disease benefit from immunosuppressive therapy, e.g., with high-dose corticosteroids, cytotoxic drugs or plasmapheresis (Gedalia, 2004; Ting and Hashkes, 2004). The overall prognosis is excellent even though one-third to one-half of patients will have one or more recurrences of symptoms, usually within 6 weeks, but rarely years later (Gedalia, 2004).

BEHÇET'S DISEASE

Clinical, Pathologic, and Epidemiologic Features

Behçet's disease is a multisystem, recurrent inflammatory disease that was described by Behçet (1937; 1940) on the basis of a triad of symptoms—oral ulcers, genital ulcers, and uveitis. However, virtually any organ system can be involved. SVV is the underlying lesion in many tissues (Figure 65.7B). In addition to small vessels, medium-sized and large arteries and veins may be involved, resulting in arterial aneurysms and thrombophlebitis (Ehrlich, 1997). Even here, the initial lesions appear to be in small vessels within the adventitia or muscularis of the larger vessels (Kurokawa et al., 2004). Patients with Behçet's disease are predisposed to venous and arterial thrombosis. Criteria for the diagnosis of Behçet's disease have been established by the International Study Group for Behçet's Disease (1990).

Behçet's disease ("Silk Road disease") has a unique geographic distribution, with highest prevalence centering on the ancient Silk Road from the Mediterranean to Far-East Asia. The prevalence ranges from approximately 400 in 100,000 in Turkey to 100 in 100,000 in Japan to 1 in 100,000 in Western Europe and the US (Watts and Scott, 2004).

The onset of Behçet's disease is usually in the fourth decade of life and rarely before puberty or after the age of 50 years. Male sex, HLA-B51 positivity, and a younger age of onset are associated with more severe disease and more vasculitis (Alpsoy et al., 2005).

Autoimmune Features

Patients with Behçet's disease have T and B cells that are specific for self-peptides derived from heat-shock protein 60 (HSP60) that are highly homologous to bacterial HSP65 (Stanford et al., 1994; Hu et al., 1998). These autoreactive

lymphocytes may play a role in pathogenesis, as described below. AECAs have been reported in Behçet's disease (Direskeneli et al., 1995), but their pathogenic significance is unknown.

Mor et al. (2002) have identified autoantibodies to tropomyosin in some patients with Behçet's disease, but this has not been confirmed.

Genetic Features

Behçet's disease is strongly associated with HLA-B51 in many different ethnic groups between the Middle East and Japan (Mizuki et al., 1997). Interestingly, the distribution of populations with a high frequency of HLA B51 parallels the distribution of Behçet's disease. The association between HLA-B51 and Behçet's disease does not hold true outside the Silk Road area (Sakane et al., 1999).

Environmental Influences

Infections have been implicated in the induction of Behçet's disease; however, a causal relationship has not been elucidated (Sakane et al., 1999). One possible link is through an immune response to microbial HSPs that stimulates an autoimmune response to homologous self-HSPs (Direskeneli, 2001)

Animal Models

Stanford et al. (1994) injected eight HSP peptides into the footpads of Lewis rats. Anterior uveitis, which is a major manifestation of Behçet's disease, was induced with six of the eight peptides. The most pathogenic peptides were derived from the sequence of human 60-kDa HSP and induced uveitis in 64–75% of rats.

Hu et al. (1998) injected a human 60-kDa HSP-derived peptide subcutaneously into rats and induced uveitis in 80%. Uveitis also was induced in 75% of rats given the peptide orally, 75% given the peptide nasally, and 92% given the peptide by both routes. Treatment with monoclonal antibody to CD4 T-lymphocytes or with IL-4 caused a decrease in uveitis. Conversely, treatment of the rats with monoclonal antibody to CD8 lymphocytes greatly enhanced the speed of onset of uveitis.

Mor et al. (2002), who have identified autoantibodies to tropomyosin in the sera of patients with Behçet's disease, immunized Lewis rats with bovine α-tropomyosin. The immunized rats developed uveitis and skin lesions with neutrophil-rich inflammation. Tropomyosin is found in many cell types, including endothelial cells, which raises the possibility that antibodies to tropomyosin can function as AECAs (Kaneko et al., 2004).

Pathogenic Mechanisms

The cause of Behçet's disease has not been completely elucidated. Acute inflammation lesions, including the vasculitis lesions, show intense infiltration of neutrophils. Circulating neutrophils from patients with Behçet's disease have evidence for increased priming (activation), such as increased production of oxygen radicals and enhanced chemotaxis (Sakane et al., 1999). There are increased circulating cytokines that activate neutrophils and endothelial cells, such as TNF-α, IL-1β, and IL-8 (Sakane et al., 1999). This enhanced activation of neutrophils and endothelium could be acting in concert with other acquired or innate immunologic mechanisms to cause vasculitis. Jorizzo et al. (1985) examined the earliest acute lesions in small vessels and suggested an immune complex pathogenesis, but there is no definitive confirmation for participation of vessel wall immune complexes in disease induction. An autoimmune response to HSPs could be playing a primary or secondary role in the inflammation. Direskeneli (2001) has proposed that streptococcal and human HSP60 expression may be upregulated in oral and genital mucosa and skin after nonspecific minor injuries stimulate self-HSP60 reactive clones. These T-cells might then traffic to other organs to cause systemic inflammation, e.g., in the ocular compartment where they could interact with retinal HSP60.

Immunologic Markers in Diagnosis

There is no specific immunologic marker for Behçet's disease. Lehner (1997) has proposed that the specific proliferative response of T-lymphocytes to four peptides derived from HSP65 can be used as a laboratory test for the diagnosis of Behçet's disease, but this approach has not been validated.

The skin pathergy test is a classical marker for Behçet's disease. A positive result is an area of erythematous induration 2 mm or larger in diameter at the site of a sterile needle stick. Typically, a positive reaction peaks at 48 h and resolves in 4–5 days.

Treatment and Outcome

The treatment and outcome of Behçet's disease is dependent on the nature and severity of the clinical manifestations, which vary from very mild and limited to the classical triad locations to very severe with life-threatening involvement of the CNS, gastrointestinal tract or large vessels (Sakane et al., 1999). Anti-TNF-α therapy has been used successfully for refractory or sight-threatening ocular disease, especially uveitis (Murray and Sivaraj, 2005). Topical treatment with corticosteroids usually is sufficient for mucocutaneous lesions unless eye or vital organs are affected. High-dose

corticosteroids sometimes combined with immunosuppresive drugs are required to manage CNS disease or arteritis. Surgical intervention may be required for large vessel disease with aneurysms or occlusion.

CONCLUDING REMARKS

A variety of pathogenic immunologic mechanisms, including autoimmune processes, mediate necrotizing arteritis and SVV. Clinically, and even pathologically, identical disease can be produced by distinctly different mechanisms; and a given pathogenic mechanism can produce more than one clinical and pathologic pattern of vasculitis. Because different organs can be affected in different patients, the clinical manifestations of even relatively specific types of vasculitis are extremely variable among patients. Therefore, the diagnosis of systemic vasculitis, including autoimmune vasculitis, is difficult, and requires skillful integration of clinical, pathologic, and laboratory data. Although difficult, precise diagnosis is essential for proper management, because the prognosis and appropriate treatment vary substantially among different categories of vasculitis. As knowledge of pathogenic immunologic mechanisms and inflammatory mediator systems increases, more effective treatments for immune-mediated vasculitis will emerge, which will make precise diagnosis even more important.

References

Agard, C., Mouthon, L., Mahr, A., and Guillevin, L. (2003). Microscopic polyangiitis and polyarteritis nodosa: how and when do they start? *Arthritis Rheum. 49*, 709–715.

Agnello, V. (1995). The aetiology of mixed cryoglobulinaemia associated with hepatitis C virus infection. *Scand. J. Immunol. 42*, 179–184.

Alarcon Segovia, D. and Brown, A.L., Jr. (1964). Classification and etiologic aspects of necrotizing angiitides: an analytic approach to a confused subject with a critical review of the evidence for hypersensitivity in polyarteritis nodosa. *Mayo Clin. Proc. 39*, 205–222.

Allen, A.C., Willis, F.R., Beattie, T.J., and Feehally, J. (1998). Abnormal IgA glycosylation in Henoch-Schönlein purpura restricted to patients with clinical nephritis. *Nephrol. Dial. Transplant. 13*, 930–934.

Alpsoy, E., Elpek, G.O., Yilmaz, F., Ciftcioglu, M.A., Akman, A., Uzun, S., and Karakuzu, A. (2005). Androgen receptor levels of oral and genital ulcers and skin pathergy test in patients with Behçet's disease. *Dermatology 210*, 31–35.

Amoli, M.M., Alansari, A., El Magadmi, M., Thomson, W., Hajeer, A.H., Calvino, M.C., Garcia-Porrua, C., Ollier, W.E., and Gonzalez-Gay, M.A. (2002a). Lack of association between A561C E-selectin polymorphism and large and small-sized blood vessel vasculitides. *Clin. Exp. Rheumatol. 20*, 575–576.

Amoli, M.M., Thomson, W., Hajeer, A.H., Calvino, M.C., Garcia-Porrua, C., Ollier, W.E., and Gonzalez-Gay, M.A. (2002b). Interleukin 1 receptor antagonist gene polymorphism is associated with severe renal involvement and renal sequelae in Henoch-Schönlein purpura. *J. Rheumatol. 29*, 1404–1407.

Amoli, M.M., Calvino, M.C., Garcia-Porrua, C., Llorca, J., Ollier, W.E., and Gonzalez-Gay, M.A. (2004). Interleukin 1beta gene polymorphism association with severe renal manifestations and renal sequelae in Henoch-Schönlein purpura. *J. Rheumatol. 31*, 295–298.

Amore, A., Coppo, R., Nedrud, J.G., Sigmund, N., Lamm, M.E., and Emancipator, S.N. (2004). The role of nasal tolerance in a model of IgA nephropathy induced in mice by Sendai virus. *Clin. Immunol. 113*, 101–108.

Amoroso, A., Berrino, M., Canale, L., Cornaglia, M., Guarrera, S., Mazzola, G., Savoldi, S., Scolari, F., Sallberg, M., Clementi, M., and Gabrielli, A. (1998). Are HLA class II and immunoglobulin constant region genes involved in the pathogenesis of mixed cryoglobulinemia type II after hepatitis C virus infection? *J. Hepatol. 29*, 36–44.

Arkin, A. (1930). A clinical and pathological study of periarteritis nodosa. A report of five cases, one histologically healed. *Am. J. Pathol. 6*, 401–426.

Baart de la Faille-Kuyber, E.H., Kater, L., Kooiker, C.J., and Dorhout Mees, E.J. (1973). IgA-deposits in cutaneous blood-vessel walls and mesangium in Henoch-Schönlein syndrome. *Lancet 1*, 892–893.

Bansal, P.J. and Tobin, M.C. (2004). Neonatal microscopic polyangiitis secondary to transfer of maternal myeloperoxidase-antineutrophil cytoplasmic antibody resulting in neonatal pulmonary hemorrhage and renal involvement. *Ann. Allergy Asthma Immunol. 93*, 398–401.

Bartunkova, J., Kolarova, I., Sediva, A., and Holzelova, E. (2002). Antineutrophil cytoplasmic antibodies, anti-Saccharomyces cerevisiae antibodies, and specific IgE to food allergens in children with inflammatory bowel diseases. *Clin. Immunol. 102*, 162–168.

Behçet, H. (1937). Uber rezidivierende apthose, durch ein virus verus-achte geschwure am mund, und an den genitale. *Dermatol. Wochenschr. 105*, 1152–1157.

Behçet, H. (1940). Some observations on the clinical picture of the so-called triple sympton complex. *Dermatologia 81*, 73–82.

Borgmann, S. and Haubitz, M. (2004). Genetic impact of pathogenesis and prognosis of ANCA-associated vasculitides. *Clin. Exp. Rheumatol. 22*(Suppl 36), S79–S86.

Burns, J.C., Cayan, D.R., Tong, G., Bainto, E.V., Turner, C.L., Shike, H., Kawasaki, T., Nakamura, Y., Yashiro, M., and Yanagawa, H. (2005). Seasonality and temporal clustering of Kawasaki syndrome. *Epidemiology 16*, 220–225.

Chan, T.M., Frampton, G., Jayne, D.R., Perry, G.J., Lockwood, C.M., and Cameron, J.S. (1993). Clinical significance of anti-endothelial cell antibodies in systemic vasculitis: a longitudinal study comparing anti-endothelial cell antibodies and anti-neutrophil cytoplasm antibodies. *Am. J. Kidney Dis. 22*, 387–392.

Charles, L.A., Falk, R.J., and Jennette, J.C. (1989). Reactivity of anti-neutrophil cytoplasmic autoantibodies with HL-60 cells. *Clin. Immunol. Immunopathol. 53*, 243–253.

Choi, H.K., Merkel, P.A., Walker, A.M., and Niles, J.L. (2000). Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titers of antimyeloperoxidase antibodies. *Arthritis Rheum. 43*, 405–413.

Churg, J. and Strauss, L. (1951). Allergic granulomatosis, allergic angiitis and periarteritis nodosa. *Am. J. Pathol. 27*, 277–301.

Clark, E. and Kaplan, B. (1937). Endocardial, arterial and other mesenchymal alterations associated with serum disease in man. *Arch. Pathol. 24*, 458–475.

Clayton, A.R. and Savage, C.O. (2000). What you should know about PR3-ANCA. Evidence for the role of T cells in the pathogenesis of systemic vasculitis. *Arthritis Res. 2*, 260–262.

Congia, M., Clemente, M.G., Dessi, C., Cucca, F., Mazzoleni, A.P., Frau, F., Lampis, R., Cao, A., Lai, M.E., and De Virgiliis, S. (1996). HLA class II genes in chronic hepatitis C virus-infection and associated immunological disorders. *Hepatology 24*, 1338–1341.

Coppo, R., Cirina, P., Amore, A., Sinico, R.A., Radice, A., and Rollino, C. (1997). Properties of circulating IgA molecules in Henoch-Schönlein purpura nephritis with focus on neutrophil cytoplasmic antigen IgA binding (IgA-ANCA): new insight into a debated issue. Italian Group of Renal Immunopathology Collaborative Study on Henoch-Schonlein purpura in adults and in children. *Nephrol. Dial. Transplant. 12*, 2269–2276.

Coppo, R., Chiesa, M., Cirina, P., Peruzzi, L., and Amore, A. (2002). In human IgA nephropathy uteroglobin does not play the role inferred from transgenic mice. *Am. J. Kidney Dis. 40*, 495–503.

Cunningham, M.W., Meissner, H.C., Heuser, J.S., Pietra, B.A., Kurahara, D.K., and Leung, D.Y. (1999). Anti-human cardiac myosin autoantibodies in Kawasaki syndrome. *J. Immunol. 163*, 1060–1065.

D'Amico, G. and Fornasieri, A. (1995). Cryoglobulinemic glomerulonephritis: a membranoproliferative glomerulonephritis induced by hepatitis C virus. *Am. J. Kidney Dis*. *25*, 361–369.

Davies, D.J., Moran, J.E., Niall, J.F., and Ryan, G.B. (1982). Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *BMJ Clin. Res. Ed*. *285*, 606.

Davin, J.C., Malaise, M., Foidart, J., and Mahieu, P. (1987). Anti-alpha-galactosyl antibodies and immune complexes in children with Henoch-Schönlein purpura or IgA nephropathy. *Kidney Int. 31*, 1132–1139.

Davson, J., Ball, J., and Platt, R. (1948). The kidney in periarteritis nodosa. *Q. J. Med*. *17*, 75–202.

Del Papa, N., Guidali, L., Sironi, M., Shoenfeld, Y., Mantovani, A., Tincani, A., Balestrieri, G., Radice, A., Sinico, R.A., and Meroni, P.L. (1996). Anti-endothelial cell IgG antibodies from patients with Wegener's granulomatosis bind to human endothelial cells *in vitro* and induce adhesion molecule expression and cytokine secretion. *Arthritis Rheum*. *39*, 758–766.

Direskeneli, H. (2001). Behçet's disease: infectious aetiology, new autoantigens, and HLA-B51. *Ann. Rheum. Dis. 60*, 996–1002.

Direskeneli, H., Keser, G., D'Cruz, D., Khamashta, M.A., Akoglu, T., Yazici, H., Yurdakul, S., Hamuryudan, V., Ozgun, S., and Goral, A.J. (1995). Anti-endothelial cell antibodies, endothelial proliferation and von Willebrand factor antigen in Behçet's disease. *Clin. Rheumatol. 14*, 55–61.

Dixon, F.J., Wazuez, J.J., and Weigle, W.O. (1958). Pathogenesis of serum sickness. *Arch. Pathol*. *65*, 18–28.

Dolman, K.M., Gans, R.O., Vervaat, T.J., Zevenbergen, G., Maingay, D., Nikkels, R.E., Donker, A.J., von dem Borne, A.E., and Goldschmeding, R. (1993). Vasculitis and antineutrophil cytoplasmic autoantibodies associated with propylthiouracil therapy. *Lancet 342*, 651–652.

Duong, T.T., Silverman, E.D., Bissessar, M.V., and Yeung, R.S. (2003). Superantigenic activity is responsible for induction of coronary arteritis in mice: an animal model of Kawasaki disease. *Int. Immunol. 15*, 79–89.

Ehrlich, G.E. (1997). Vasculitis in Behçet's disease. *Int. Rev. Immunol. 14*, 81–88.

Elkayam, O., Yaron, M., and Caspi, D. (1996). Minocycline induced arthritis associated with fever, livedo reticularis, and pANCA. *Ann. Rheum. Dis. 55*, 769–771.

Elzouki, A.N., Segelmark, M., Wieslander, J., and Eriksson, S. (1994). Strong link between the alpha 1-antitrypsin PiZ allele and Wegener's granulomatosis. *J. Intern. Med. 236*, 543–548.

Esnault, V.L., Testa, A., Audrain, M., Roge, C., Hamidou, M., Barrier, J.H., Sesboue, R., Martin, J.P., and Lesavre, P. (1993). Alpha 1-antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis. *Kidney Int. 43*, 1329–1332.

Ewert, B.H., Jennette, J.C., and Falk, R.J. (1992a). Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int*. *41*, 375–383.

Ewert, B.H., Becker, M., Jennette, J.C., and Falk, R.J. (1992b). Anti-myeloperoxidase antibodies stimulate neutrophils to adhere to cultured human endothelial cells utilizing the beta-2 integrin CD 11/18. *J. Am. Soc. Nephrol*. *3*, 585.

Falk, R.J. and Jennette, J.C. (1988). Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N. Engl. J. Med*. *318*, 1651–1657.

Falk, R.J., Hogan, S., Carey, T.S., and Jennette, J.C. (1990a). Clinical course of anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. *Ann. Intern. Med*. *113*, 656–663.

Falk, R.J., Terrell, R.S., Charles, L.A., and Jennette, J.C. (1990b). Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. *Proc. Natl. Acad. Sci. USA 87*, 4115–4119.

Fauci, A.S., Haynes, B., and Katz, P. (1978). The spectrum of vasculitis: clinical, pathologic, immunologic and therapeutic considerations. *Ann. Intern. Med*. *89*, 660–676.

Ferri, C., Sebastiani, M., Giuggioli, D., Cazzato, M., Longombardo, G., Antonelli, A., Puccini, R., Michelassi, C., and Zignego, A.L. (2004). Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. *Semin. Arthritis Rheum. 33*, 355–374.

Fujita, Y., Nakamura, Y., Sakata, K., Hara, N., Kobayashi, M., Nagai, M., Yanagawa, H., and Kawasaki, T. (1989). Kawasaki disease in families. *Pediatrics 84*, 666–669.

Furusho, K., Kamiya, T., Nakano, H., Kiyosawa, N., Shinomiya, K., Hayashidera, T., Tamura, T., Hirose, O., Manabe, Y., and Yokoyama, T. (1984). High-dose intravenous gammaglobulin for Kawasaki disease. *Lancet 2*, 1055–1058.

Gardner-Medwin, J.M., Dolezalova, P., Cummins, C., and Southwood, T.R. (2002). Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. *Lancet 360*, 1197–1202.

Gedalia, A. (2004). Henoch-Schönlein purpura. *Curr. Rheumatol. Rep. 6*, 195–202.

Gencik, M., Meller, S., Borgmann, S., and Fricke, H. (2000). Proteinase 3 gene polymorphisms and Wegener's granulomatosis. *Kidney Int. 58*, 2473–2477.

Germuth, F.G., Jr. (1953). A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J. Exp. Med*. *97*, 257–282.

Godman, G.C. and Churg, J. (1954). Wegener's granulomatosis. Pathology and review of the literature. *Arch. Pathol. Lab. Med. 58*, 533–553.

Goldschmeding, R., van der Schoot, C.E., ten Bokkel Huinink, D., Hack, C.E., van den Ende, M.E., Kallenberg, C.G., and von dem Borne, A.E. (1989). Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J. Clin. Invest*. *84*, 1577–1587.

Gonzalez-Gay, M.A., Calvino, M.C., Vazquez-Lopez, M.E., Garcia-Porrua, C., Fernandez-Iglesias, J. L., Dierssen, T., and Llorca, J. (2004). Implications of upper respiratory tract infections and drugs in the clinical spectrum of Henoch-Schönlein purpura in children. *Clin. Exp. Rheumatol. 22*, 781–784.

Gorevic, P.D., Kassab, H.J., Levo, Y., Kohn, R., Meltzer, M., Prose, P., and Franklin, E.C. (1980). Mixed cryoglobulinemia: clinical aspects and long-term follow-up of 40 patients. *Am. J. Med*. *69*, 287–308.

Gower, R.G., Sausker, W.F., Kohler, P.F., Thorne, G.E., and McIntosh, R.M. (1978). Small vessel vasculitis caused by hepatitis B virus immune complexes. Small vessel vasculitis and HBsAG. *J. Allergy Clin. Immunol. 62*, 222–228.

Gregorini, G., Ferioli, A., Donato, F., Tira, P., Morassi, L., Tardanico, R., Lancini, L., and Maiorca, R. (1993). Association between silica exposure and necrotizing crescentic glomerulonephritis with p-ANCA and

anti-MPO antibodies: a hospital-based case-control study. *Adv. Exp. Med. Biol. 336*, 435–440.

Grey, H.M. and Kohler, P.F. (1973). Cryoimmunoglobulins. *Semin. Hematol. 10*, 87–112.

Griffith, M.E., Coulthart, A., and Pusey, C.D. (1996). T cell responses to myeloperoxidase (MPO) and proteinase 3 (PR3) in patients with systemic vasculitis. *Clin. Exp. Immunol. 103*, 253–258.

Grunebaum, E., Blank, M., Cohen, S., Afek, A., Kapolovic, J., Meroni, P.L., Youinou, P., and Schoenfeld, Y. (2002). The role of anti-endothelial cell antibodies in Kawasaki disease—*in vitro* and *in vivo* studies. *Clin. Exp. Immunol. 130*, 233–240.

Guillevin, L. (1999). Treatment of classic polyarteritis nodosa in 1999. *Nephrol. Dial. Transplant. 14*, 2077–2079.

Guillevin, L., Lhote, F., Brauner, M., and Casassus, P. (1995). Antineutrophil cytoplasmic antibodies (ANCA) and abnormal angiograms in polyarteritis nodosa and Churg-Strauss syndrome: indications for the diagnosis of microscopic polyangiitis. *Ann. Med. Interne (Paris) 146*, 548–550.

Gyotoku, Y., Abdelmoula, M., Spertini, F., Izui, S., and Lambert, P.H. (1987). Cryoglobulinemia induced by monoclonal immunoglobulin G rheumatoid factors derived from autoimmune MRL/MpJ-lpr/lpr mice. *J. Immunol. 138*, 3785–3792.

Han, S.H. (2004). Extrahepatic manifestations of chronic hepatitis B. *Clin. Liver Dis. 8*, 403–418.

Han, W.K., Choi, H.K., Roth, R.M., McCluskey, R.T., and Niles, J.L. (2003). Serial ANCA titers: useful tool for prevention of relapses in ANCA-associated vasculitis. *Kidney Int. 63*, 1079–1085.

Haubitz, M. and Woywodt, A. (2004). Circulating endothelial cells and vasculitis. *Intern. Med. 43*, 660–667.

Henoch, E. (1868). Uber den zusammenhang von purpura und intestinal-stoerungen. *Berl. Klin. Wochenschur. 5*, 517–519.

Henoch, E. (1882). *Lectures on diseases of children: a handbook for physicians and students.* New York: W. Wood & Co.

Hogan, S.L., Satterly, K.K., Dooley, M.A., Nachman, P.H., Jennette, J.C., and Falk, R.J. (2001). Silica exposure in anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and lupus nephritis. *J. Am. Soc. Nephrol. 12*, 134–142.

Holmen, C., Christensson, M., Pettersson, E., Bratt, J., Stjarne, P., Karrar, A., and Sumitran-Holgersson, S. (2004). Wegener's granulomatosis is associated with organ-specific antiendothelial cell antibodies. *Kidney Int. 66*, 1049–1060.

Hu, W., Hasan, A., Wilson, A., Stanford, M.R., Li-Yang, Y., Todryk, S., Whiston, R., Shinnick, T., Mizushima, Y., *van der*, Z.R., and Lehner, T. (1998). Experimental mucosal induction of uveitis with the 60-kDa heat shock protein-derived peptide 336–351. *Eur. J. Immunol. 28*, 2444–2455.

Huugen, D., Xiao, H., an Esch, A., Falk, R.J., Peutz-Kootstra, C.J., Buurman, W.A., Cohen Tervaert, J.W., Jennette, J.C., and Heeringa, P. (2005). Aggravation of anti-myeloperoxidase antibody induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor alpha. *Am. J. Pathol. 167*, 47–58.

International Study Group for Behçet's Disease (1990). Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. *Lancet 335*, 1078–1080.

Jagiello, P., Gencik, M., Arning, L., Wieczorek, S., Kunstmann, E., Csernok, E., Gross, W.L., and Epplen, J.T. (2004). New genomic region for Wegener's granulomatosis as revealed by an extended association screen with 202 apoptosis-related genes. *Hum. Genet. 114*, 468–477.

Janssen, H.L., van Zonneveld, M., van Nunen, A.B., Niesters, H.G., Schalm, S.W., and de Man, R.A. (2004). Polyarteritis nodosa associated with hepatitis B virus infection. The role of antiviral treatment and mutations in the hepatitis B virus genome. *Eur. J. Gastroenterol. Hepatol. 16*, 801–807.

Jayne, D. (2003). Current attitudes to the therapy of vasculitis. *Kidney Blood Press. Res. 26*, 231–239.

Jayne, D.R., Chapel, H., Adu, D., Misbah, S., O'Donoghue, D., Scott, D., and Lockwood, C.M. (2000). Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. *Q. J. Med. 93*, 433–439.

Jennette, J.C. (1991). Antineutrophil cytoplasmic autoantibody-associated diseases: a pathologist's perspective. *Am. J. Kidney Dis. 18*, 164–170.

Jennette, J.C. (2002). Implications for pathogenesis of patterns of injury in small- and medium-sized-vessel vasculitis. *Cleve. Clin. J. Med. 69*(Suppl 2), SII33–SII38.

Jennette, J.C. (2003). Rapidly progressive crescentic glomerulonephritis. *Kidney Int. 63*, 1164–1177.

Jennette, J.C. and Falk, R.J. (1993). Antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am. J. Clin. Pathol. 99*, 221–223.

Jennette, J.C. and Falk, R.J. (1994). Pathogenic potential of anti-neutrophil cytoplasmic autoantibodies. *Lab. Invest. 70*, 135–137.

Jennette, J.C. and Falk, R.J. (1995). Anti-neutrophil cytoplasmic autoantibodies: Discovery, specificity, disease associations and pathogenic potential. *Adv. Pathol. Lab. Med. 8*, 363–377.

Jennette, J.C. and Falk, R.J. (1997). Small-vessel vasculitis. *N. Engl. J. Med. 337*, 1512–1523.

Jennette, J.C. and Falk, R.J. (1998) Pathogenesis of the vascular and glomecular damage in ANCA-positive vasculitis. *Nephrol. Dial. Transplant. 13* (Suppl 11) 16–20.

Jennette, J.C., Hoidal, J.R., and Falk, R.J. (1990). Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood 75*, 2263–2264.

Jennette, J.C., Wieslander, J., Tuttle, R., and Falk, R.J. (1991). Serum IgA-fibronectin aggregates in patients with IgA nephropathy and Henoch-Schönlein purpura: diagnostic value and pathogenic implications. The Glomerular Disease Collaborative Network. *Am. J. Kidney Dis. 18*, 466–471.

Jennette, J.C., Falk, R.J., Andrassy, K., Bacon, P.A., Churg, J., Gross, W.L., Hagen, E.C., Hoffman, G.S., Hunder, G.G., Kallenberg, C.G., McCluskey, R.T., Sinico, R.A., Rees, A.J., van Es, L.A., Waldherr, R., and Wiik, A. (1994). Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum. 37*, 187–192.

Jordan, S.C. (1995). Treatment of systemic and renal-limited vasculitic disorders with pooled human intravenous immune globulin. *J. Clin. Immunol. 15*(Suppl.), 76S–85S.

Jorizzo, J.L., Solomon, A.R., and Cavallo, T. (1985). Behçet's syndrome. Immunopathologic and histopathologic assessment of pathergy lesions is useful in diagnosis and follow-up. *Arch. Pathol. Lab. Med. 109*, 747–751.

Kallenberg, C.G., Brouwer, E., Weening, J.J., and Tervaert, J.W. (1994). Anti-neutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. *Kidney Int. 46*, 1–15.

Kaneko, M., Ono, T., Matsubara, T., Yamamoto, Y., Ikeda, H., Yoshiki, T., Furukawa, S., and Nakayama, E. (2004). Serological identification of endothelial antigens predominantly recognized in Kawasaki disease patients by recombinant expression cloning. *Microbiol. Immunol. 48*, 703–711.

Kawasaki, T. (1967). Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi Jpn. J. Allergol. 16*, 178–222.

Kettritz, R., Jennette, J.C., and Falk, R.J. (1997). Crosslinking of ANCA-antigens stimulates superoxide release by human neutrophils. *J. Am. Soc. Nephrol. 8*, 386–394.

Kikuchi, S., Pastore, Y., Fossati-Jimack, L., Kuroki, A., Yoshida, H., Fulpius, T., Araki, K., Takahashi, S., Lemoine, R., Reininger, L., and Izui, S. (2002). A transgenic mouse model of autoimmune glomerulo-

nephritis and necrotizing arteritis associated with cryoglobulinemia, *J. Immunol. 169*, 4644–4650.

Klinger, H. (1931). Grenzformen der Periarteritis nodosa. *Frankf. Ztschr. Pathol. 42*, 455–480.

Korbet, S.M., Schwartz, M.M., and Lewis, E.J. (1984). Immune complex deposition and coronary vasculitis in systemic lupus erythematosus. Report of two cases. *Am. J. Med. 77*, 141–146.

Kurokawa, M.S., Yoshikawa, H., and Suzuki, N. (2004). Behçet's disease. *Semin. Resp. Crit. Care 25*, 557–568.

Kussmaul, A. and Maier, R. (1866). Uber eine bisher nicht beschreibene eigenthumliche Arterienerkrankung (Periarteritis nodosa), die mit Morbus Brightii und rapid fortschreitender allgemeiner Muskellahmung einhergeht. *Dtsch. Arch. Klin. Med. 1*, 484–518.

Lane, S.E., Watts, R.A., Bentham, G., Innes, N.J., and Scott, D.G. (2003). Are environmental factors important in primary systemic vasculitis? A case-control study. *Arthritis Rheum. 48*, 814–823.

Lehner, T. (1997). The role of heat shock protein, microbial and autoimmune agents in the aetiology of Behçet's disease. *Int. Rev. Immunol. 14*, 21–32.

Lenzi, M., Frisoni, M., Mantovani, V., Ricci, P., Muratori, L., Francesconi, R., Cuccia, M., Ferri, S., and Bianchi, F.B. (1998). Haplotype HLA-B8-DR3 confers susceptibility to hepatitis C virus-related mixed cryoglobulinemia. *Blood 91*, 2062–2066.

Lerner, R.A., Glassock, R.J., and Dixon, F.J. (1967). The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J. Exp. Med. 126*, 989–1004.

Leung, D.Y., Collins, T., Lapierre, L.A., Geha, R.S., and Pober, J.S. (1986a). Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. *J. Clin. Invest. 77*, 1428–1435.

Leung, D.Y., Geha, R.S., Newburger, J.W., Burns, J.C., Fiers, W., Lapierre, L.A., and Pober, J.S. (1986b). Two monokines, interleukin 1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. *J. Exp. Med. 164*, 1958–1972.

Leung, D.Y., Cotran, R.S., Kurt-Jones, E., Burns, J.C., Newburger, J.W., and Pober, J.S. (1989). Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet 2*, 1298–1302.

Levy, J.B., Hammad, T., Coulthart, A., Dougan, T., and Pusey, C.D. (2004). Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int. 66*, 1535–1540.

Lim, L.C., Taylor, J.G., III, Schmitz, J.L., Folds, J.D., Wilkman, A.S., Falk, R.J., and Jennette, J.C. (1999). Diagnostic usefulness of antineutrophil cytoplasmic autoantibody serology. Comparative evaluation of commercial indirect fluorescent antibody kits and enzyme immunoassay kits. *Am. J. Clin. Pathol. 111*, 363–369.

Little, M.A. and Pusey, C.D. (2004). Rapidly progressive glomerulonephritis: current and evolving treatment strategies. *J. Nephrol. 17*(Suppl 8), S10–S19.

Ludemann, J., Utecht, B., and Gross, W.L. (1990). Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinolytic enzyme. *J. Exp. Med. 171*, 357–362.

Magilavy, D.B., Petty, R.E., Cassidy, J.T., and Sullivan, D.B. (1977). A syndrome of childhood polyarteritis. *J. Pediatr. 91*, 25–30.

Mahr, A., Guillevin, L., Poissonnet, M., and Ayme, S. (2004). Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg–Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum. 51*, 92–99.

Mason, J.C., Cowie, M.R., Davies, K.A., Schofield, J.B., Cambridge, J., Jackson, J., So, A., Allard, S.A., and Walport, M.J. (1994). Familial polyarteritis nodosa. *Arthritis Rheum. 37*, 1249–1253.

Masuda, M., Nakanishi, K., Yoshizawa, N., Iijima, K., and Yoshikawa, N. (2003). Group A streptococcal antigen in the glomeruli of children with Henoch-Schönlein nephritis. *Am. J. Kidney Dis. 41*, 366–370.

Meltzer, M., Franklin, E.C., Elias, K., McCluskey, R.T., and Cooper, N. (1966). Cryoglobulinemia—a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity, *Am. J. Med. 40*, 837–856.

Mizuki, N., Inoko, H., and Ohno, S. (1997). Molecular genetics (HLA) of Behçet's disease. *Yonsei Med. J. 38*, 333–349.

Mor, F., Weinberger, A., and Cohen, I.R. (2002). Identification of alpha-tropomyosin as a target self-antigen in Behçet's syndrome. *Eur. J. Immunol. 32*, 356–365.

Mulder, A.H., Herringa, P, Brouwer, E., Limburg, P.C., and Kallenberg, C.G. (1994). Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA): a Fc gamma RII-dependent process. *Clin. Exp. Immunol. 98*, 270–278.

Murray, P.I. and Sivarj, R.R. (2005). Anti-TNF-α therapy for uveitis. Behçet's and Beyond. *Eye, 19*, 831–833.

Nachman, P.H., Hogan, S.L., Jennette, J.C., and Falk, R.J. (1996). Treatment response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J. Am. Soc. Nephrol. 7*, 33–39.

Nagashima, M., Matsushima, M., Matsuoka, H., Ogawa, A., and Okumura, N. (1987). High-dose gammaglobulin therapy for Kawasaki disease. *J. Pediatr. 110*, 710–712.

Namba, M., Kohda, M., Gotoh, M., Nakagawa, S., and Ueki, H. (1977). A case of Schönlein-Henoch-purpura: an immunopathological study. *J. Dermatol. 4*, 203–206.

Newburger, J.W. and Fulton, D.R. (2004). Kawasaki disease. *Curr. Opin. Pediatr. 16*, 508–514.

Niles, J.L., McCluskey, R.T., Ahmad, M.F., and Arnaout, M.A. (1989). Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. *Blood 74*, 1888–1893.

Osler, W. (1895). On the visceral complications of erythema eudativum multiforme. *Am. J. Med. Sci. 2*, 629–647.

Osler, W. (1914). The visceral lesions of purpura and allied conditions. *BMJ 1*, 517–525.

Ostergaard, J.R., Storm, K., and Lamm, L.U. (1990). Lack of association between HLA and Schoenlein-Henoch purpura. *Tissue Antigens 35*, 234–235.

Pendergraft, W.F., III, Preston, G.A., Shah, R.R., Tropsha, A., Carter, C.W., Jr., Jennette, J.C., and Falk, R.J. (2004). Autoimmunity is triggered by cPR–3(105–201), a protein complementary to human autoantigen proteinase–3. *Nat. Med. 10*, 72–79.

Pendergraft, W.F., III, Pressler, B.M., Jennette, J.C., Falk, R.J., and Preston, G.A. (2005). Autoantigen complementarity: a new theory implicating complementary proteins as initiators of autoimmune disease. *J. Mol. Med. 83*, 12–25.

Pober, J.S. (1988). Warner-Lambert/Parke-Davis award lecture. Cytokine-mediated activation of vascular endothelium. Physiology and pathology. *Am. J. Pathol. 133*, 426–433.

Porges, A.J., Redecha, P.B., Kimberly, W.T., Csernok, E., Gross, W.L., and Kimberly, R.P. (1994). Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. *J. Immunol. 153*, 1271–1280.

Radford, D.J., Savage, C.O., and Nash, G.B. (2000). Treatment of rolling neutrophils with antineutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum. 43*, 1337–1345.

Rarok, A.A., Limburg, P.C., and Kallenberg, C.G. (2003). Neutrophil-activating potential of antineutrophil cytoplasm autoantibodies. *J. Leukoc. Biol. 74*, 3–15.

Rauch, A.M. and Hurwitz, E.S. (1985). Centers for Disease Control (CDC) case definition for Kawasaki syndrome. *Pediatr. Infect. Dis. J. 4*, 702–703.

Reveille, J.D., Goodman, R.E., Barger, B.O., and Acton, R.T. (1989). Familial polyarteritis nodosa: a serologic and immunogenetic analysis. *J. Rheumatol. 16*, 181–185.

Rich, A.R. (1942). The role of hypersensitivity in periarteritis nodosa. As indicated by seven cases developing during serum sickness and sulfonamide therapy. *Bull. Johns Hopkins Hosp. 71*, 123–140.

Rich, A.R. and Gregory, J.E. (1943). The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp. 72*, 65–88.

Robson, W.L. and Leung, A.K. (1994). Henoch-Schönlein purpura. *Adv. Pediatr. 41*, 163–194.

Ronco, P., Verroust, P., Mignon, F., Kourilsky, O., Vanhille, P., Meyrier, A., Mery, J.P., and Morel-Maroger, L. (1983). Immunopathological studies of polyarteritis nodosa and Wegener's granulomatosis: a report of 43 patients with 51 renal biopsies. *Q. J. Med. 52*, 212–223.

Rowley, A.H., Duffy, C.E., and Shulman, S.T. (1988). Prevention of giant coronary artery aneurysms in Kawasaki disease by intravenous gamma globulin therapy. *J. Pediatr. 113*, 290–294.

Sakane, T., Takeno, M., Suzuki, N., and Inaba, G. (1999). Behçet's disease. *N. Engl. J. Med. 341*, 1284–1291.

Savage, C.O., Pottinger, B.E., Gaskin, G., Pusey, C.D., and Pearson, J.D. (1992). Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am. J. Pathol. 141*, 335–342.

Savige, J., Gillis, D., Benson, E., Davies, D., Esnault, V., Falk, R.J., Hagen, E.C., Jayne, D., Jennette, J.C., Paspaliaris, B., Pollock, W., Pusey, C., Savage, C.O., Silvestrini, R., van der, W.F., Wieslander, J., and Wiik, A. (1999). International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am. J. Clin. Pathol. 111*, 507–513.

Saxena, R., Bygren, P., Rasmussen, N., and Wieslander, J. (1991). Circulating autoantibodies in patients with extracapillary glomerulonephritis. *Nephrol. Dialysis Transpl. 6*, 389–397.

Schönlein, J.L. (1837). *Allgemeine und specielle Pathologie und Therapie*, 3rd edn. Herisau: Literatur-Comptoir.

Stanford, M.R., Kasp, E., Whiston, R., Hasan, A., Todryk, S., Shinnick, T., Mizushima, Y., Dumonde, D.C., van der, Z.R., and Lehner, T. (1994). Heat shock protein peptides reactive in patients with Behçet's disease are uveitogenic in Lewis rats. *Clin. Exp. Immunol. 97*, 226–231.

Stegeman, C.A., Tervaert, J.W., de Jong, P.E., and Kallenberg, C.G. (1996). Trimethoprim-sulfamethoxazole (co-trimoxazole) for the prevention of relapses of Wegener's granulomatosis. Dutch Co-Trimoxazole Wegener Study Group. *N. Engl. J. Med. 335*, 16–20.

Sturgill, B.C. and Westervelt, F.B. (1965). Immunofluorescence studies in a case of Goodpasture's syndrome. *JAMA 194*, 914–916.

Takahashi, K., Oharaseki, T., Wakayama, M., Yokouchi, Y., Naoe, S., and Murata, H. (2004). Histopathological features of murine systemic vasculitis caused by *Candida albicans* extract—an animal model of Kawasaki disease. *Inflamm. Res. 53*, 72–77.

Tanaka, N., Naoe, S., and Kawasaki, T. (1971). Pathological study on autopsy cases of mucocutaneous lymph node syndrome. *J. Jpn. Red Cross Cent. Hosp. 2*, 85–94.

Tanemoto, M., Miyakawa, H., Hanai, J., Yago, M., Kitaoka, M., and Uchida, S. (1995). Myeloperoxidase-antineutrophil cytoplasmic antibody-positive crescentic glomerulonephritis complicating the course of Graves' disease: report of three adult cases. *Am. J. Kidney Dis. 26*, 774–780.

Ting, T.V. and Hashkes, P.J. (2004). Update on childhood vasculitides. *Curr. Opin. Rheumatol. 16*, 560–565.

Uehara, R., Yashiro, M., Nakamura, Y., and Yanagawa, H. (2003). Kawasaki disease in parents and children. *Acta Paediatr. 92*, 694–697.

van der Woude, F.J., Rasmussen, N., Lobatto, S., Wiik, A., Permin, H., van Es, L.A., van der Giessen, M., van der Hem, G.K., and The, T.H. (1985). Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet 1*, 425–429.

Vargunam, M., Adu, D., Taylor, C.M., Michael, J., Richards, N., Neuberger, J., and Thompson, R.A. (1992). Endothelium myeloperoxidase–antimyeloperoxidase interaction in vasculitis. *Nephrol Dialysis Transpl 7*, 1077–1081.

Vogt, B.A., Kim, Y., Jennette, J.C., Falk, R.J., Burke, B.A., and Sinaiko, A. (1994). Antineutrophil cytoplasmic autoantibody-positive crescentic glomerulonephritis as a complication of treatment with propylthiouracil in children. *J. Pediatr 124*, 986–988.

Watts, R.A. and Scott, D.G. (2004). Epidemiology of the vasculitides. *Semin. Respir. Crit. Care Med. 25*, 455–464.

Wegener, F. (1939). Uber eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arteriensystems under der Nieren. *Beitrage Zur Pathologieschen Anatomie 102*, 36–68.

Weiss, M.A. and Crissman, J.D. (1984). Renal biopsy findings in Wegener's granulomatosis: segmental necrotizing glomerulonephritis with glomerular thrombosis. *Hum. Pathol. 15*, 943–956.

Willan, R. (1808). *On Cutaneous Diseases* (pp. 452–471). Kimber & Conrad.

Williams, J.M. and Savage, C.O. (2005). Characterization of the Regulation and Functional Consequences of p21ras Activation in Neutrophils by Antineutrophil Cytoplasm Antibodies. *J. Am. Soc. Nephrol. 16*, 90–96.

Williams, J.M., Kamesh, L., and Savage, C.O. (2005). Translating basic science into patient therapy for ANCA-associated small vessel vasculitis. *Clin. Sci. (Lond) 108*, 101–112.

Winkelmann, R.K. (1958). Clinical and pathologic findings in the skin in anaphylactoid purpura (allergic purpura). *Proc. Mayo Clin. 33*, 277–288.

Xiao, H., Heeringa, P., Hu, P., Liu, Z., Zhao, M., Aratani, Y., Maeda, N., Falk, R.J., and Jennette, J.C. (2002). Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J. Clin. Invest. 110*, 955–963.

Xiao, H., Heeringa, P., Liu, Z., Huugen, D., Hu, P., Falk, R.J., and Jennette, J.C. (2005). The role for neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibody. *Am. J. Pathol. 167*, 39–45.

Zeek, P.M. (1952). Periarteritis nodosa: a critical review. *Am. J. Clin. Pathol. 22*, 777–790.

Zeek, P.M., Smith, C.C., and Weeter, J.C. (1948). Studies on periarteritis nodosa. III. The differentiation between the vascular lesions of periarteritis nodosa and of hypersensitivity. *Am. J. Pathol. 24*, 889–917.

Zhang, L., Jayne, D.R., Zhao, M.H., Lockwood, C.M., and Oliveira, D.B. (1995). Distribution of MHC class II alleles in primary systemic vasculitis. *Kidney Int. 47*, 294–298.

Zheng, F., Kundu, G.C., Zhang, Z., Ward, J., DeMayo, F., and Mukherjee, A.B. (1999). Uteroglobin is essential in preventing immunoglobulin A nephropathy in mice. *Nat. Med. 5*, 1018–1025.

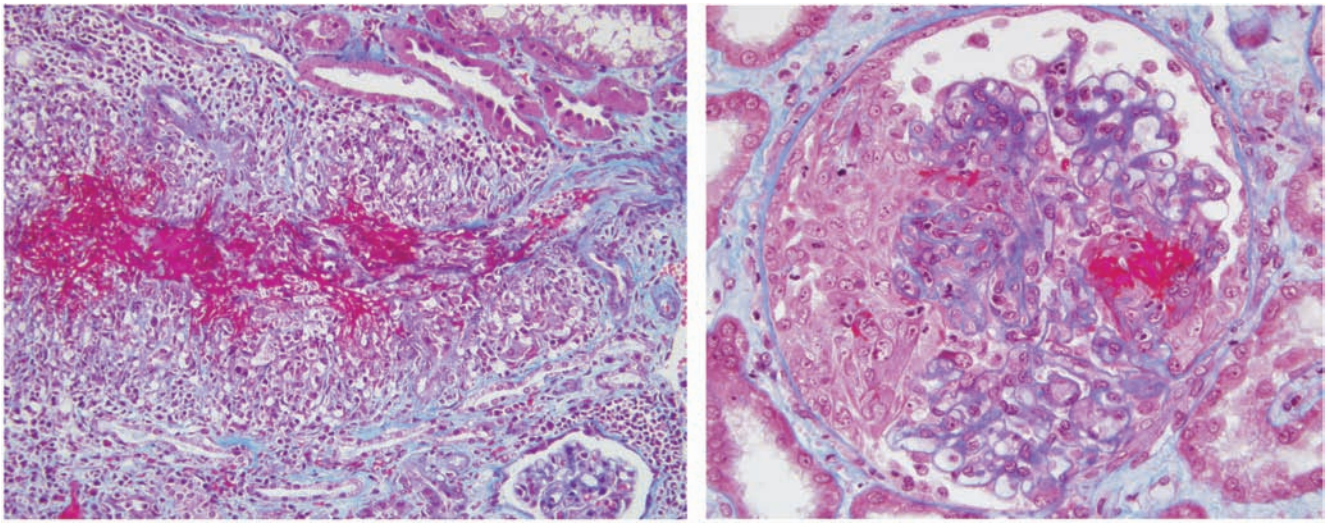


FIGURE 65.3 *A*, Necrotizing arteritis in a small artery and *B*, necrotizing glomerulonephritis in a patient with microscopic polyangiitis. The artery and glomerulus have bright red staining for fibrinoid necrosis in this Masson trichrome stain. The artery and adjacent tissue are infiltrated by neutrophils and mononuclear leukocytes. The glomerulus has a cellular crescent.

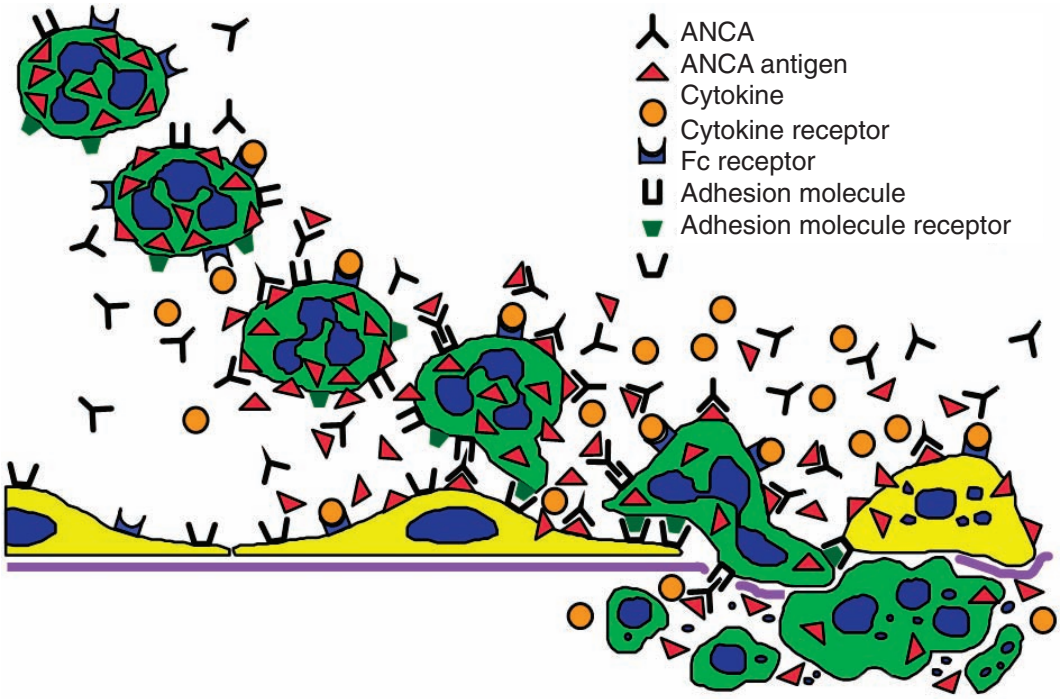


FIGURE 65.6 Putative pathogenic mechanism for antineutrophil cytoplasmic autoantibody (ANCA)-induced vasculitis. ANCA antigens (granule proteins) that are normally within the cytoplasm of neutrophils are transferred to the surface by cytokine priming, where they can interact with ANCAs. Some antigens also are released and can bind to endothelial cells. These free and bound antigens can also react with ANCAs. The interaction of neutrophils with ANCAs induces full activation with respiratory burst and degranulation, which causes vascular injury (vasculitis).

Reproduced with permission from Jennette and Falk (1998).

CHAPTER

66

Large and Medium Vessel Vasculitides

CORNELIA M. WEYAND AND JÖRG J. GORONZY

Department of Medicine, Kathleen B. and Mason I. Lowance Center for Human Immunology, Emory University School of Medicine, Room 1003 Woodruff Memorial Research Building, 101 Woodruff Circle, Atlanta, GA

VASCULITIDES OF LARGE AND MEDIUM-SIZED BLOOD VESSELS

Vasculitides stand out among the immune-mediated diseases for their potential to rapidly progress to life-threatening complications. Inflammation in the wall of medium-sized and large blood vessels is considered a medical emergency that requires prompt diagnosis and therapeutic management. Arteritides threaten the host through two different mechanisms (Weyand and Goronzy, 2003a). Destruction of the vessel wall leads to aneurysm formation, rupture, and hemorrhage. Alternatively, arteritis initiates luminal stenosis with subsequent occlusion, tissue ischemia, and infarct.

Inflammation in venous vessels is rare, whereas the layered wall structure of large arteries appears to be a preferred site for granulomatous vasculitis, including giant cell arteritis (GCA) and Takayasu's arteritis (TA) (Table 66.1). Both target the aorta and its major branches. However, the overlap in affected vascular territories is only partial, and each entity also manifests in unique arterial beds. Aortitides can be associated with other syndromes, such as relapsing polyarthritis, sarcoidosis, inflammatory bowel disease, and infections; however, GCA and TA are the most frequent causes of aortic wall inflammation (Rojo-Leyva et al., 2000). Notably, age is a strong risk factor for each for these vasculitides with the old susceptible to GCA and the young susceptible to TA. The pathologic and radiographic features can be indistinguishable, and clinical information is necessary to allow for proper diagnosis and classification (Gravanis, 2000).

GIANT CELL ARTERITIS

Giant cell arteritis is also known as temporal arteritis, cranial arteritis, granulomatous arteritis, polymyalgia arteritis, and Horton's disease. Typically, granulomatous lesions are formed in the wall of medium-sized arteries, particularly in the upper extremity and cranial branches of the aorta. $CD4^+$ T cells are recruited to the adventitia of affected blood vessels where they interact with tissue-residing dendritic cells (DC) and undergo *in situ* activation. Activated T cells regulate the differentiation of macrophages into tissue-injurious effector cells and orchestrate the production of growth factors intimately involved in the process of intimal hyperplasia. Vessel stenosis/occlusion leads to ischemia, and blindness-inducing ischemic optic neuropathy is one of the most feared clinical manifestations. Variations exist in the vascular inflammation, which translate into differences of the clinical disease profile. The diagnosis can be made by biopsy of the temporal artery. Almost all patients are older than 50 years of age, and the highest incidence has been reported in persons of Northern European ancestry. If

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

TABLE 66.1 Principal features of giant cell arteritis and Takayasu's arteritis

	Giant cell arteritis	Takayasu's arteritis
Clinical	Subacute disease onset Restricted to individuals older than 50 years Highest incidence in individuals with Northern European ancestry Often combined with polymyalgia rheumatica	Subacute disease onset 90% of patients are female Highest risk in Asian and South American populations Systemic inflammatory syndrome with malaise, fever, arthralgias, and chest pain
Preferred vascular territories	Extracranial branches of the carotid artery Subclavian–axillary junction Aorta	Aorta Innominate, carotid, subclavian, mesenteric, renal arteries
Severe complications	Blindness Stroke Aortic aneurysm Wasting	Stroke Pulselessness Visual impairment Aortic regurgitation Hypertension
Pathogenesis	Key cellular elements in vasculitic lesions: arterial wall dendritic cells, interferon-γ-producing $CD4^+$ T cells, tissue injurious macrophages Trigger of immune-mediated vascular inflammation unknown Intense systemic inflammation with highly elevated interleukin-6 and hepatic acute phase reactants	Key cellular elements in the vascular lesions: perforin-producing T cells and natural killer cells Instigator of vessel wall inflammation unknown Systemic inflammatory syndrome

diagnosed early and treated appropriately, the clinical outcome is excellent (Weyand and Goronzy, 2003a; 2003b).

Historic Background

In 1932, Bayard Horton and colleagues (Horton et al., 1932) reported on two patients admitted to the Mayo Clinic with "fever, weakness, anorexia, weight loss, anemia and painful, tender areas over the scalp and along the temporal vessels." Histomorphology of the removed temporal artery described periarteritis and arteritis with granulation tissue in the adventitia. Horton recognized that this type of arteritis was distinct from all other vasculitic syndromes and named it "temporal arteritis." Prior descriptions of temporal artery disease suggest that GCA may be a very old disease. In 1890, the English surgeon Jonathan Hutchinson described a patient with a red and swollen temporal artery who had difficulties wearing his hat (Hutchinson, 1890). In the grave of Pa-Aton-Ern-Hebs, built during the Amarna period (around 1350 AD), a blind harpist was shown with nodularity and swelling of the temporal artery. And Ali Ibn Isa (940–1010 BC), an ophthalmologist in Baghdad, recommended removal of the temporal artery not only to treat headaches, but also inflammation of the scalp muscles associated with blindness (Henriet et al., 1989).

Horton deserves credit for recognizing the connection between the constitutional symptoms and the arteritis of the temporal vessels and for introducing temporal artery biopsy as a diagnostic test.

Clinical, Pathologic, and Epidemiologic Features

The clinical manifestations of GCA are characterized by two major dimensions: 1) arterial stenosis and occlusion of arteries causing impaired blood flow, ischemia, and tissue infarction; and 2) systemic inflammation leading to a massive increase in acute phase proteins, liver abnormalities, anemia, weight loss, malaise, and fever (Table 66.2) (Evans et al., 2000; Weyand and Goronzy, 2003). Evidence suggests that the systemic inflammatory syndrome precedes rather than follows vascular inflammation. Clinical symptoms vary depending on the nature of the vascular lesions and the vascular bed preferentially affected. For clinical purposes it is helpful to subdivide the GCA syndrome into different subtypes, including cranial GCA, non-stenosing GCA, large vessel GCA, and polymyalgia rheumatica (PMR).

In cranial GCA, the major focus of the vasculopathy lies in the branches of the aorta that supply the head and the neck. Most often affected are the superficial temporal artery, the vertebral artery, the ophthalmic and posterior ciliary arteries, and, less frequently, the internal and external carotid

TABLE 66.2 The clinical profile for giant cell arteritis

Sign/Symptom	Frequency (%)	Pathology
Headaches	80	
Scalp tenderness	70	
Jaw claudication	30	Vascular stenosis
Ocular symptoms (blindness, amaurosis fugax, motor deficit)	<20	Vascular stenosis
Painful dysphagia	<10	Vascular stenosis
Cough	<10	Vascular stenosis
Limb claudication	10	Vascular stenosis
Absent pulses	<10	Vascular stenosis
Asymmetric blood pressure readings	<10	Vascular stenosis
CNS ischemia	<5	Vascular stenosis
Peripheral neuropathy	<5	Vascular stenosis
Aortic regurgitation	<5	Vascular dilatation
Intense acute phase response (elevated ESR, CRP, IL-6)	90	Systemic inflammation
Anemia	70	Systemic inflammation
Polymyalgia rheumatica	40	Systemic inflammation
Wasting syndrome	30	Systemic inflammation
Synovitis	<5	Systemic inflammation

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6.

arteries. Clinical consequences of vascular insufficiency include headaches, scalp tenderness, claudication of the masseter muscles, and vision loss due to ischemia in the visual pathway. The headaches are often intense, throbbing, and sharp in character, and are combined with temporal tenderness. On physical examination, the temporal vessels are thickened, with nodules and absence of pulses. Other arteries of the scalp can be involved. Vision loss is one of the reasons that GCA is considered a prime ophthalmic emergency. Occlusion in branches of the ophthalmic artery results in ischemic optic neuropathy that causes sudden and pain-free blindness. Amaurosis fugax can precede complete vision loss. Patients with blindness in one eye are at high risk to suffer additional visual loss in the other eye. Vascular insufficiency of arteries supplying the orbita can cause a wide spectrum of ophthalmic complications, but ischemic optic neuropathy remains the most frequent ophthalmic manifestation (Glutz von Blotzheim and Borruat, 1997). Intermittent claudication of the masseter and temporal muscles are relatively disease-specific symptoms but only affect a fraction of patients (Smetana and Shmerling, 2002). Usually, jaw claudication is induced by chewing or prolonged talking. Claudication of the tongue and painful dysphagia can occur. Impaired blood flow in the vertebrobasilar and carotid arteries causes ischemia of the central nervous system, e.g., transient ischemic attacks or stroke. Chronic nonproductive cough has been attributed to vasculitis of pulmonary artery branches.

In a subset of patients, the primary targets of vasculitis are the subclavian, axillary, and carotid arteries, and the aorta itself (Brack et al., 1999; Salvarani, 2003). Presenting symptoms of upper extremity vasculitis are claudication of the arms, loss of pulses, paresthesisas, and, only rarely, frank gangrene. Temporal artery biopsies are often negative, and, in the majority of patients, symptoms indicative of cranial ischemia are lacking (Brack et al., 1999). Aortitis can coexist with cranial arteritis or large vessel involvement and predominantly manifests in the thoracic aorta. Silent aneurysm, aortic dissection, or rupture is a consequence of destruction of the elastic membranes (Evans et al., 1984; Lie, 1995). Not unusually, histomorphologic evidence of vasculitis is found during surgical repair of aortic aneurysm. Aortic involvement is typically seen in patients many years after the initial diagnosis of GCA, suggesting a smoldering disease process.

Inflammation of medium-sized arteries in GCA does not necessarily lead to vascular stenosis but may manifest as non-stenosing vasculitis. Only in recent years has it become clear that patients with non-stenosing GCA present with a different clinical profile than those who have lumenal occlusion (Weyand et al., 1997). In non-stenosing GCA, the dominant features are those of intense systemic inflammation, often manifesting as fever and wasting. Frequently such patients seek medical attention because of nonspecific symptoms such as malaise, depression, anorexia, and weight loss and are subjected to a work-up for underlying malignancy. Lack of cranial symptoms, such as headaches, scalp tenderness, and abnormal temporal artery, can generate a challenging clinical scenario in which only the experienced clinician will search actively for vasculitis. Temporal artery biopsy is the diagnostic procedure of choice.

The vasculitic component of GCA may remain subclinical with the systemic inflammatory syndrome appearing in isolation (Weyand and Goronzy, 2003b). Signs and symptoms of impaired blood flow are missing, and clinical findings are dominated by myalgias, stiffness, and laboratory signs of heightened acute phase response. This subtype of GCA is best known as PMR, a clinical syndrome that is about two- to threefold more frequent in incidence than GCA (Doran et al., 2002; Salvarani et al., 2002). Polymyalgia rheumatica and GCA are closely related entities, affecting the same patient population. They can occur together, and PMR can precede or follow GCA. Histologic examination of arterial biopsies is unrevealing, but molecular analysis of arterial tissues demonstrates the activation of immune responses in the vessel wall (Weyand et al., 1994a). Lead symptoms are those of shoulder girdle and pelvic girdle pain and stiffness. Synovitis of hip and shoulder joints has been described in some patients with PMR (Salvarani et al., 1999; Cantini et al., 2001). It has been suggested that arteritic and synovitic forms of PMR exist. However, the diagnosis of PMR is founded on nonspecific findings, such as pain and

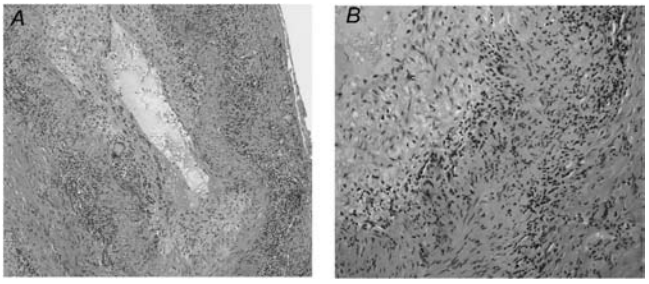


Figure 66.1 Giant cell arteritis. Cross-section of the temporal artery from a 78-year-old patient. *A,* Note transmural inflammation with granuloma formation in the inner media and along the intimal–medial junction. Partial occlusion of the lumen by thickened intima. *B,* Detailed view of several multinucleated giant cells, close to the fragmented internal elastic lamina.

Reproduced with permission from Weyand and Goronzy, 2003a.

elevated acute phase proteins, making it currently impossible to arrive at an objective case definition.

The Vascular Lesion

In its typical form, GCA is a panarteritis with T lymphocytes and macrophages infiltrating into all layers of the vessel wall (Figure 66.1) (Gravanis, 2000). In about half of the cases, granulomatous formations are seen. Giant cells, which give the name to the syndrome, are not necessarily present. They have a tendency to accumulate at the media–intima border, often closely related to the fragmented internal elastic lamina. Inflammatory infiltrates are usually segmental but can be circumferential. Most typical is the focal placement of lesions producing the so-called "skip" pattern of vasculitis. In some patients, the infiltrates are predominately composed of lymphocytes without granuloma formation. The intima is hyperplastic, obstructing the lumen. Frequently, the adventitia is infiltrated by T cells and macrophages. Clusters of lymphocytes and macrophages in the adventitia, at times arranged as cuffs around vasa vasorum, should be considered sufficient to establish the diagnosis of GCA (Björnsson, 2002).

Epidemiology

Among the vasculitic syndromes, GCA is the most frequent in the Western world. Age is the most significant risk factor; the disease almost exclusively occurs in individuals older than 50 years of age, and susceptibility increases as individuals get older (Machado et al., 1988; Salvarani et al., 2004). The female-to-male ratio of patients is 3 : 1. A characteristic feature of GCA is pronounced geographical variations in prevalence. The range of incidence rates spans between 1 and 25 cases in 100,000 persons aged 50 years and older. Highest incidence rates have been reported in Northern Europe, including Iceland, Denmark, and Sweden (Baldursson et al., 1994; Nordborg et al., 2003), as well as in areas of North America settled by populations of Scandinavian origin (Machado et al., 1988), such as Minnesota. Conversely, blacks and Hispanics are infrequently affected (Smith et al., 1983; Gonzalez et al., 1989).

Genetic Features

Giant cell arteritis is an HLA class II-associated disease (Weyand et al., 1992; 1994b). HLA-DR4 confers the highest risk to develop arteritis. Several allelic variants of HLA DR B1*04 are overrepresented among affected individuals. Sharing of sequence polymorphisms in the antigen-binding groove of HLA-DR molecules have been cited as evidence for a role of antigenic peptides in disease initiation. HLA class II polymorphisms seem not to influence the patterning or severity of the disease. Minor effects of polymorphisms in multiple non-HLA genes, such as corticotrophin-releasing hormone, CCL5 (RANTES), intracellular adhesion molecule 1 (ICAM-1), and interleukin 6 (IL-6), have been suggested, some with controversial results (Gonzalez-Gay et al., 2003). It is possible that the marked geographical variations in disease susceptibility are related to differences in population genetics, for example, the representation of HLA class II haplotypes.

Pathogenic Mechanisms

Progress has been made in deciphering the pathogenic mechanisms relevant for the inflammatory injury of the vessel wall (Weyand, 2000; Weyand and Goronzy, 2003a). Data on the systemic component with the massive upregulation of acute phase responses are more limited. A comprehensive pathogenic model would have to explain why individuals of Scandinavian origin develop granulomatous lesions in the walls of selected medium-sized arteries when they reach the seventh to eighth decades of their lives; how panarteritis translates into occlusive vasculopathy; and how vascular insufficiency is related to the clinical profile of the syndrome. Over the last decade, much progress has been made in understanding the pathways of immunostimulation in the artery and how the immune system regulates vessel stenosis/occlusion. A concept explaining the target tissue susceptibility of selected arterial territories is emerging.

Principally, GCA is a chronic inflammatory disease in which $CD4^+$ T lymphocytes establish stable lymphoid microstructures in the arterial wall and orchestrate the differentiation of tissue-injurious macrophages (Figure 66.2). The response-to-injury program of the vessel is maladaptive and leads to overshooting proliferation of the intimal layer resulting in luminal occlusion, ischemia, and infarct of dependent organ structures.

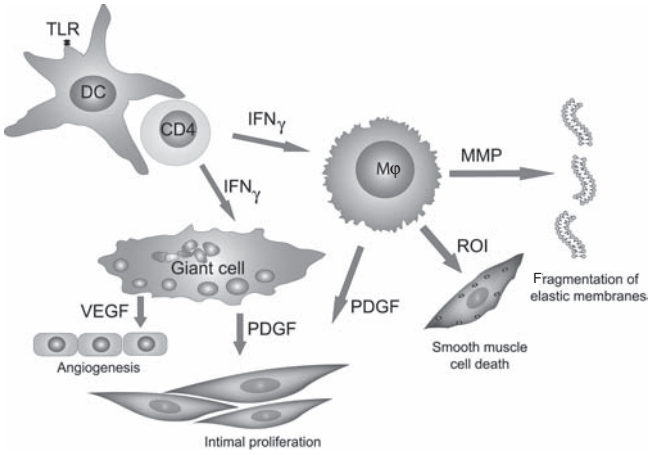


Figure 66.2 Pathogenic pathways in giant cell arteritis. Medium-sized and large arteries possess a population of indigenous dendritic cells (DC) that are located in the inner adventitia and respond to triggering of Toll-like receptors (TLR). Once activated, these DCs recruit and stimulate $CD4^+$ T cells. By producing interferon-γ (IFN-γ), activated $CD4^+$ T-cells orchestrate the effector functions of tissue-injurious macrophages (Mφ) and of multinucleated giant cells. Vessel wall damage is mediated through reactive oxygen intermediates (ROI) and matrix metalloproteinases (MMP). $CD4^+$ T cells also regulate the production of growth and angiogenesis factors (PDGF, VEGF), which facilitate capillary growth and intimal hyperplasia.

T cells and Antigen-presenting Cells in Giant Cell Arteritis

The vast majority of T cells in the lesions are $CD4^+$ T cells of the memory phenotype (Andersson et al., 1987). All evidence suggests that $CD4^+$ T cells enter the vessel wall through the vasa vasorum and not from the main lumen. They infiltrate into the adventitia recruited by chemokines that derive from DCs positioned at the adventitia–media border (Krupa et al., 2002). Adventitial DCs are an indigenous cell population that was only recently described (Ma-Krupa et al., 2004). They form a circumferential ring along the lamina elastica externa. They express typical DC markers, such as CD11c, fascin, and S-100 but lack CD1a and thus are distinct from Langerhans cells in the skin. Functional profiling has demonstrated that they possess Toll-like receptors (TLR) and respond to pathogen-associated molecular patterns.

TLRs are characteristically found on cells of the innate immune system; they are specific for molecular patterns that are generated by pathogens or by tissue injury related to infection (Banchereau and Steinman, 1998; Kaisho and Akira, 2003). Dendritic cells expressing TLRs are placed in strategically important sites, e.g., in the skin and below mucosal surfaces, where they constantly probe their microenvironment for TLR ligands. Classical examples of such ligands are lipopolysaccharides, flagellin, viral RNA, bacterial DNA, and also heat-shock proteins (see Chapter 9). The triggering of TLRs, together with additional signals, initiates the maturation of immature DCs. Upon maturation, such DCs switch their chemokine receptor profile, become migratory, and travel via lymphatic vessels to secondary lymphoid tissues, such as lymph nodes. There they home to the T-cell zones, become stationary, start producing T-cell-attracting chemokines, and upregulate the expression of antigen-presenting HLA molecules and co-stimulatory molecules.

It is not known what the physiological role of adventitial DCs is and what they are checking for. Experiments that utilized the implantation of human temporal arteries into immunodeficient mice have revealed that such adventitial DCs respond to LPS and undergo rapid activation when triggered by blood-borne mediators (Ma-Krupa et al., 2004). In GCA, almost all DCs in the wall are highly activated and have adapted a functional profile reminiscent of that in lymphnode-positioned DCs (Krupa et al., 2002). They produce high amounts of chemokines, are no longer migratory, and can present antigens to T cells. It has been proposed that one of the pathomechanisms in GCA is the trapping of arterial wall DCs in the tissue instead of allowing them to migrate to lymph nodes, which leads to a misplaced immune response.

Experiments in patients with PMR who lack T-cell infiltrates in the temporal artery have supported the concept that DC activation is a very early step in the disease process (Ma-Krupa et al., 2004). In fact, DCs in PMR arteries have already acquired the capability to produce T-cell-attracting chemokines. If appropriate T cells are made available, they will initiate T-cell recruitment and *in situ* activation. How patients with PMR are protected from accumulating T cells in the artery, although tissue-residing DCs have already matured, is not understood.

Once $CD4^+$ T-cells have made their entrance into the adventitial layer of medium-sized arteries, they undergo local activation (Weyand et al., 1994c; Brack et al., 1997). The major consequence is production of interferon-γ (IFN-γ), a key cytokine in the disease (Wagner et al., 1996). Interferon-γ production remains localized in the adventitia, even when the disease progresses and panarteritic infiltrates form. $CD4^+$ T cells undergo clonal expansion and regulate the attraction of macrophages into the lesions. Studies in right-sided and left-sided temporal artery specimens from the same patient have shown that identical T-cell clonotypes emerge in physically separated lesions (Weyand et al., 1994c), providing strong support for antigen-driven selection (Weyand and Goronzy, 1995).

The intensity of IFN-γ production, which could be viewed as a measure of T-cell activation in the vasculitic lesions, has been correlated with the clinical manifestations of this disease. Patients with high production of IFN-γ in the artery have more intense intimal hyperplasia, more visual symptoms, and cranial ischemia. Patients with distinctly low production of IFN-γ in the temporal artery sample often lack

vessel occlusion and tend to present with fever of unknown origin or other constitutional symptoms (Weyand et al., 1997).

Depletion experiments have indicated that $CD83^+$ DCs are the critical antigen-presenting cells and thus have gatekeeper function in the disease (Ma-Krupa et al., 2004). Since DCs sit in a unique position in the artery (outside of the lamina elastica externa), their absence or presence may dictate whether a particular artery is susceptible to be targeted by GCA. This concept pulls together structural characteristics of blood vessels with susceptibility for immune-mediated attack and, for the first time, provides a molecular basis for the unusual patterning of temporal arteritis.

Macrophages in Giant Cell Arteritis

There are no data to suggest that T lymphocytes accumulating in the vasculitic infiltrates have direct involvement in wall injury (Weyand and Goronzy, 1999). Rather, multiple pathways have been identified through which macrophages mediate tissue damage. Macrophage function is closely related to the positioning in the wall, suggesting a tight interaction between infiltrating macrophages and the cellular and matrix components of the particular microenvironment (Weyand et al., 1996).

Macrophages placed in the adventitia usually focus on the production of pro-inflammatory cytokines. IL-1β and IL-6 have been demonstrated in this macrophage population. Tumor necrosis factor-α (TNF-α) has been reported to be present in inflamed arteries, but the cellular source has not been determined. The staining pattern suggests that most of the TNF-α is produced in the media (Hernandez-Rodriguez et al., 2004). Macrophages in the media specialize in generating reactive oxygen intermediates (Rittner et al., 1999a). Numerous lines of evidence have demonstrated the contribution of oxidative stress to the disease process. Gene-expression profiling has guided interest towards oxidative damage. Toxic aldehydes, products of lipid peroxidation, have been detected in the medial smooth muscle cell layer (Rittner et al., 1999a). The formation of nitrotyrosine, a marker of nitrosative stress, has also been localized to the media (Borkowski et al., 2002). Interestingly, only endothelial cells of medial neovessels are affected by nitrotyrosine formation. These studies have pinpointed tissue injury pathways, identified the cellular players, and at least partially described target structures incurring damage.

A noteworthy aspect of the presence of oxidative stress in the media is the induction of protective mechanisms, such as the induction of aldose reductase, a ketoreductase with a role in metabolizing toxic aldehydes (Rittner et al., 1999b). Blocking aldose reductase in GCA lesions increased apoptosis rates of smooth muscle cells, demonstrating a protective role of the enzyme. There is no information about whether patients actually vary in the induction of such protective mechanisms, making them more or less susceptible to oxidative damage.

Other functional properties of media-located macrophages include the secretion of matrix metalloproteinases (MMPs) (Sorbi et al., 1996; Tomita and Imakawa, 1998). CD68-expressing macrophages in the vicinity of the lamina elastica interna are prone to produce MMP-2 as well as MMP-9. Such metalloproteinases almost certainly are the mechanisms underlying the typical fragmentation of the elastic membranes.

So far, macrophages captured in the intima have not been assigned to particular injury pathways (Weyand et al., 1996). They have been described to stain positive for NOS-2, but downstream effects of nitrosative stress have not been detected in the intima itself in GCA.

Intimal Hyperplasia

Blockage of the vascular lumen by hyperplastic intima is the mechanism through which GCA causes vascular insufficiency and tissue ischemia. Myofibroblasts in the intima proliferate and deposit matrix. Whether they originate from undifferentiated precursors or de-differentiate from myocytes is not known. They have migrated to the intima from deeper wall layers, either the media or the adventitia. Factors regulating myofibroblasts migration and differentiation thus have gatekeeper function in the disease process.

Inflamed temporal artery walls produce platelet-derived growth factor (PDGF), which derives from at least two cells types, $CD68^+$ macrophages and resident wall cells (Kaiser et al., 1999). Numerically, the most important sources are macrophages in the media, often close to the lamina elastica interna. Multinucleated giant cells can also produce this growth factor. Fibroblast growth factor has been described to be present in vasculitic lesions; its function is unexplored.

The outgrowth of the expanding intimal layer needs to be supported by the formation of new blood vessels (Kaiser et al., 1998; Cid, 2002). Neoangiogenesis typically affects the media and the hyperplastic intima. Newly formed blood vessels are highly organized. Multinucleated giant cells have been found to secrete vascular endothelial growth factor (VEGF) (Kaiser et al., 1998). In essence, tissue-infiltrating macrophages appear to have a critical role in regulating the process of intimal proliferation and neoangiogenesis. Interestingly, VEGF production is correlated with IFN-γ production, thereby connecting the wall remodeling in the intima and media with the adaptive immune responses in the adventitia.

The Systemic Inflammatory Syndrome

Mechanisms driving the systemic inflammatory component of GCA are less well understood. Patients with PMR

in whom vascular inflammatory infiltrates are undetectable by standard histology are equally affected by systemic inflammation as GCA patients with full-fledged vasculitis (Wagner et al., 1994; Weyand et al., 1999). This clinical constellation has supported the concept that systemic inflammation is not a "spillover" from the vasculitis; instead, it precedes vascular inflammation and has pathogenic roots that do not necessarily lead to arteritis.

Typical findings include a massive acute phase response with manifestations in multiple organ systems (Weyand and Goronzy, 2003b). Patients show signs of abnormalities in hematopoiesis, such as anemia and thrombocytosis. Liver function is often abnormal and shows elevation of alkaline phosphatase levels. By affecting the central nervous system, the acute-phase response causes fever, malaise, and depression. Profound elevation of the erythrocyte sedimentation rate (ESR), a typical laboratory marker of GCA, is also a consequence of excessive production of acute phase proteins.

The only known pathomechanism related to the systemic inflammatory syndrome is the production of high amounts of IL-6 (Roche et al., 1993; Wagner et al., 1994; Weyand et al., 2000), a cytokine that has been implicated in inducing acute phase proteins in hepatocytes. In patients with GCA, as well as those with PMR, circulating monocytes are found to be spontaneously activated and produce IL-6 (Wagner et al., 1994). These data suggest initiator function of an activated innate immune system in the syndrome.

Treatment, Monitoring, and Outcome

The golden standard for treatment of GCA is corticosteroids. Patients respond explicitly well, with prompt and substantial improvement of symptoms within 24–48 hours. Initial doses of 60 mg of prednisone (or approx. 1 mg per kg body weight) have been found to be effective in almost all patients. Indeed, current discussions center on the question of whether at least some patients could be successfully treated with lower doses (Chevalet et al., 2000).

Once patients are stabilized on high doses of corticosteroids, daily prednisone doses should be tapered. A reduction of 10–20% every 2 weeks has proven to be a clinically useful guidance. Both clinical symptoms and laboratory markers of inflammation are monitored to guide the tapering process. There is preliminary evidence that IL-6 is a more sensitive marker of disease activity than ESR, but properly designed clinical trials have not been performed (Weyand et al., 2000). A frequent clinical dilemma is a discrepancy between clinical and laboratory findings. To avoid overutilization of corticosteroids, it has been recommended that treatment decisions should not be solely based on laboratory results.

Many patients develop signs of flaring disease when corticosteroids are reduced. Fortunately, severe manifestations, such as sight-threatening ischemic complications, appear to be rare (Aiello et al., 1993; Weyand et al., 2000). Instead, most disease flares present as PMR or constitutional symptoms. Dose adjustments of corticosteroids can effectively recapture disease control.

Corticosteroids can be discontinued in most patients after approximately 2 years of therapy. There is evidence that the disease process does not enter remission but continues with smoldering activity (Uddhammar, 2000; Weyand et al., 2000). Whether that chronic state of inflammation requires continued immunosuppression or can be managed by watchful monitoring is a matter of discussion.

One of the serious long-term consequences of GCA is the development of aortic aneurysm (Evans et al., 1994; Lie, 1995). Patients need to be informed about this complication, and monitoring for aortic wall thickness and aortic dimension has been recommended. It is unknown whether continuous immunosuppression can prevent aortic wall damage.

With an average age of almost 75 years at disease onset and the duration of therapy for several years, patients are prone to show steroid side effects (Proven et al., 2003). Monitoring of blood glucose and blood pressure is obvious. Also, bone-sparing therapy with calcium and vitamin substitutions (and other agents if necessary) should be a fixed component of management.

Given the high rate of steroid side effects, efforts have been made to identify steroid-sparing therapies. In an experimental model of GCA with human temporal arteries implanted into immunodeficient mice, aspirin effectively suppressed IFN-γ production and augmented the anti-inflammatory effects of corticosteroids (Weyand et al., 2002). In contrast to other chronic inflammatory diseases, patients with GCA seem not to benefit from methotrexate as an immunosuppressant. Although results of clinical trials have been controversial, a well-designed clinical study with a large cohort of patients could not demonstrate any advantage for methotrexate/corticosteroid combination therapy compared with corticosteroids alone (Jover et al., 2001; Spiera et al., 2001; Hoffman et al., 2002). New biologic agents, such as cytokine inhibitors, are in the process of being tested for their steroid-sparing activity in GCA.

Overall, the outcome of GCA is good. No shortening of life expectancy has been reported, emphasizing that highly aggressive therapies may have to be used with caution (Matteson et al., 1996; Gran et al., 2001).

TAKAYASU'S ARTERITIS

Takayasu's arteritis is a systemic arteritis that predominantly manifests in the aorta and its major branches. It is also known as pulseless disease or occlusive thromboaortopathy. The typical patient is a female of Asian or South American origin presenting with vascular insufficiency and

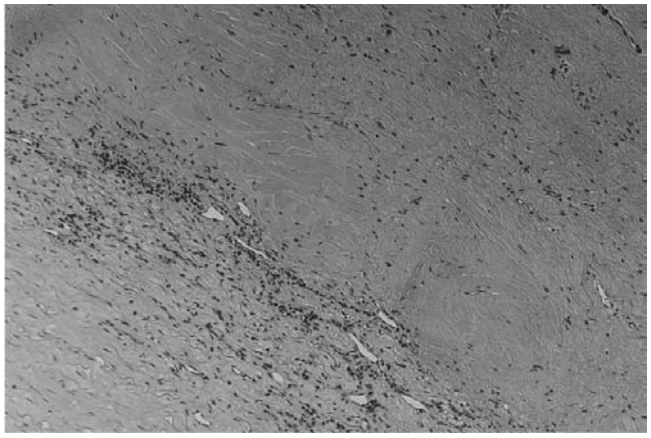


Figure 66.3 Takayasu's arteritis. Cross-section of an innominate artery. Note transmural round cell infiltration with dominance of inflammation along the media–adventitia border. Extensive neovascularization has occurred in the media. The adventitia shows fibrotic expansion.

generalized inflammation in the second or third decades of life. But care has to be taken not to miss the diagnosis in males and middle-aged individuals. Inflammatory infiltrates in the wall of large elastic arteries induce thickening of the adventitia, destruction of the media, and hyperplasia of the intima (Figure 66.3) (Gravanis, 2000). Recently, diagnosis and management of TA have benefited from advances in non-invasive imaging methods and more aggressive use of surgical procedures.

Historic Background

In 1830, Yamamoto reported the first case of TA. In 1908, M. Takayasu was the first to describe peculiar optic fundus abnormalities with coronal anastomosis (Takayasu, 1908), which 40 years later were interpreted as neovascularization and anastomosis secondary to ischemia caused by the occlusion of cervical vessels. In 1951, Shimizu and Sano described a cohort of 31 cases and made the connection between pulselessness, coronal anastomosis of retinal vessels, and carotid abnormalities and called it pulseless disease (Shimizu and Sano, 1951).

Clinical, Pathologic, and Epidemiologic Features

Takayasu's arteritis is a rare disease with incidence rates of 1–2 cases in 1 million individuals per year (Watts and Scott, 1997). The most significant risk factors are female sex, age less than 40 years, and selected ethnic origin. The highest prevalence rates have been reported for Asian countries, including Japan, Korea, China, India, Thailand, and Turkey (Koide, 1992). South American countries, such as Mexico, Brazil, Columbia, and Peru, are now also considered higher incidence areas (Dabague and Reyes, 1996). The disease does occur in whites, can affect males, and needs to be kept in mind as a differential diagnosis in older individuals with aortitis or large vessel vasculitis.

Prominent histomorphologic findings include the thickening of the aortic wall which may involve all three layers (see Figure 66.3) but often is most significant in the adventitia (Gravanis, 2000; Björnsson, 2002). Extensions of inflammatory infiltrates and fibrosis into the periaortic tissues are not unusual. Most often, the aortic lumen is compromised, but stenotic lesions can alternate with aneurysmal dilatation giving rise to fusiform or saccular aneurysms. Also characteristic are "skipped" areas with normal vessel wall next to densely inflamed regions. Besides the aorta and its primary branches, coronary and pulmonary arteries can be involved.

Microscopic examination shows inflammatory infiltrates composed of lymphocytes and plasma cells primarily around the vasa vasorum, causing vasa vasoritis with lumenal stenosis. Lymphocytes, plasma cells, and occasional giant cells accumulate in the media, sometimes complicating the distinction between GCA and TA (Gravanis, 2000). Patches of medial necrosis can occur, and destruction of elastic membranes is typical. Growth of fibroblasts and smooth muscle cells and deposition of acid mucopolysaccharides result in widening of the intimal layer, similar to that in fibromuscular hyperplasia. Vascular insufficiency in the aortic branches is related to ostial stenosis or direct involvement.

Available data suggest that the clinical spectrum of TA is different in distinct geographic areas (Numano, 1997). Japanese, Korean, and North American patients are more likely to present with impairment of cervical, cerebral, and upper extremity blood flow and aortic regurgitation, due to involvement of the ascending aorta and the primary aortic arch branches. Conversely, in Indian patients, the abdominal aorta, including the renal arteries, appears to be the preferred target, resulting in renovascular hypertension. Overall, blindness and severe retinal ischemia are less common now than they used to be (Numano, 2002).

Initial presentation may be dominated by a generalized inflammatory syndrome with fever, night sweats, weakness, arthralgias, and chest pain (Kerr et al., 1994). Direct complications of vessel wall inflammation include headaches, syncope, visual disturbances, and face and neck pain from insufficient blood flow in the cervical vessels (see Table 66.3). Stenotic lesions in the brachiocephalic and subclavian arteries lead to arm claudication, pulselessness, and discrepant blood pressure measurements (Figure 66.4). Ischemic heart disease is a result of reduction in coronary blood flow. A feared complication of TA is aortic regurgitation caused by dilatation of the ascending aorta. Congestive heart failure is not unusual (Endo et al., 2003).

TABLE 66.3 The clinical spectrum for Takayasu's arteritis

Organ system	Sign/symptom	Frequency (%)
Vascular	Bruit	70
	Claudication	70
	Reduced/absent pulses	60
	Asymmetrical blood pressure	50
CNS	Dizziness	40
	Visual abnormalities	30
	Stroke/TIA	10
Constitutional	Malaise	70
	Fever	30
Cardiac	Weight loss	20
	Aortic regurgitation	20
	Angina	10
	Congestive heart failure	<5

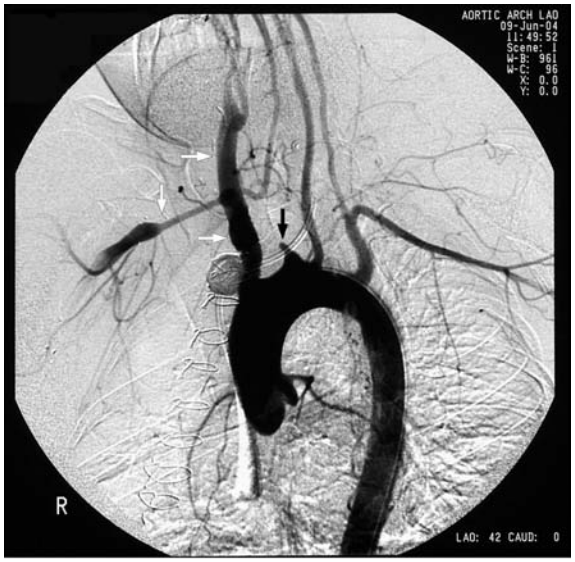


Figure 66.4 Takayasu's arteritis. Angiography of the aortic arch and the cervical vessels. The native brachiocephalic artery *(black arrow)* is occluded just distal to its origin. A graft has been placed *(white arrows)* from the ascending aorta to the right common carotid artery with a separate limb connecting to the right subclavian artery. The left carotid artery and both vertebral arteries are normal.

Marked progress has been made in imaging modalities, compensating at least partially for the difficulties in accessing tissue to establish the diagnosis of TA and follow its course for patients receiving therapy. Besides conventional angiography, which has been the standard imaging tool for diagnosing and evaluating patients with TA (see Figure 66.4), a number of alternatives have emerged, including magnetic resonance (MR) imaging and MR angiography, computed tomography (CT), Doppler ultrasound, and metabolic imaging with positron emission tomography (PET) (Kissin and Merkel, 2004). Ultrasound is particularly useful in evaluating the carotid arteries, with high sensitivity for submillimeter changes in wall thickness. Both CT and MR imaging provide excellent visualization of vessels and their relationship to neighboring structures and demonstrate both lumenal and mural abnormalities. Contrast enhancement is necessary for CT to depict the vessel lumen, but it can provide fast information about the aorta and its wall; MR imaging has inherent multiplanar imaging capabilities, can be used with or without contrast enhancement, and lends itself for the evaluation of the aorta ands its major branches (Matsunaga et al., 2003; Nastri et al., 2004). With the potential to assess mural edema and vascularity, it has been hoped that it would be an ideal instrument to monitor disease activity and progress in patients on immunosuppression. However, a recent study has cast doubt on the utility of edema-weighted MR as a sole means to estimate disease activity (Tso et al., 2002). A role for PET, which detects areas of active glucose metabolism in the vascular wall in identifying early disease, has been suggested (Webb et al., 2004), but no large-scale controlled studies are available.

Genetic Features

Like GCA, TA is an HLA-associated disease (Kerr et al., 1994; Salazar et al., 2000). More than half of the Japanese patients carry the HLA A24-B52-DR2 haplotype (Kimura et al., 1996). Non-polymorphic HLA molecules, specifically MICA, have also been reported to be enriched among patients (Kimura et al., 1998).

Pathogenic Mechanisms

Despite considerable overlap in the histomorphology and clinical presentation of GCA and TA, evidence suggests that pathogenic mechanisms are distinct (Seko, 2002). Whereas $CD4^+$ T cells producing IFN-γ have been identified as key regulators in the vascular lesions of GCA, they appear to be less important in TA. In the vascular infiltrates of TA, γδ T cells account for 31% of the cells, natural killer cells for 20% and $CD8^+$ cytotoxic T cells for 15% (Seko, 2000). $CD4^+$ T cells, macrophages, and B cells were less frequent. In support for the concept that cytotoxic cells directly damage resident cells in the aorta, Seko et al. (Seko et al., 1994) have shown cellular expression of perforin and deposition of perforin directly onto wall resident cells (Figure 66.5). Heat-shock proteins, in particular HSP65, have been proposed as the antigen stimulating cytotoxic lymphocytes (Seko et al., 2000). Restricted usage of T-cell receptor AV and BV genes in tissue infiltrating T cells also support the concept of selective expansion of antigen-reactive T cells in the vessel wall infiltrates (Seko et al., 1996). Dense expression of co-stimulatory molecules is in line with a role for adaptive immune responses driving the disease process. Recent data suggesting a possible role of metalloproteinases as

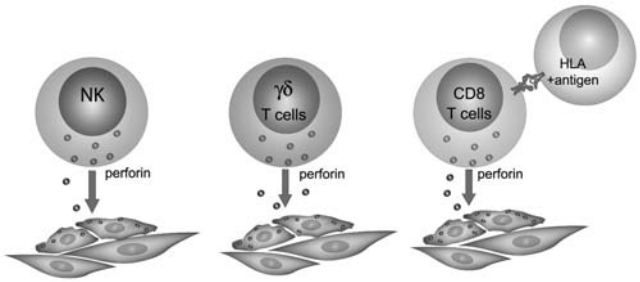


Figure 66.5 Schematic model of pathogenic pathways in Takayasu's arteritis. Vascular infiltrates are predominantly cytotoxic $CD8^+$ T cells, γδ T cells, and natural killer (NK) cells, all of which produce the pore-forming enzyme perforin and, upon activation, release perforin to kill vessel wall cells, such as vascular smooth muscle cells. HLA molecules, which serve as the ligand for $CD8^+$ T cells, and co-stimulatory molecules, which amplify T-cell activation, are both highly expressed in the vascular lesions of Takayasu's arteritis.

biomarkers of disease activities refocus interest on macrophage-dependent disease mechanisms (Matsuyama et al., 2003). Destruction of elastic membranes points towards release of elastolytic enzymes, likely derived from tissue-infiltrating macrophages.

Much less is known about the pathomechanism of the systemic inflammatory syndrome. Cytokine levels of IL-6 and RANTES have been reported to be elevated and to correlate with clinical activity (Noris et al., 1999).

Treatment and Outcome

Immunosuppressive therapy remains the mainstay of treatment for this arteritis, but, in contrast to GCA, surgical procedures are gaining in importance for managing patients with TA (Tada, 1994).

As with most rare disease, randomized controlled treatment trials are explicitly difficult to perform, are missing, or are based on small patient numbers. The immunosuppressants of choice are corticosteroids, which are started at a dose of 40–60 mg/day prednisone. Clinicians in Japan have advocated doses of only 20–30 mg/day (Numano, 2002). Once acute disease activity is controlled, an effort needs to be made to reduce corticosteroids. Tapering by 5 mg/day every 2–3 weeks has been adopted as a useful guideline. However, there has been agreement that a target maintenance dose of 5–10 mg/day should be kept stable over a prolonged period to avoid exacerbation of vascular and generalized inflammation. Although not formally tested, most patients receive aspirin or an alternative agent to reduce platelet aggregation and thrombus formation.

About 50% of patients may be considered to be insufficiently treated with corticosteroid monotherapy (Kerr et al., 1994). Some of these patients may benefit from methotrexate as a steroid-sparing agent (Hoffman et al., 1994). Mycophenolate mofetil has been reported to show clinical efficiency in a small patient cohort (Daina et al., 1999). A recent report suggests that blocking TNF-α may be helpful in treating patients with persistent disease activity (Hoffman et al., 2004).

Monitoring for and managing hypertension is prudent in patients with TA. There is an ongoing discussion about whether vascular inflammation predisposes for the accelerated development and progression of atherosclerosis, although it may be difficult to separate these disease processes (Numano et al., 2000). Accelerated atherosclerosis demands appropriate monitoring for risk factors and treatment of dyslipidemia.

Critical renal artery stenosis, limiting claudication of extremities, cerebrovascular ischemia, coronary ischemia, and aortic regurgitation may represent indications for surgical intervention. If clinically possible, quiescence of vascular inflammation should be aimed for prior to surgery. Prevention of stroke may be possible if critical stenosis of cervical vessels is bypassed with grafts originating from the ascending aorta. Percutaneous transluminal angioplasty has emerged as an alternative to bypass surgery, specifically for renal artery stenosis (Weaver et al., 2004), and may be useful for preserving the competence of vessels in other territories.

The best outcome data for TA are available from Japan where all patients with TA are registered by the government. Analysis of 897 patients through 1998 showed that more than 70% of patients had well-controlled disease, enjoying almost normal lives. Twenty-five percent of patients had severe complications. Cardiac manifestations have become the most common cause of death among TA patients (Numano, 2002).

CONCLUDING REMARKS— FUTURE PERSPECTIVES

Vasculitides are infrequent yet clinically challenging diseases because they threaten blood supply to vital organs. Damage to large and medium-sized arteries immediately puts the host at risk for severe clinical consequences, as compensatory mechanisms for losing the function of the aorta and its major branches are very limited. In contrast to non-inflammatory vasculopathies, arteritides are characterized by a combination of ischemic tissue damage and a syndrome of generalized inflammation. Pathogenic mechanisms relevant for these two dimensions of disease may be distinct and respond differentially to therapy. Systemic inflammation is caused by the excessive production of cytokines. In the cases of GCA and TA, induction of an abrupt and massive acute phase response is typical. As part of the acute phase response, patients produce hepatic acute phase proteins that give rise to diagnostically important laboratory abnormalities, such as increases in ESR and C-reactive protein (CRP).

In both GCA and TA, T lymphocytes are the key regulators of tissue injury in the blood vessel wall. They either mediate direct cellular damage, as in the case of TA, or orchestrate the functional activity of tissue-injurious macrophages, as in GCA. Arterial wall injury causes hyperproliferation of myofibroblasts and results in thickening of the intima, the underlying mechanism of vessel stenosis/occlusion. In GCA, tissue-resident dendritic cells have been implicated in initiating T-cell activation, possibly after being triggered by blood-borne stimuli. There is no evidence that autoantibodies or other B-cell-dependent functions contribute to large vessel vasculitis (Martinez-Taboada et al., 1996).

Giant cell arteritis and TA are unusual among the chronic inflammatory diseases in that they respond very well to corticosteroids. However, even prolonged therapy can usually not induce complete remission, and side effects are common. So far, alternative immunosuppressive agents have been amazingly ineffective in treating GCA but may have a role in TA. Major progress could be made by targeting disease pathways, such as the oxidative damage of vessel wall resident cells, and the process of intimal hyperplasia (Weyand and Goronzy, 2003a).

Despite advances in non-invasive imaging methods, it has proven difficult to monitor patients for disease activity. Cytokines may be useful biomarkers in assessing the "burden of disease," at least as far as the systemic inflammatory syndrome is concerned. Well-designed clinical trials are necessary to validate the use of cytokine levels, particularly for IL-6, as a marker of disease in patients.

The ultimate challenge in understanding GCA and TA remains the identification of the initial triggers of the disease processes. Although infections have been suspected, scientific evidence for their role in starting arteritis is lacking (Regan et al., 2002; Nordborg and Nordborg, 2003). Local factors intrinsic to the artery itself, such as dendritic cells positioned in the adventitia of medium-sized and large arteries, may be instrumental in breaking tolerance and giving rise to misplaced immune responses (Ma-Krupa et al., 2004). Also, age is a major risk factor in GCA and TA, suggesting that age-related changes in T-cell function may be critical determinants of disease susceptibility (Goronzy and Weyand, 2003).

Acknowledgments

Supported in part by grants from the National Institutes of Health (AR42527, HL63919, AR41974, AI44142, AI57266, EY11916, and AG15043).

References

Aiello, P.D., Trautmann, J.C., McPhee, T.J., Kunselman, A.R., and Hunder, G.G. (1993). Visual prognosis in giant cell arteritis. *Ophthalmology 100*, 550–555.

Andersson, R., Jonsson, R., Tarkowski, A., Bengtsson, B.A., and Malmvall, B.E. (1987). T cell subsets and expression of immunological activation markers in the arterial walls of patients with giant cell arteritis. *Ann. Rheum. Dis. 46,* 915–923.

Baldursson, O., Steinsson, K., Bjornsson, J., and Lie, J.T. (1994). Giant cell arteritis in Iceland. An epidemiologic and histopathologic analysis. *Arthritis Rheum. 37,* 1007–1012.

Banchereau, J. and Steinman, R.M. (1998). Dendritic cells and the control of immunity. *Nature 392,* 245–252.

Björnsson, J. (2002). Histopathology of primary vasculitic disorders. In G.S. Hoffman (ed.), *Inflammatory Diseases of Blood Vessels. (*pp. 255–265). New York: Marcel Dekker.

Borkowski, A., Younge, B.R., Szweda, L., Mock, B., Bjornsson, J., Moeller, K., Goronzy, J.J., and Weyand, C.M. (2002). Reactive nitrogen intermediates in giant cell arteritis: selective nitration of neocapillaries. *Am. J. Pathol. 161,* 115–123.

Brack, A., Geisler, A., Martinez-Taboada, V.M., Younge, B.R., Goronzy, J.J., and Weyand, C.M. (1997). Giant cell vasculitis is a T cell-dependent disease. *Mol. Med. 3,* 530–543.

Brack, A., Martinez-Taboada, V., Stanson, A., Goronzy, J.J., and Weyand, C.M. (1999). Disease pattern in cranial and large-vessel giant cell arteritis. *Arthritis Rheum. 42,* 311–317.

Cantini, F., Salvarani, C., Olivieri, I., Niccoli, L., Padula, A., Macchioni, L., Boiardi, L., Ciancio, G., Mastrorosato, M., Rubini, F., Bozza, A., and Zanfranceschi, G. (2001). Shoulder ultrasonography in the diagnosis of polymyalgia rheumatica: a case–control study. *J. Rheumatol. 28,* 1049–1055.

Chevalet, P., Barrier, J.H., Pottier, P., Magadur-Joly, G., Pottier, M.A., Hamidou, M., Planchon, B., El Kouri, D., Connan, L., Dupond, J.L., De Wazieres, B., Dien, G., Duhamel, E., Grosbois, B., Jego, P., Le Strat, A., Capdeville, J., Letellier, P., and Agron, L. (2000). A randomized, multicenter, controlled trial using intravenous pulses of methylprednisolone in the initial treatment of simple forms of giant cell arteritis: a one year followup study of 164 patients. *J. Rheumatol. 27,* 1484–1491.

Cid, M.C. (2002). Endothelial cell biology, perivascular inflammation, and vasculitis. *Cleve. Clin. J. Med. 69*(Suppl 2), SII45–49.

Dabague, J. and Reyes, P.A. (1996). Takayasu arteritis in Mexico: a 38-year clinical perspective through literature review. *Int. J. Cardiol. 54*(Suppl), S103–109.

Daina, E., Schieppati, A., and Remuzzi, G. (1999). Mycophenolate mofetil for the treatment of Takayasu arteritis: report of three cases. *Ann. Intern. Med. 130,* 422–426.

Doran, M.F., Crowson, C.S., O'Fallon, W.M., Hunder, G.G., and Gabriel, S.E. (2002). Trends in the incidence of polymyalgia rheumatica over a 30 year period in Olmsted County, Minnesota, USA. *J. Rheumatol. 29,* 1694–1697.

Endo, M., Tomizawa, Y., Nishida, H., Aomi, S., Nakazawa, M., Tsurumi, Y., Kawana, M., and Kasanuki, H. (2003). Angiographic findings and surgical treatments of coronary artery involvement in Takayasu arteritis. *J. Thorac. Cardiovasc. Surg. 125,* 570–577.

Evans, J.M. and Hunder, G.G. (2000). Polymyalgia rheumatica and giant cell arteritis. *Rheum. Dis. Clin. N. Am. 26,* 493–515.

Evans, J.M., Bowles, C.A., Bjornsson, J., Mullany, C.J., and Hunder, G.G. (1994). Thoracic aortic aneurysm and rupture in giant cell arteritis. A descriptive study of 41 cases. *Arthritis Rheum. 37,* 1539–1547.

Glutz von Blotzheim, S. and Borruat, F.X. (1997). Neuro-ophthalmic complications of biopsy-proven giant cell arteritis. *Eur. J. Ophthalmol. 7,* 375–382.

Gonzalez, E.B., Varner, W.T., Lisse, J.R., Daniels, J.C., and Hokanson, J.A. (1989). Giant-cell arteritis in the southern United States. An 11-year retrospective study from the Texas Gulf Coast. *Arch. Intern. Med. 149,* 1561–1565.

Gonzalez-Gay, M.A., Amoli, M.M., Garcia-Porrua, C., and Ollier, W.E. (2003). Genetic markers of disease susceptibility and severity in giant cell arteritis and polymyalgia rheumatica. *Semin Arthritis Rheum. 33,* 38–48.

Goronzy, J.J. and Weyand, C.M. (2003). Aging, autoimmunity and arthritis: T-cell senescence and contraction of T-cell repertoire diversity—catalysts of autoimmunity and chronic inflammation. *Arthritis Res. Ther. 5,* 225–234.

Gran, J.T., Myklebust, G., Wilsgaard, T., and Jacobsen, B.K. (2001). Survival in polymyalgia rheumatica and temporal arteritis: a study of 398 cases and matched population controls. *Rheumatology (Oxford) 40,* 1238–1242.

Gravanis, M.B. (2000). Giant cell arteritis and Takayasu aortitis: morphologic, pathogenetic and etiologic factors. *Int. J. Cardiol. 75*(Suppl 1), S21–33; discussion S35–26.

Henriet, J.-P., Mann, J., Gosselin, J., Hamel-Desnos C., Ducrocq, M., Brand, G., Maiza, D., Courtheaux, P., Evrand, C., and Theron, J. (1989). Historique de la maladie de Horton. *J. Maladies Vasculaires 14,* 93–97.

Hernandez-Rodriguez, J., Segarra, M., Vilardell, C., Sanchez, M., Garcia-Martinez, A., Esteban, M.J., Queralt, C., Grau, J.M., Urbano-Marquez, A., Palacin, A., Colomer, D., and Cid, M.C. (2004). Tissue production of pro-inflammatory cytokines (IL-1beta, TNFalpha and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. *Rheumatology (Oxford) 43,* 294–301.

Hoffman, G.S., Leavitt, R.Y., Kerr, G.S., Rottem, M., Sneller, M.C., and Fauci, A.S. (1994). Treatment of glucocorticoid-resistant or relapsing Takayasu arteritis with methotrexate. *Arthritis Rheum. 37,* 578–582.

Hoffman, G.S., Cid, M.C., Hellmann, D.B., Guillevin, L., Stone, J.H., Schousboe, J., Cohen, P., Calabrese, L.H., Dickler, H., Merkel, P.A., Fortin, P., Flynn, J.A., Locker, G.A., Easley, K.A., Schned, E., Hunder, G.G., Sneller, M.C., Tuggle, C., Swanson, H., Hernandez-Rodriguez, J., Lopez-Soto, A., Bork, D., Hoffman, D.B., Kalunian, K., Klashman, D., Wilke, W.S., Scheetz, R.J., Mandell, B.F., Fessler, B.J., Kosmorsky, G., Prayson, R., Luqmani, R.A., Nuki, G., McRorie, E., Sherrer, Y., Baca, S., Walsh, B., Ferland, D., Soubrier, M., Choi, H.K., Gross, W., Segal, A.M., Ludivico, C., and Puechal, X. (2002). A multicenter, randomized, double-blind, placebo-controlled trial of adjuvant methotrexate treatment for giant cell arteritis. *Arthritis Rheum. 46,* 1309–1318.

Hoffman, G.S., Merkel, P.A., Brasington, R.D., Lenschow, D.J., and Liang, P. (2004). Anti-tumor necrosis factor therapy in patients with difficult to treat Takayasu arteritis. *Arthritis Rheum. 50,* 2296–2304.

Horton, B.T., Magath, T.B., and Brown G.E. (1932). An undescribed form of arteritis of the temporal vessels. *Mayo Clin. Proc. 7,* 700–701.

Hutchinson, J. (1890). Diseases of the arteries. On a peculiar form of thrombolic arteritis of the aged which is sometimes productive of gangrene. *Arch. Surg. 1,* 323–329.

Jover, J.A., Hernandez-Garcia, C., Morado, I.C., Vargas, E., Banares, A., and Fernandez-Gutierrez, B. (2001). Combined treatment of giant-cell arteritis with methotrexate and prednisone. A randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med. 134,* 106–114.

Kaiser, M., Weyand, C.M., Bjornsson, J., and Goronzy, J.J. (1998). Platelet-derived growth factor, intimal hyperplasia, and ischemic complications in giant cell arteritis. *Arthritis Rheum. 41,* 623–633.

Kaiser, M., Younge, B., Bjornsson, J., Goronzy, J.J., and Weyand, C.M. (1999). Formation of new vasa vasorum in vasculitis. Production of angiogenic cytokines by multinucleated giant cells. *Am. J. Pathol. 155,* 765–774.

Kaisho, T. and Akira, S. (2003). Regulation of dendritic cell function through toll-like receptors. *Curr. Mol. Med. 3,* 759–771.

Kerr, G.S., Hallahan, C.W., Giordano, J., Leavitt, R.Y., Fauci, A.S., Rottem, M., and Hoffman, G.S. (1994). Takayasu arteritis. *Ann. Intern. Med. 120,* 919–929.

Kimura, A., Kobayashi, Y., Takahashi, M., Ohbuchi, N., Kitamura, H., Nakamura, T., Satoh, M., Sasaoka, T., Hiroi, S., Arimura, T., Akai, J., Aerbajinai, W., Yasukochi, Y., and Numano, F. (1998). MICA gene polymorphism in Takayasu's arteritis and Buerger's disease. *Int. J. Cardiol. 66*(Suppl 1), S107–113; discussion S115.

Kimura, A., Kitamura, H., Date, Y., and Numano, F. (1996). Comprehensive analysis of HLA genes in Takayasu arteritis in Japan. *Int. J. Cardiol. 54*(Suppl), S61–69.

Kissin, E.Y. and Merkel, P.A. (2004). Diagnostic imaging in Takayasu arteritis. *Curr. Opin. Rheumatol. 16,* 31–37.

Koide, K. (1992). Takayasu arteritis in Japan. *Heart Vessels Suppl. 7,* 48–54.

Krupa, W.M., Dewan, M., Jeon, M.S., Kurtin, P.J., Younge, B.R., Goronzy, J.J., and Weyand, C.M. (2002). Trapping of misdirected dendritic cells in the granulomatous lesions of giant cell arteritis. *Am. J. Pathol. 161,* 1815–1823.

Lie, J.T. (1995). Aortic and extracranial large vessel giant cell arteritis: a review of 72 cases with histopathologic documentation. *Semin. Arthritis Rheum. 24,* 422–431.

Ma-Krupa, W., Jeon, M.S., Spoerl, S., Tedder, T.F., Goronzy, J.J., and Weyand, C.M. (2004). Activation of arterial wall dendritic cells and breakdown of self-tolerance in giant cell arteritis. *J. Exp. Med. 199,* 173–183.

Machado, E.B., Michet, C.J., Ballard, D.J., Hunder, G.G., Beard, C.M., Chu, C.P., and O'Fallon, W.M. (1988). Trends in incidence and clinical presentation of temporal arteritis in Olmsted County, Minnesota, 1950–1985. *Arthritis Rheum. 31,* 745–749.

Martinez-Taboada, V., Brack, A., Hunder, G.G., Goronzy, J.J., and Weyand, C.M. (1996). The inflammatory infiltrate in giant cell arteritis selects against B lymphocytes. *J. Rheumatol. 23,* 1011–1014.

Matsunaga, N., Hayashi, K., Okada, M., and Sakamoto, I. (2003). Magnetic resonance imaging features of aortic diseases. *Top. Magn. Reson. Imaging 14,* 253–266.

Matsuyama, A., Sakai, N., Ishigami, M., Hiraoka, H., Kashine, S., Hirata, A., Nakamura, T., Yamashita, S., and Matsuzawa, Y. (2003). Matrix metalloproteinases as novel disease markers in Takayasu arteritis. *Circulation 108,* 1469–1473.

Matteson, E.L., Gold, K.N., Bloch, D.A., and Hunder, G.G. (1996). Long-term survival of patients with giant cell arteritis in the American College of Rheumatology giant cell arteritis classification criteria cohort. *Am. J. Med. 100,* 193–196.

Nastri, M.V., Baptista, L.P., Baroni, R.H., Blasbalg, R., de Avila, L.F., Leite, C.C., de Castro, C.C., and Cerri, G.G. (2004). Gadolinium-enhanced three-dimensional MR angiography of Takayasu arteritis. *Radiographics 24,* 773–786.

Nordborg, E. and Nordborg, C. (2003). Giant cell arteritis: epidemiological clues to its pathogenesis and an update on its treatment. *Rheumatology (Oxford) 42,* 413–421.

Nordborg, C., Johansson, H., Petursdottir, V., and Nordborg, E. (2003). The epidemiology of biopsy-positive giant cell arteritis: special reference to changes in the age of the population. *Rheumatology (Oxford) 42,* 549–552.

Noris, M., Daina, E., Gamba, S., Bonazzola, S., and Remuzzi, G. (1999). Interleukin-6 and RANTES in Takayasu arteritis: a guide for therapeutic decisions? *Circulation 100,* 55–60.

Numano, F. (1997). Differences in clinical presentation and outcome in different countries for Takayasu's arteritis. *Curr. Opin. Rheumatol. 9,* 12–15.

Numano, F. (2002). Takayasu's arteritis: clinical aspects. In G.S. Hoffman (ed.), *Inflammatory Diseases of Blood Vessels,* (pp. 455–466). New York: Marcel Dekker.

Numano, F. and Kakuta, T. (1996). Takayasu arteritis—five doctors in the history of Takayasu arteritis. *Int. J. Cardiol. 54*(Suppl), S1–10.

Numano, F., Kishi, Y., Tanaka, A., Ohkawara, M., Kakuta, T., and Kobayashi, Y. (2000). Inflammation and atherosclerosis. Atherosclerotic lesions in Takayasu arteritis. *Ann. N. Y. Acad. Sci. 902,* 65–76.

Proven, A., Gabriel, S.E., Orces, C., O'Fallon, W.M., and Hunder, G.G. (2003). Glucocorticoid therapy in giant cell arteritis: duration and adverse outcomes. *Arthritis Rheum. 49,* 703–708.

Regan, M.J., Wood, B.J., Hsieh, Y.H., Theodore, M.L., Quinn, T.C., Hellmann, D.B., Green, W.R., Gaydos, C.A., and Stone, J.H. (2002). Temporal arteritis and *Chlamydia pneumoniae*: failure to detect the organism by polymerase chain reaction in ninety cases and ninety controls. *Arthritis Rheum. 46,* 1056–1060.

Rittner, H.L., Kaiser, M., Brack, A., Szweda, L.I., Goronzy, J.J., and Weyand, C.M. (1999a). Tissue-destructive macrophages in giant cell arteritis. *Circ. Res. 84,* 1050–1058.

Rittner, H.L., Hafner, V., Klimiuk, P.A., Szweda, L.I., Goronzy, J.J., and Weyand, C.M. (1999b). Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. *J. Clin. Invest. 103,* 1007–1013.

Roche, N.E., Fulbright, J.W., Wagner, A.D., Hunder, G.G., Goronzy, J.J., and Weyand, C.M. (1993). Correlation of interleukin-6 production and disease activity in polymyalgia rheumatica and giant cell arteritis. *Arthritis Rheum. 36,* 1286–1294.

Rojo-Leyva, F., Ratliff, N.B., Cosgrove, 3rd 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.

Salazar, M., Varela, A., Ramirez, L.A., Uribe, O., Vasquez, G., Egea, E., Yunis, E.J., and Iglesias-Gamarra, A. (2000). Association of HLA-DRB1*1602 and DRB1*1001 with Takayasu arteritis in Colombian mestizos as markers of Amerindian ancestry. *Int. J. Cardiol. 75*(Suppl 1), S113–116.

Salvarani, C. (2003). Large vessel vasculitis. *Clin. Exp. Rheumatol. 21,* S133–134.

Salvarani, C. and Hunder, G.G. (1999). Musculoskeletal manifestations in a population-based cohort of patients with giant cell arteritis. *Arthritis Rheum. 42,* 1259–1266.

Salvarani, C., Cantini, F., Boiardi, L., and Hunder, G.G. (2002). Polymyalgia rheumatica and giant-cell arteritis. *N. Engl. J. Med. 347,* 261–271.

Salvarani, C., Crowson, C.S., O'Fallon, W.M., Hunder, G.G., and Gabriel, S.E. (2004). Reappraisal of the epidemiology of giant cell arteritis in Olmsted County, Minnesota, over a fifty-year period. *Arthritis Rheum. 51,* 264–268.

Seko, Y. (2000). Takayasu arteritis: insights into immunopathology. *Jpn Heart J. 41,* 15–26.

Seko, Y. (2002). Takayasu's arteritis: pathogenesis. In G. S. Hoffman (ed.), *Inflammatory Diseases of Blood Vessels,* (pp. 443–453). New York: Marcel Dekker.

Seko, Y., Minota, S., Kawasaki, A., Shinkai, Y., Maeda, K., Yagita, H., Okumura, K., Sato, O., Takagi, A., Tada, Y., and Yazaki, Y. (1994). Perforin-secreting killer cell infiltration and expression of a 65-kD heat-shock protein in aortic tissue of patients with Takayasu's arteritis. *J. Clin. Invest. 93,* 750–758.

Seko, Y., Sato, O., Takagi, A., Tada, Y., Matsuo, H., Yagita, H., Okumura, K., and Yazaki, Y. (1996). Restricted usage of T-cell receptor Valpha-Vbeta genes in infiltrating cells in aortic tissue of patients with Takayasu's arteritis. *Circulation 93,* 1788–1790.

Seko, Y., Takahashi, N., Tada, Y., Yagita, H., Okumura, K., and Nagai, R. (2000). Restricted usage of T-cell receptor Vgamma-Vdelta genes and expression of costimulatory molecules in Takayasu's arteritis. *Int. J. Cardiol. 75*(Suppl 1), S77–83; discussion S85–77.

Shimizu, K. and Sano, K. (1951). Pulseless disease. *J. Neuropath. Clin. Neuro. 1*, 1.37–1.47.

Smetana, G.W. and Shmerling, R.H. (2002). Does this patient have temporal arteritis? *JAMA 287,* 92–101.

Smith, C.A., Fidler, W.J., and Pinals, R.S. (1983). The epidemiology of giant cell arteritis. Report of a ten-year study in Shelby County, Tennessee. *Arthritis Rheum. 26,* 1214–1219.

Sorbi, D., French, D.L., Nuovo, G.J., Kew, R.R., Arbeit, L.A., and Gruber, B.L. (1996). Elevated levels of 92-kd type IV collagenase (matrix metalloproteinase 9) in giant cell arteritis. *Arthritis Rheum. 39,* 1747–1753.

Spiera, R.F., Mitnick, H.J., Kupersmith, M., Richmond, M., Spiera, H., Peterson, M.G., and Paget, S.A. (2001). A prospective, double-blind, randomized, placebo controlled trial of methotrexate in the treatment of giant cell arteritis (GCA). *Clin. Exp. Rheumatol. 19,* 495–501.

Tada, Y. (1994). [Surgical treatment of intractable vasculitis syndromes—with special reference to Buerger disease, Takayasu arteritis, and so-called inflammatory abdominal aortic aneurysm]. *Nippon Rinsho 52,* 2192–2202.

Takayasu, M. (1908). A case with peculiar changes of the central retinal vessels. *Acta Soc. Ophthal. Japan 12,* 554–555.

Tomita, T. and Imakawa, K. (1998). Matrix metalloproteinases and tissue inhibitors of metalloproteinases in giant cell arteritis: an immunocytochemical study. *Pathology 30*, 40–50.

Tso, E., Flamm, S.D., White, R.D., Schvartzman, P.R., Mascha, E., and Hoffman, G.S. (2002). Takayasu arteritis: utility and limitations of magnetic resonance imaging in diagnosis and treatment. *Arthritis Rheum. 46,* 1634–1642.

Uddhammar, A.C. (2000). Von Willebrand factor in polymyalgia rheumatica and giant cell arteritis. *Clin. Exp. Rheumatol. 18,* S32–33.

Wagner, A.D., Goronzy, J.J., and Weyand, C.M. (1994). Functional profile of tissue-infiltrating and circulating CD68+ cells in giant cell arteritis. Evidence for two components of the disease. *J. Clin. Invest. 94,* 1134–1140.

Wagner, A.D., Bjornsson, J., Bartley, G.B., Goronzy, J.J., and Weyand, C.M. (1996). Interferon-gamma-producing T cells in giant cell vasculitis represent a minority of tissue-infiltrating cells and are located distant from the site of pathology. *Am. J. Pathol. 148,* 1925–1933.

Watts, R.A. and Scott, D.G. (1997). Classification and epidemiology of the vasculitides. *Baillières Clin. Rheumatol. 11,* 191–217.

Webb, M., Chambers, A., Al-Nahhas, A., Mason, J.C., Maudlin, L., Rahman, L., and Frank, J. (2004). The role of 18F-FDG PET in characterising disease activity in Takayasu arteritis. *Eur. J. Nucl. Med. Mol. Imaging 31,* 627–634.

Weyand, C.M. (2000). The Dunlop-Dottridge Lecture: The pathogenesis of giant cell arteritis. *J. Rheumatol. 27,* 517–522.

Weyand, C.M. and Goronzy, J.J. (1999). Arterial wall injury in giant cell arteritis. *Arthritis Rheum. 42,* 844–853.

Weyand, C.M. and Goronzy J.J. (2003a). Medium- and large-vessel vasculitis. *N. Engl. J. Med. 349,* 160–169.

Weyand, C.M. and Goronzy J.J. (2003b). Giant-cell arteritis and polymyalgia rheumatica. *Ann. Intern. Med. 139,* 505–515.

Weyand, C.M., Hicok, K.C., Hunder, G.G., and Goronzy, J.J. (1992). The HLA-DRB1 locus as a genetic component in giant cell arteritis. Mapping of a disease-linked sequence motif to the antigen binding site of the HLA-DR molecule. *J. Clin. Invest. 90,* 2355–2361.

Weyand, C.M., Hicok, K.C., Hunder, G.G., and Goronzy, J.J. (1994a). Tissue cytokine patterns in patients with polymyalgia rheumatica and giant cell arteritis. *Ann. Intern. Med. 121,* 484–491.

Weyand, C.M., Hunder, N.N., Hicok, K.C., Hunder, G.G., and Goronzy, J.J. (1994b). HLA-DRB1 alleles in polymyalgia rheumatica, giant cell arteritis, and rheumatoid arthritis. *Arthritis Rheum. 37,* 514–520.

Weyand, C.M., Schonberger, J., Oppitz, U., Hunder, N.N., Hicok, K.C., and Goronzy, J.J. (1994c). Distinct vascular lesions in giant cell arteritis share identical T cell clonotypes. *J. Exp. Med. 179,* 951–960.

Weyand, C.M. and Goronzy, J.J. (1995). Giant cell arteritis as an antigen-driven disease. *Rheum. Dis. Clin. North Am. 21,* 1027–1039.

Weyand, C.M., Wagner, A.D., Bjornsson, J., and Goronzy, J.J. (1996). Correlation of the topographical arrangement and the functional pattern of tissue-infiltrating macrophages in giant cell arteritis. *J. Clin. Invest. 98,* 1642–1649.

Weyand, C.M., Tetzlaff, N., Bjornsson, J., Brack, A., Younge, B., and Goronzy, J.J. (1997). Disease patterns and tissue cytokine profiles in giant cell arteritis. *Arthritis Rheum. 40,* 19–26.

Weyand, C.M., Fulbright, J.W., Evans, J.M., Hunder, G.G., and Goronzy, J.J. (1999). Corticosteroid requirements in polymyalgia rheumatica. *Arch. Intern. Med. 159,* 577–584.

Weyand, C.M., Fulbright, J.W., Hunder, G.G., Evans, J.M., and Goronzy, J.J. (2000). Treatment of giant cell arteritis: interleukin–6 as a biologic marker of disease activity. *Arthritis Rheum. 43,* 1041–1048.

Weyand, C.M., Kaiser, M., Yang, H., Younge, B., and Goronzy, J.J. (2002). Therapeutic effects of acetylsalicylic acid in giant cell arteritis. *Arthritis Rheum. 46,* 457–466.

Weaver, F.A., Kumar, S.R., Yellin, A.E., Anderson, S., Hood, D.B., Rowe, V.L., Kitridou, R.C., Kohl, R.D., and Alexander, J. (2004). Renal revascularization in Takayasu arteritis-induced renal artery stenosis. *J. Vasc. Surg. 39,* 749–757.

CHAPTER

67

Idiopathic Bronchiolitis Obliterans, Idiopathic Pulmonary Fibrosis, and Autoimmune Disorders of the Lung

BRIAN D. GELBMAN* AND RONALD G. CRYSTAL*†

**Division of Pulmonary and Critical Care Medicine and †Department of Genetic Medicine, Weill Medical College of Cornell University, New York, New York, USA*

Idiopathic bronchiolitis obliterans (IBO), a disorder of the small airways, and idiopathic pulmonary fibrosis (IPF), a disorder that affects primarily the alveoli, are chronic inflammatory/fibrotic disorders that are typically progressive and often fatal. Although both are classified as "lung disorders of unknown etiology," there are multiple clues suggesting that autoimmune mechanisms play a role in the pathogenesis of both disorders. The focus of this chapter is to explore the link between autoimmune mechanisms underlying IBO and IPF as models for autoimmune disorders of the lung. To do so, we will first discuss the history and the clinical features of IBO and IPF, followed by a summary of the autoimmune, genetic, environmental features, current concepts of pathogenesis, and the therapies currently available and under investigation for both disorders.

HISTORY

Although both IBO and IPF are inflammatory/fibrotic disorders, because they are centered on the bronchioles and alveoli, respectively, and have different clinical, radiologic, and pathologic features, they have always been considered to be different disorders.

Idiopathic Bronchiolitis Obliterans

The term "bronchiolitis obliterans" was first used by Lange in 1901 when he described two patients in whom the bronchioles were blocked by plugs of granulation tissue (Lange, 1901), though those patients likely had what is now known as "cryptogenic organizing pneumonia" (COP). In 1966, Baar and Galindo used the term "bronchiolitis fibrosa obliterans" to describe concentric rings of fibrotic tissue in the wall of the airways (Baar and Galindo, 1966). In 1973, Gosink et al. employed "bronchiolitis obliterans" to describe a group of patients that had submucosal and peribronchiolar infiltrate of granulation tissue resulting in extrinsic narrowing of the bronchiolar lumen (Gosink et al., 1973). In 1981, Turton et al. added the term "cryptogenic obliterative bronchiolitis" to patients that had progressive airflow obstruction

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

with no identifiable cause, such as asthma, chronic bronchitis, or emphysema (Turton et al., 1981).

During the 1970s, several rheumatologic conditions, particularly rheumatoid arthritis (RA), were recognized to be associated with bronchiolitis obliterans, as were adverse reactions to therapies used for RA such as gold and penicillamine (Geddes et al., 1977). In 1982, Roca et al. described the first case of bronchiolitis obliterans as a complication of bone marrow transplantation in a patient who developed graft-versus-host disease following an allogeneic bone marrow transplant for aplastic anemia (Roca et al., 1982). In 1984, Burke et al. characterized the first case of bronchiolitis obliterans associated with a heart-lung transplant (Burke et al., 1984).

In this chapter, we will focus on "idiopathic bronchiolitis obliterans," the form of bronchiolitis obliterans that is not associated with any known condition or cause, although we will use information regarding other forms of bronchiolitis obliterans to develop the general concepts of the autoimmune features of these disorders.

Idiopathic Pulmonary Fibrosis

The first recognition of the broad category of idiopathic interstitial lung diseases, which includes IPF, is credited to Osler in 1892, when he described a chronic fibrinoid change occurring between the alveolus and the blood vessels (Osler, 1892). Osler demonstrated great foresight by also noting that there were diverse patterns of the disease that made classification difficult.

Idiopathic pulmonary fibrosis, also referred to as "cryptogenic fibrosing alveolitis," "idiopathic interstitial pneumonitis," "usual interstitial pneumonitis," and "idiopathic interstitial pneumonia," was first described by the Czech pathologist Sandoz in 1907, but went largely unnoticed (Sandoz, 1907). Hamman and Rich are frequently credited with the first description of IPF in 1935 (Hamman and Rich, 1935), despite the fact that their initial cases were more acute in presentation, unlike classic IPF, and may have been descriptions of acute respiratory distress syndrome. In 1964, Scadding coined the term "cryptogenic fibrosing alveolitis" to describe IPF as a diffuse inflammatory and fibrotic lung disease affecting primarily the alveoli (Scadding, 1964). Scadding advanced the concept that IPF was a slow, progressive disease that was distinctly different from the relatively acute process described by Hamman and Rich.

Idiopathic pulmonary fibrosis was further defined by Liebow and Carrington in the 1960s using histologic criteria. These investigators separated IPF into two morphologic patterns, which they referred to as "usual interstitial pneumonia" (UIP; now often referred to as "usual interstitial pneumonitis") and "desquamative interstitial pneumonia" (DIP; (Liebow and Carrington, 1969). The inflammatory component of IPF was characterized by our group using bronchoalveolar lavage to distinguish IPF from other interstitial lung disorders (Crystal et al., 1976; Reynolds et al., 1977). The pathologic pattern of UIP was further refined over the next several decades, including the description of nonspecific interstitial pneumonia (NSIP) by Katzenstein and Fiorelli in 1994 as a distinct entity (Katzenstein and Fiorelli, 1994). The classification system developed by the American Thoracic Society and European Respiratory Society in 2001, separates IPF from NSIP and DIP based primarily on pathologic findings (ATS, 2002).

CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC FEATURES

Idiopathic Bronchiolitis Obliterans

Bronchiolitis refers to nonspecific small airway inflammation and consequent damage to the airway epithelium. The current classification of the bronchiolar diseases separates constrictive bronchiolitis, characterized by concentric narrowing of the walls of the bronchioles with luminal obliteration, and proliferative bronchiolitis, characterized by an organizing intraluminal exudate fills and obstructs the bronchioles (Figure 67.1). When proliferative bronchiolitis is associated with diffuse alveolar disease, it is considered a distinct disease entity and is referred to as "cryptogenic organizing pneumonia" or "cryptogenic organizing pneumonitis" (ATS 2002; King, 2003a). The category of constrictive bronchiolitis is subcategorized depending on whether the dominant pathology is "inflammatory" or "fibrotic." The "inflammatory" group of constructive bron-

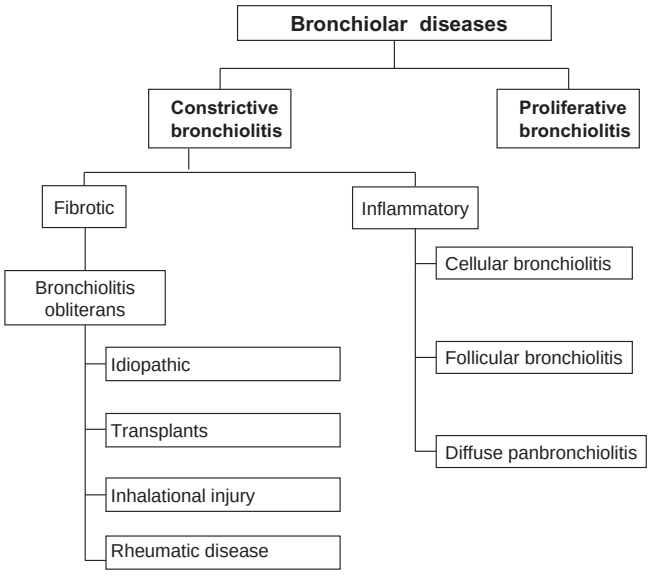


Figure 67.1 Relationship of idiopathic bronchiolitis obliterans to other bronchiolar diseases.

chiolitis disorders includes cellular bronchiolitis, follicular bronchiolitis, and diffuse pan-bronchiolitis. The "fibrotic" group of constrictive bronchiolitis disorders are referred to as "bronchiolitis obliterans," a generic term that describes a bronchiolar disorder with airflow obstruction, in which the primary pathologic features are bronchiolar fibrosis with limited associated bronchiolar inflammation. Bronchiolitis obliterans is associated with several clinical settings, including IBO (the focus of our discussion) as well as bronchiolitis obliterans associated with transplants, inhalational injury, and multisystem autoimmune diseases.

The lack of an accepted classification scheme has led to the continued use of the generic term "bronchiolitis obliterans" to encompass all of these clinicopathologic conditions, independent of whether it is "idiopathic" or associated with another disorder or known cause. For the purpose of this review, we will use the term "idiopathic bronchiolitis obliterans" or IBO to distinguish the idiopathic form from bronchiolitis obliterans associated with transplantation, inhalational injury, and the multisystem autoimmune diseases.

Idiopathic bronchiolitis obliterans is uncommon. It is more prevalent in women (King, 2003a; Kraft et al., 1993). The clinical and pathologic features of bronchiolitis obliterans commonly seen in association with the multisystem autoimmune diseases are identical to the idiopathic form of the disease (King, 2003a; Ryu et al., 2003). A form of bronchiolitis obliterans clinically indistinguishable from the idiopathic form is believed to occur following viral infection; for the purpose of this review, the post-viral form of bronchiolitis obliterans will be considered the same disease entity as IBO.

Individuals with IBO usually present with persistent cough and worsening dyspnea. Physical exam is notable for an end-expiratory wheeze or high pitched end-inspiratory "squeak." Most patients will have an obstructive pattern on pulmonary function testing, although the disease can present with normal, restrictive, or mixed pattern (Markopoulo et al., 2002).

Routine chest radiographs may reveal hyperinflation or reticulonodular opacities, but routine chest X-rays are frequently normal in IBO and are, therefore, limited in utility. High-resolution computed tomography (HRCT), with inspiratory and expiratory views, provides direct and indirect signs suggestive of bronchiolitis (Franquet and Stern, 1999; Markopoulo et al., 2002; Ryu et al., 2003). The only direct sign, bronchiole thickening, is uncommon. The indirect signs include bronchiolar dilatation (bronchiolectasis), and the "mosaic pattern," a heterogeneous display of different densities of the lung parenchyma observed in expiratory views (Figure 67.2). The HRCT mosaic pattern is believed to result from relatively lucent regions that develop from a combination of air-trapping in the alveoli that are unable to collapse during exhalation (because the bronchiole lumens

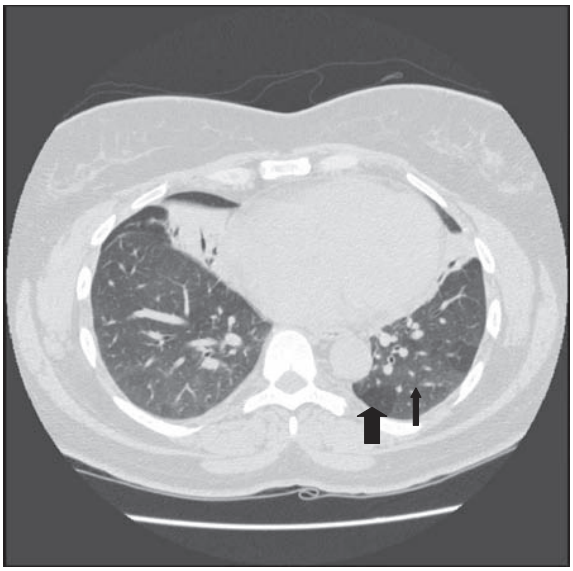


Figure 67.2 Mosaic pattern on high resolution chest tomography taken after expiration typical of idiopathic bronchiolitis obliterans. The thick arrow points to an area of low density, reflecting air-trapping. The thin arrow points to a high density area of lung, which represents normal alveolar collapse during exhalation.

are obstructed) and a decrease in blood flow to the alveoli that remain inflated (Franquet and Stern, 1999; Hansell, 2001). High-resolution computed tomography also is useful for differentiating IBO from disease of the lung parenchyma, such as the interstitial lung diseases of unknown etiology like IPF.

The classic pathologic findings of IBO include extrinsic compression of the bronchiolar lumen caused by surrounding inflammation and fibrosis in the bronchiolar wall (Figure 67.3) (Colby, 1998). In contrast to the "inflammatory" category of constrictive bronchiolitis, in IBO, the fibrosis dominates over the inflammation. As the compression worsens, the airway lumen may become obliterated and scarred. Inflammation and fibrosis can occur in the subepithelial, mural, or adventitial layer of the bronchiole, and usually varies depending on the underlying cause. Other morphologic findings of IBO include smooth muscle hypertrophy, bronchiolectasis, mucostasis, and bronchiolarization of adjacent alveolar spaces (Colby, 1998; Myers and Colby, 1993). The areas affected are scattered and patchy in distribution. For this reason, transbronchial lung biopsy is insensitive as a diagnostic tool for IBO, as it often misses the involved area, and, therefore, a surgical lung biopsy is often necessary to make the diagnosis.

There have been no epidemiologic studies performed to quantify the overall prevalence of IBO. Although it is generally considered a rare disease, it is likely that IBO is underdiagnosed and may account for a small percentage of cases misdiagnosed as asthma or chronic obstructive pulmonary disease (COPD). The prevalence of bronchiolitis obliterans

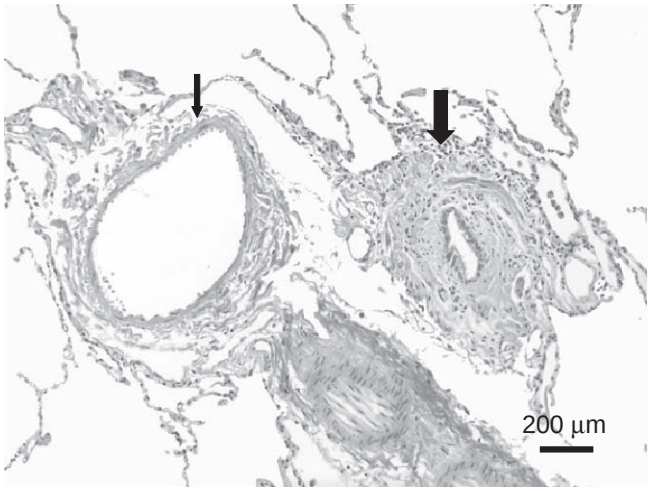


Figure 67.3 Idiopathic bronchiolitis obliterans. The thick arrow points to a narrowed bronchiole, which should have a similar sized lumen as the accompanying blood vessel *(thin arrow)*. The concentric narrowing of the bronchiole is due to fibrosis and scarring in the bronchiole wall. Bronchial smooth muscle hyperplasia is also apparent, while the surrounding alveoli are relatively normal (hematoxylin and eosin, bar = 200 μm).

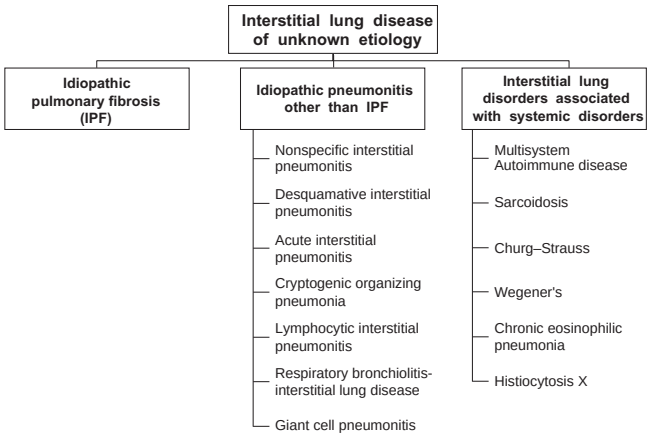


Figure 67.4 Relationship of idiopathic pulmonary fibrosis to other idiopathic interstitial disorders. Some of the rare disorders have been left off the lists (see Schoenberger and Crystal, 1983; Crystal, 1985; ATS 2002 for further details).

in association with RA is less than 1% (King, 2003a). The reported incidence of bronchiolitis obliterans following allogeneic bone marrow transplant is 2–10% of patients who develop graft-versus-host disease (Paz et al., 1993). The incidence in lung transplant recipients is significantly higher, 28, 49, 56, and 71% for individuals at 1, 2, 3, and 5 years post-transplant (Reichenspurner et al., 1996).

Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (often referred to as "cryptogenic fibrosing alveolitis" in Europe) refers to a distinctive type of chronic inflammatory/fibrotic interstitial lung disorder of unknown cause that is limited to the lungs and associated with a histologic pattern of UIP (Figure 67.4) (ATS, 2002). The pattern of UIP on surgical lung biopsy is also seen in patients with multisystem autoimmune disease who present with similar pulmonary-related clinical features to IPF (Harrison et al., 1991).

Individuals with IPF typically present with the gradual onset of symptoms, most commonly dyspnea on exertion and non-productive cough. Most will have had at least 6 months of symptoms before presentation, with an average duration of 24 months (ATS, 2002). The clinical course typically involves gradual deterioration, occasionally punctuated by periods of rapid decline, although some of the disease stabilizes in some individuals. The best estimates for life expectancy from time of diagnosis to death are derived from case–control cohort studies, because they are not biased like prevalence studies, which tend to be over-represented by survivors. According to two such studies, the average survival from the time of diagnosis to death is 3–4 years (Hubbard et al., 1998; Mapel et al., 1998).

On physical exam, patients with IPF may have digital clubbing (25–50%) and fine, inspiratory, velcro-like crackles confined to the bases of the lungs. As there is progressive loss of alveoli, and the diffusing capacity drops below 50% predicted, pulmonary hypertension develops, first with exercise, and later at rest (Crystal et al., 1976; McLees et al., 1979). Once established at rest, the pulmonary hypertension is associated with an increased pulmonary component of the second sound on cardiac exam, and eventual fixed split of the second sound. In the late stages of the disease, there can be signs of right heart failure such as peripheral edema.

Pulmonary function testing usually shows a restrictive pattern of ventilatory defects and a decrease in diffusing capacity, although in early stages of the disease, these tests can be normal (Fulmer et al., 1979; Keogh and Crystal, 1980; Cherniack et al., 1995). Individuals with IPF typically have mild-to-moderate hypoxemia with concomitant low resting oxygen saturation that falls with exercise (Keogh and Crystal, 1980).

Chest radiographs of individuals with IPF are routinely abnormal with a reticular pattern seen at the periphery and in the bases. Later in the disease, there is honeycombing and volume loss in the lower lobes (Staples et al., 1987). In early stages of the disease, chest radiographs may be normal. The preferred imaging modality is HRCT as it is more sensitive than chest radiograph. The typical HRCT findings are reticular-nodular opacities (Figure 67.5). Later in the disease, traction bronchiectasis is commonly seen in the peripheral and basal segments of the lower lobes. Volume loss, and ground glass opacities are frequently present. As the disease progresses, honeycomb cysts develop and enlarge over time

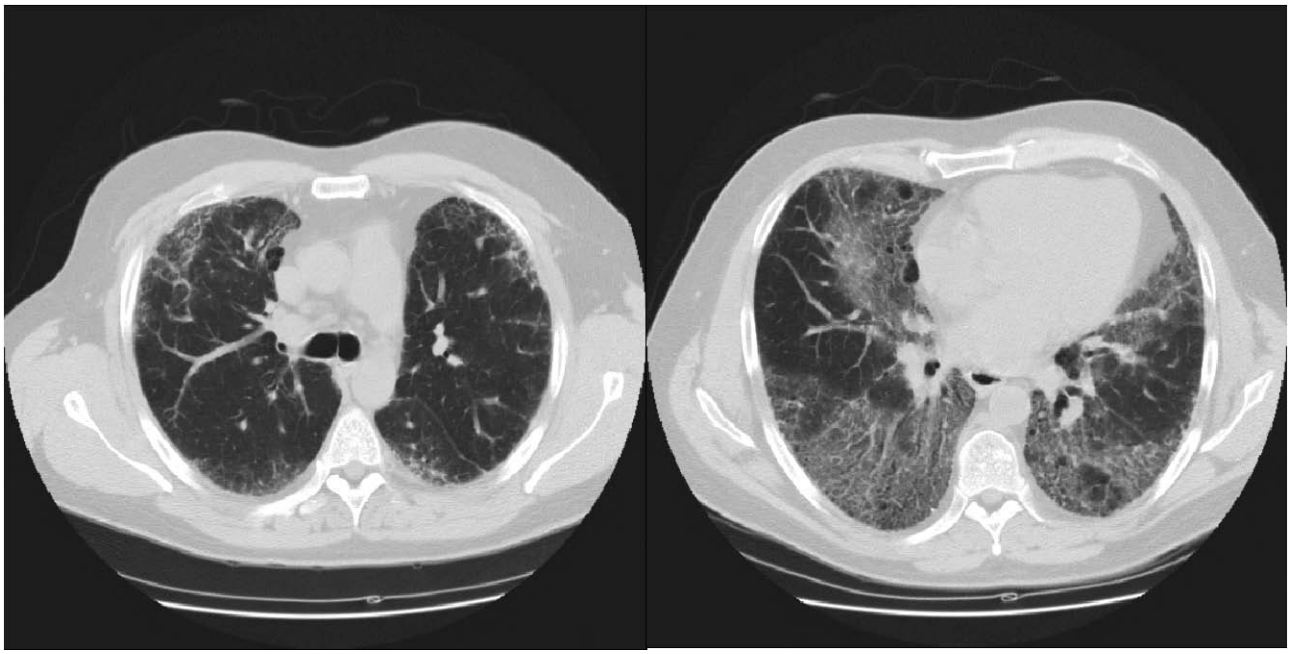


Figure 67.5 Diffuse reticular-nodular pattern on high resolution chest tomography commonly observed in idiopathic pulmonary fibrosis. Note areas of honeycombing in some areas.

(Staples et al., 1987). The chest X-ray and HRCT appearance of IPF is nearly identical to that seen in pulmonary fibrosis associated with the rheumatic disorders, with the lone exception of possibly more basal involvement seen on HRCT in IPF (Chan et al., 1997).

Transbronchial biopsies are insufficient to diagnose IPF because the biopsy specimen is too small and does not preserve the architecture of the lung. However, transbronchial biopsies may be useful to rule out other conditions, such as sarcoidosis, that can mimic IPF. A surgical lung biopsy has been required in the past to firmly establish the diagnosis. However, the recent consensus statement by the American Thoracic Society (ATS) and European Respiratory Society (ERS) permits the diagnosis of IPF when there are typical clinical and HRCT findings of IPF (Hunninghake et al., 2001; ATS, 2002). Surgical lung biopsies are still recommended whenever there are atypical clinical or HRCT features.

The histologic pattern characteristic for IPF is referred to as UIP (Figure 67.6). This pattern is distinct from the other forms of idiopathic interstitial lung disorders such as desquamative interstitial pneumonia, lymphocytic interstitial pneumonia, cryptogenic organizing pneumonia, nonspecific interstitial pneumonia and acute interstitial pneumonia (Liebow and Carrington, 1969; Katzenstein and Fiorelli, 1994; ATS, 2002). There is typically a heterogeneous distribution of interstitial fibrosis interspersed within areas of normal lung, suggesting different temporal stages of involvement. There is patchy inflammation, dominated by alveolar macrophages, and to a lesser extent, lymphocytes, neutrophils, and sometimes eosinophils. The alveolar epithelium undergoes marked changes, referred to as "cuboidalization," with a loss of type I epithelial cells, and their replacement with cuboidal cells, both alveolar type II epithelial cells and bronchiolar epithelium. One hallmark finding in IPF is the subepithelial "fibroblastic focus," which is a nodule of spindle-shaped, mesenchymal cells that produce an abundant deposition of extracellular matrix. These foci are believed to be the "leading edge" of the fibrotic process. Occasionally, the interstitial fibrosis extends through breaks in the epithelium into the alveolar space to form intra-alveolar fibrosis (Basset et al., 1986). Later in the disease there is architectural destruction and fibrosis with honeycombing. Frequently, the subpleural parenchyma is the most severely involved region. When a biopsy shows areas of both UIP and nonspecific interstitial pneumonitis, the default pathologic diagnosis becomes the clinical diagnosis IPF, as these patients have been shown to behave similarly to IPF with UIP-only pattern (Flaherty et al., 2001).

The histologic features of pulmonary fibrosis associated with multisystem autoimmune disease can be classified into the same pathologic descriptions of idiopathic interstitial pneumonias put forth by the ATS and ERS, including UIP, NSIP, DIP, and COP (see Figure 67.4). The UIP pattern that is seen with multisystem autoimmune diseases is essentially identical to the pattern seen in the IPF. In polymyositis, dermatomyositis, and scleroderma, NSIP is the most frequently

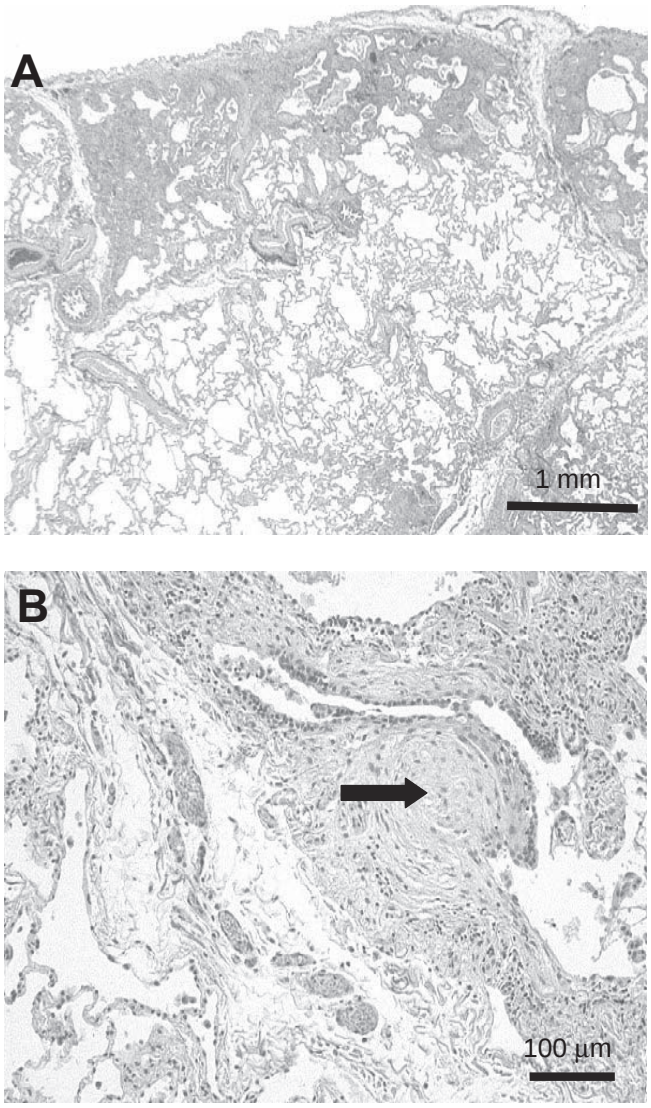


Figure 67.6 Histologic pattern of idiopathic pulmonary fibrosis. The patterns are often referred to as "usual interstitial pneumonitis." *A,* Subpleural, paraseptal and interstitial collagen deposition together with areas of mildly abnormal alveolar parenchyma (hematoxylin and eosin, bar = 1 mm). *B,* Thickened interstitium with collagen deposition, mild to moderate alveolar septal mononuclear cell infiltrates, mainly lymphocytes, cuboidalization of the epithelium and large fibroblastic foci *(thick arrow).* The fibroblastic foci are composed of spindled mesenchymal cells and are thought to represent the "leading edge" of the progressive fibroinflammatory process (hematoxylin and eosin; bar = 100 μm).

seen histologic pattern, followed by UIP (Douglas et al., 2001; Bouros et al., 2002). The prevalence of the different histologic subtypes of idiopathic interstitial pneumonias has not been well characterized in the other multisystem autoimmune diseases.

The estimated annual incidence of idiopathic pulmonary fibrosis is 7 in 100,000 for women and 10 in 100,000 for men. Most patients present between 50 and 70 years of age, however, the incidence, prevalence, and death rate rise with age (Coultas et al., 1994).

Several studies have tried to establish the prevalence of pulmonary fibrosis in patients with the multisystem autoimmune diseases, but the results have varied depending on the method by which patients are screened for pulmonary involvement. Studies that utilize autopsy or lung biopsy data are subject to over-diagnosis since they have been shown to detect subclinical disease that can fail to progress (Cervantes-Perez et al., 1980). Likewise, HRCT screening studies may also overestimate the prevalence of disease by detecting parenchymal changes that may not be clinically significant. In RA, cross-sectional studies using chest radiographs estimate the prevalence to be less than 5% of patients (Hyland et al., 1983). When HRCT is used to screen patients, the prevalence appears to be between 20% and 50%, although these studies are limited by selection bias (Remy-Jardin et al., 1994; Gabbay et al., 1997). Pulmonary fibrosis is most prevalent in scleroderma, where autopsy series have shown it to be present in 70% of cases and chest radiograph series estimate the prevalence between 25% and 65% (Minai et al., 1998; Wiedemann and Matthay, 1989). The prevalence of clinically overt pulmonary fibrosis is 30% in polymyositis and dermatomyositis, 5% in systemic lupus erythematosus, and 10% in Sjögren syndrome (Eisenberg et al., 1973; Gardiner, 1993; Schwarz, 1998).

AUTOIMMUNE FEATURES

Idiopathic Bronchiolitis Obliterans

There are no data regarding autoimmune phenomena in IBO, although bronchiolitis obliterans has been reported in association with many different types of autoimmune disorders, including RA, lupus erythematosus, ankylosing spondylitis, Sjögren syndrome, and systemic sclerosis (Colby, 1998; Ryu et al., 2003). In RA, bronchiolar involvement typically presents in the fifth and sixth decades of life and is more frequently seen in women (Geddes et al., 1977; Epler et al., 1979; Herzog et al., 1981). Most patients with IBO in association with RA have long-standing symptoms of arthritis, though occasionally the pulmonary involvement predates the rheumatologic disease. Immunofluorescence stains in affected lung areas of RA patients have shown IgM and IgG depositions in the alveolar septum (Begin et al., 1982; Yousem et al., 1985).

Further evidence for autoimmune reactivity in bronchiolitis obliterans was demonstrated in bronchiolitis obliterans association with paraneoplastic pemphigus, in which the deposition of IgG antibodies was observed on the surface of bronchial epithelial cells (Nousari et al., 1999). The autoantibodies are reactive against plakins, the cytoskeletal

adhesion molecules (Nousari et al., 1999). These patients also have an inflammatory infiltrate in the bronchiole submucosa consisting of lymphocytes, neutrophils, and plasma cells. Nousari et al. (1999) report that 30% of patients with paraneoplastic pemphigus develop respiratory failure with features of bronchiolitis obliterans.

Indirect evidence for autoimmune mechanisms in IBO comes from the bronchiolitis obliterans syndrome associated with both lung and allogeneic stem cell transplants. These cases, which can be viewed as in-vivo human models of bronchiolar disease, have been more extensively studied than IBO or rheumatic disease-associated form bronchiolitis obliterans. The bronchiolitis obliterans syndrome in association with lung transplantation is the main cause of morbidity and mortality (Arcasoy and Kotloff, 1999). It is a form of chronic lung rejection that is mediated by B-cell and T-cell activation against mismatched HLA class I and II antigens (Kelly and Hertz, 1997). Bronchiolitis obliterans associated with allogeneic bone marrow transplants is also thought to represent graft-versus-host disease. The disease progresses from inflammatory changes around the bronchioles to fibrosis and scarring (Ratanatharathorn et al., 2001). It is interesting to note that in bronchiolitis obliterans syndrome associated with lung transplant or with bone marrow transplant, the bronchioles are the more frequent site for chronic rejection, not the lung parenchyma.

Idiopathic Pulmonary Fibrosis

Although the etiology of IPF remains unknown, it has been hypothesized for 30 years that autoimmune mechanisms to external stimuli are the driving force for repeated injury and inflammation that ultimately leads to fibrosis (Crystal et al., 1976; 1981; 1984). Although no clear infectious or environmental agent has been found as the etiology of IPF (Gross and Hunninghake, 2001), there are extensive data regarding autoimmune cellular and humoral processes ongoing in this disorder.

Initial studies on IPF relevant to autoimmunity focused on identifying autoantibodies that target the pulmonary parenchyma. Turner-Warwick demonstrated that 40% of patients with IPF had circulating, nonspecific autoantibodies such as antinuclear antibodies (ANA) and rheumatoid factor (Turner-Warwick and Doniach, 1965). Increases in circulating ANA and rheumatoid factor were observed in 30% of our patients (Crystal et al., 1976). Similar studies that evaluated the presence of autoantibodies to nuclear antigens, DNA topoisomerase, and cytokeratin detected the antibodies in some patients (Table 67.1). More recent studies have focused on antibodies that are specific to lung proteins, although these studies have also shown variable expression of autoantibodies (Robinson et al., 2001; Wallace et al., 1994). These results raise the possibility that autoantibodies are a secondary consequence of the ongoing immune activation in the setting of inflammation and tissue damage, rather than the causative mechanism.

Similarly, investigations have evaluated the role that immune complex deposition plays in the pathogenesis of inflammation and fibrosis in IPF. Early studies observed elevated levels of circulating immune complexes in certain subsets of patients with IPF, however, these were performed prior to the current classification system and included patients with other forms of interstitial lung disease (Dreisin et al., 1978; Haslam et al., 1979). Hunninghake et al. (1981) demonstrated a correlation between the level of immune complexes in bronchoalveolar lavage fluid and the levels of neutrophil chemotactic factor released by alveolar macrophages in patients with IPF. Other studies have also seen elevated levels of immune complex in lavage fluid, but the levels are variable and do not correlate with disease activity.

The lack of clear evidence for a humoral mechanism as the primary cause for IPF shifted the focus towards cellular immunity. One popular theory is that an unknown stimulus triggers a dysregulated activation of cell-mediated immune response that results in the fibrotic process analogous to that observed in abnormal wound healing. This theory rests on the belief that a normal cell-mediated defense to a pulmonary insult would be a Th1 response, as seen in most infections and in hypersensitivity pneumonitis (Wallace et al., 1995; Lukacs et al., 2001; Kunkel, 2004). The Th1 response is characterized by the release of interferon-γ and the activation of neutrophils and macrophages for efficient clearing of the antigen, as well as suppressing fibroblast activation and collagen deposition. Cytokine profiles in IPF are closer to the Th2 response, which is typified by elevated levels of the interleukins IL-4, IL-5, and IL-13, resulting in fibroblast activation (Hancock et al., 1998; Wallace and Howie, 1999). Early evidence for this T-cell-mediated mechanism in IPF was demonstrated by Kravis et al. (1976), who discovered that circulating lymphocytes from patients with IPF would release migration inhibitor factor after exposure to collagen and would lyse collagen-coated sheep red blood cells.

One experimental model used to demonstrate a cell-mediated autoimmune mechanism is the adoptive transfer, hapten immune pulmonary interstitial fibrosis model (Stein-Streilein et al., 1987). In this model, donor mice are sensitized by a hapten, and then lymph nodes and spleen are transferred to recipient mice. When the recipient mice are then challenged with intratracheal administration of the hapten, they develop pulmonary fibrosis in 7–14 days. Interestingly, when the adoptive transfer with Th1 cells was used, an alveolitis developed, but not fibrosis, thus supporting the Th2 theory regarding the pathogenesis of the fibrotic component of IPF (Irifune et al., 2003).

In pulmonary fibrosis associated with the multisystem autoimmune disease, several serologic and genetic markers

TABLE 67.1 Autoantibodies associated with idiopathic pulmonary fibrosis*

Antigens	Number of patients	Result	Reference
Fresh unfixed lung tissue, rheumatoid factor, nuclear, thyroglobulin, gastric parietal cells	48 IPF	Sensitivity: Positive titers for: RF 49%, ANA 28%, rat liver homogenate 19%, gastric cells 0%; no autoantibodies specific to lung were detected by immunofluorescence	Turner-Warwick and Doniach, 1965
Nuclear: ANA, dsDNA, ssDNA, RF	53 IPF 33 SLE 50 controls	Sensitivity: IPF patients had 42% ANA (titer > 1:10), 25% anti-dsDNA and 100% anti-ssDNA titers that were two standard deviations above the normal range, levels did not correlate with disease activity	Holgate et al., 1983
Nuclear: ANA, nRNP, dsDNA, Sm, SS-A and SS-B	68 IPF 54 PF-AID 47 controls	Sensitivity: ANA present in 21% of IPF and 46% of PF-AID. Anti-nRNP present in 15% of IPF and PF-AID. Other antibodies were not significantly different from control group Specificity: ANA 94%, anti-nRNP 98%	Chapman et al., 1984
Histidyl-tRNA synthetase (Jo-1 antigen)	62 IPF 19 PF-AID (myositis) 53 myositis alone	Sensitivity: Antibody present in 68% of PF-AID (myositis) patients compared to 3% IPF alone and 7.5% myositis alone Specificity: >99% normal and 98% autoimmune controls	Bernstein et al., 1984
Topoisomerase II	41 IPF	Sensitivity: 44% positive. Remained elevated in 17 of 19 follow-up patients. Did not correlate with disease activity	Meliconi et al., 1993
Collagen type I, II, III, IV	16 IPF 29 controls	Sensitivity: 75% of IPF patients had antibody titers to at least one type of collagen > 1:16. Specificity: 83% using cut-off titer 1:16. Negative correlation between antibody level and duration of disease.	Nakos et al., 1993
Lung proteins derived from IPF, sarcoid, and normal lung	17 IPF 17 controls	Sensitivity: 71% of IPF patients had IgG that reacted to lung proteins in the 70–90 kDa range by Western blot; IgG reacted to alveolar lining cells Specificity: 82%	Wallace et al., 1994
Cytokeratin 19	26 IPF 11 PF-AID 52 controls	Significantly higher mean serum levels in IPF compared with control. No cut-off value determined by ELISA because of overlap between groups	Fujita et al., 1999
Expressed cDNA library from lung cancer cell line	11 IPF	Serum from index patient used to probe expressed cDNA library. Antigens recognized were unique to index patient (including anti-alanyl tRNA synthetase), but not shared with sera from other 10 patients	Robinson et al., 2001
Endothelial cell protein extract	45 PF-AID (scleroderma) 16 controls	Sensitivity: 93% PF-AID had positive staining using an indirect immunofluorescent assay against rodent lung tissue Specificity: 88%	Wusirika et al., 2003

*Summary of autoantibody studies in patients with idiopathic pulmonary fibrosis.

ANA, anti-nuclear antibodies; ELISA, enzyme-linked immunosorbent assay; IPF, idiopathic pulmonary fibrosis; nRNP, nuclear ribonucleoprotein; PF-AID, pulmonary fibrosis associated with autoimmune disease; RF, rheumatoid factor; SS-A and SS-B, Sjögren syndrome A and B antigens; SLE, systemic lupus erythematosus; Sm, Smith antigen.

are linked to the development of lung disease. For instance, patients with RA who have high levels of rheumatoid factor and prominent rheumatoid nodules are at increased risk for developing pulmonary fibrosis (Hyland et al., 1983). In systemic sclerosis, the presence of anti-topoisomerase antibodies and diffuse cutaneous involvement is associated with pulmonary fibrosis (Fanning et al., 1998).

Perhaps the best evidence for associating autoantibodies to pulmonary fibrosis is found in patients with polymyositis and dermatomyositis, in which antibodies against the aminoacyl-tRNA synthetases have been shown to be highly correlated with pulmonary fibrosis (Bernstein et al., 1984; Targoff, 1993). At least five known forms of the autoantibodies have been identified, which include anti-alanyl-tRNA synthetase (PL), anti-histidyl-tRNA synthetase (Jo-1), anti-isoleucyl-tRNA synthetase (OJ), anti-glycyl-tRNA synthetase, and anti-threonyl-tRNA synthetase. The strongest correlation appears to be with anti-Jo-1 antibodies, which have been reported to have a frequency in interstitial lung disease of between 50% (Hochberg et al., 1984) and 100% (Yoshida et al., 1983). Patients with polymyositis or dermatomyositis can frequently present with the "antisynthetase syndrome" characterized by pulmonary fibrosis in 50–75% of patients, arthritis, Raynaud phenomenon, and fevers. Occasionally, the antisynthetase syndrome can occur in the absence of clinical myositis (Marguerie et al., 1990). There are also several case series of patients with isolated pulmonary fibrosis occurring in association with aminoacyl-tRNA synthetase antibodies (Friedman et al., 1996; Sauty et al., 1997). These patients have been shown to have a $CD8^+$-lymphocyte predominant bronchoalveolar lavage and nonspecific interstitial pneumonitis pattern on lung biopsy. Most of the patients described in these case series have been more responsive to therapy with cyclosporine and azathioprine than patients with IPF.

GENETIC FEATURES

Idiopathic Bronchiolitis Obliterans

Given the rarity of IBO, there are no studies that demonstrate a genetic predisposition to this condition. The same is true for the autoimmune-associated forms of bronchiolitis obliterans. There is some preliminary work to investigate genetic susceptibility to developing bronchiolitis obliterans after lung transplant, with identification of polymorphisms for genes encoding key cytokines, including tumor necrosis factor-α, transforming growth factor-β, interferon-γ and IL-6 and IL-10, in association with this disorder (Estenne et al., 2002).

Idiopathic Pulmonary Fibrosis

Up to 3% of cases of IPF occur in clusters of families, suggesting that a genetic predisposition may be responsible for susceptibility to disease (Hodgson et al., 2002). A simple pattern of Mendelian inheritance has not been observed. Familial cases appear to be inherited in an autosomal dominant pattern, although the penetrance is variable. In familial cases of IPF, children of individuals with fibrotic lung disease can have evidence of inflammatory alveolitis in bronchial lavage fluid, without having clinical evidence of disease (Bitterman et al., 1986). There have been several reports of pulmonary fibrosis occurring in separately raised monozygotic twins, underscoring the role of genetic factors (Peabody et al., 1950; Javaheri et al., 1980). Using a candidate gene approach in one large family kindred with IPF, Thomas et al. (2002) identified a mutation in the highly conserved coding region of surfactant protein C, resulting in its aberrant cellular distribution in the lung tissue of affected individuals.

In addition to the familial form of the disease, there is evidence for a genetic basis for the more common, sporadic form of the disease. A study from Finland demonstrated a clustering of sporadic cases of IPF in areas where familial cases were found, suggesting a founder's effect (Hodgson et al., 2002). One prevailing hypothesis is that susceptibility does not lie in one gene locus, but rather within multiple genes that interact through inflammatory and fibrotic mechanisms, creating a background genotype of fibrotic potential, yet still requiring an insult to create the profibrotic phenotype. This theory would explain why the familial form of the disease has an earlier age of onset (55 vs. 67 years), yet still present relatively later in life, implying that a reduced threshold for the development of fibrosis exists in familial types (Marshall et al., 2000). This theory is also supported by the fact that only a small proportion of individuals who receive drugs known to cause pulmonary fibrosis (such as bleomycin or amiodarone) actually develop the disease (Tisdale et al., 1995).

One major limitation in the search for genetic sources for susceptibility is that the sizes of the association studies are limited because of the rarity of the disease. Polymorphisms in several genes have been explored as potential causes for the inherited susceptibility to IPF, however, they utilize a traditional candidate gene approach, which is limited by our current understanding of the disease and may lead to false positive associations. These studies are typically focused on genes involved in the inflammatory pathways, such as IL-1α and tumor necrosis factor-β, but they have shown conflicting results (Pantelidis et al., 2001; Whyte et al., 2000). Xaubet et al. (2003), recently demonstrated that transforming growth factor-β polymorphisms were related to disease progression, but they found no association to susceptibility to IPF.

Given recent advances in genomics, microarray analysis, and proteomics, there is an excellent opportunity to perform genome-wide searches for linkage in patients with IPF to identify novel genes that are involved in pathogenesis and may represent therapeutic targets. Many of these genetic

approaches are simply hypothesis generators; however, this may be a necessary first step to decipher the complex interactions of multiple loci. Additionally, microarray technology can be used for "molecular fingerprinting" to identify subclassifications within the general category of IPF.

ENVIRONMENTAL INFLUENCES

Idiopathic Bronchiolitis Obliterans

Environmental factors are clearly linked to the development of bronchiolitis obliterans in general, although specific causative environmental factors for IBO are unknown. Most notably, inhalational injuries can progress into constrictive bronchiolitis. The mechanism is believed to be due to inflammation caused by the irritant in the bronchioles that progresses into irreversible fibrosis and airway obstruction. Frequently, this occurs after exposure to water-insoluble gases (such as oxides of nitrogen in the case of silo-fillers lung) and organic dusts or fumes, which are not rapidly absorbed in the mucus membranes of the upper airways (Ramirez and Dowell, 1971; Fleming et al., 1979). Nitrous fumes are a significant industrial hazard and can be found in agriculture, fire fighting, and chemical industries. The timing of the injury can be delayed from the exposure because the gases are slowly hydrolyzed into acids that act as powerful oxidants, which eventually penetrate the small airways and cause severe tissue injury. Clinical symptoms may present as acute, subacute, or chronic. The chronic form usually presents as a new clinical illness, weeks to months after recovery from the initial acute illness (King, 2003a).

There have been many case reports of industrial exposures followed by the development of bronchiolitis obliterans in people working in unrelated industries, such as battery workers, food flavoring workers, and nylon flock workers (Konichezky et al., 1993; Eschenbacher et al., 1999; Kreiss et al., 2002). It is difficult to prove causation when only a few workers are affected. Kreiss et al. (2002) reported a high incidence of bronchiolitis obliterans in 7% of workers (8 out of 117) at a microwave popcorn plant, which was attributed to inhalation of the volatile agent, diacetyl in the butter flavoring. This observation was supported by toxicity studies of diacetyl inhalation in rats (Hubbs et al., 2002). Bronchiolitis obliterans has also been described in a truck driver who inhaled fly ash as well as in a young man with smoke inhalation from a fire (Boswell and McCunney, 1995; Tasaka et al., 1995). Both cases recovered from the acute illness only to have their symptoms recur and progress weeks to months later.

Bronchiolitis obliterans can also occur as a sequela from prior pulmonary infections, most commonly seen with adenovirus, but also associated with other viruses (RSV, influenza) and mycoplasma (Penn and Liu, 1993; Wright et al., 1992; Chan et al., 1997). Swyer–James syndrome is one variation of bronchiolitis obliterans that occurs in response to infection in children (Swyer and James, 1953). The affected lung fails to develop properly, with scarring of the bronchioles resulting in air-trapping and decreased blood flow to the affected lung. Chest radiograph is notable for a unilateral hyperlucent lung with normal or reduced volume during inspiration and air-trapping during expiration (Muller and Miller, 1995).

Some medications have been implicated as etiologic agents in the development of bronchiolitis obliterans. Both gold and penicillamine have been suspected; however, it is difficult to determine if the bronchiolitis obliterans results from the medication or the underlying disease (Geddes et al., 1977).

Idiopathic Pulmonary Fibrosis

Several environmental factors have been investigated for an etiologic role in IPF. The premise of an environmental stimulus that leads to repeated lung injury followed by abnormal wound healing fits nicely into one theory of the pathogenesis of IPF (see below). The search for these stimuli is challenging because the offending agent may be different between individuals based on individual differences in genetic susceptibility. Nevertheless, epidemiologic studies have identified links between occupational exposures, medications, and infectious agents and IPF.

Four separate case-controlled studies have looked at the association between occupational exposure and IPF. Although these studies are limited because of recall bias, there were some consistent observations. Metal dust exposure was significantly associated with IPF in every study and in a dose-response relationship (Hubbard et al., 1996; Iwai et al., 1994; Baumgartner et al., 1997; 2000). Two studies showed a significant association with livestock exposure, farming, and agricultural exposure (Baumgartner et al., 2000; Iwai et al., 1994). Paraquat, a herbicide used in agriculture, has been found to cause fatal pulmonary fibrosis in humans and experimental animals after oral, inhalational, or cutaneous exposure (Schoenberger et al., 1984). Anecdotal case reports have linked cases of IPF with occupations that result in toxic dust or fume exposure such as diamond polishing, dairy work, welding, gold extraction, and dental work (Baumgartner et al., 1997).

Pulmonary fibrosis is a well-known side effect of several medications, most notably bleomycin, amiodarone, methotrexate, and nitrofurantoin (Holmberg and Boman, 1981; Schoenberger and Crystal, 1983; Martin and Rosenow, 1988; Israel-Biet et al., 1991; Sleijfer, 2001). It has also been found as a rare complication of more commonly used medications such as beta-blockers, antidepressants, anticonvulsants, and nonsteroidal anti-inflammatory

drugs. However, these associations have been difficult to prove given the ubiquity of these medications and the relative rarity of IPF (Coultas et al., 1994).

The effect of smoking on the natural history of IPF is controversial, but of four case–control studies examining the association of smoking with pulmonary fibrosis (Iwai et al., 1994; Hubbard et al., 1996; Baumgartner et al., 1997; 2000), three show a significant link between smoking and the development of IPF. Some studies have shown improved survival in smokers with IPF compared with nonsmokers (Cherniack et al., 1995; King, et al., 2001). However, this observation may be due to lead time bias in the smoking group, and simply reflect a greater severity of disease in the nonsmoking groups.

ANIMAL MODELS

Animal models of the classical human Mendelian genetic diseases are relatively easy to produce because the genotype typically involves one affected gene, and knockout models of that gene, in mice for instance, can usually approximate the phenotype. Generating an animal model of a complex polygenic disease poses far more challenges because it involves multiple genes each exerting a relatively small effect.

Idiopathic Bronchiolitis Obliterans

Most of the animal models for bronchiolitis obliterans are designed to mimic the transplant-associated form of the disease. One model frequently used is the heterotopic murine tracheal transplant, whereby grafts of trachea and main bronchi are placed subcutaneously into allogeneic mismatched recipients (Hertz et al., 1993). By 21 days, grafts demonstrate fibroproliferation in the airway lumen, a characteristic for the human chronic rejection process. Subsequent studies on this model have shown that this is mediated by cellular and humoral immunity (Kelly and Hertz, 1997). Although this model can successfully mimic the phenotypic changes of IBO, it has limited applicability to understanding IBO because the entire tracheobronchial graft is an immunogenic stimulus, which is not likely the case in the idiopathic or autoimmune associated forms of the disease.

Adams et al. (2000) demonstrated that by removing the airway epithelium in rat trachea isografts that were implanted into the omentum, the grafts would develop pathologic changes characteristic of obliterative airways disease. Interestingly, when the airway epithelium was reseeded, the amount of obliteration significantly decreased.

Idiopathic Pulmonary Fibrosis

There is no animal model for IPF that clearly represents human IPF. However, there are several animal models for susceptibility to developing experimental pulmonary fibrosis. The susceptibility to bleomycin-induced interstitial lung disease has been correlated with both immune related (Th2 type) and non-immune related genes in different strains of mice (Rossi et al., 1987). Transforming growth factor-β has been shown to induce pulmonary fibrotic changes when overexpressed via intratracheal administration of an adenoviral vector (Liu et al., 2001). Likewise, when mice are treated with an adenoviral vector expressing SMAD-7, an inhibitory regulator of TGF-β production, they appear to be protected against bleomycin-induced lung injury (Nakao et al., 1999).

Many different transgenic and knockout mice have also been developed to investigate the molecular pathways that lead to pulmonary fibrosis. One example is mice that overexpress TNF-α will develop pulmonary fibrosis and have some of the features of human IPF (Miyazaki et al., 1995). Knockout mice with targeted deletions of genes necessary for fibrosis (e.g., adhesion molecules, ICAM-1 and L-selectin, which facilitate the accumulation of leukocytes) show significantly decreased fibrosis in response to bleomycin (Hamaguchi et al., 2002).

Many of these models are focused on only a select number of candidate genes, which may or may not have relevance to human disease. While these studies have been useful to identify genes that may play a role in susceptibility, the actual human form of the disease is likely far more complex and polygenic, which may prohibit creating a comprehensive animal model that reflects the underlying pathogenesis.

PATHOGENIC MECHANISMS

Idiopathic Bronchiolitis Obliterans

The pathogenesis underlying bronchiolitis obliterans varies according to the clinical condition with which it is associated. Differences in the initial insulting agent, location of the insult, and length of exposure can account for the differences in pathology and severity. The general sequence of events, however, is believed to be similar in order to result in a histopathologic pattern of constrictive bronchiolitis.

Airway epithelial injury followed by an inflammatory cellular infiltrate, predominantly neutrophils, is believed to be the initiating event (King, 2003a). The subsequent release of inflammatory cytokines results in bronchiolar inflammation. If the offending agent is removed and the inflammation resolves, there may be clinical recovery and resolution. Conversely, repetitive stimuli followed by abnormal repair processes and fibrosis are what likely leads to bronchiole luminal fibrosis and the typical findings of constrictive bronchiolitis. This theory of fibrosis is derived from studies of bronchiolitis obliterans in the lung transplant population,

where the disease represents a form of chronic rejection. Transplant patients who develop bronchiolitis obliterans have been shown to have higher levels of neutrophils and IL-8 in bronchial lavage fluid (DiGiovine et al., 1996).

Idiopathic Pulmonary Fibrosis

The original theory for the pathogenesis of IPF rested on the premise that inflammation of the alveoli was followed by fibrosis (Crystal et al., 1976; 1981; 1984; Hunninghake et al., 1979). There are many observations that support this hypothesis. Early studies that used bronchial lavage demonstrated increased numbers of alveolar macrophages, neutrophils, eosinophils, and lymphocytes (Crystal et al., 1976; Reynolds et al., 1977; Weinberger et al., 1978). Gallium scans, which are nonspecific markers of inflammation with macrophages and neutrophils, are positive in about 70% of all patients with IPF (Crystal et al., 1976; Line et al., 1978). Biopsies that are performed in early stages of the disease reveal large amounts of inflammation and alveolar wall derangement compared with biopsies taken in the later stages of disease where fibrosis predominates (Carrington et al., 1978). Alveolar macrophages are believed to play a central role in the inflammatory process through the release of cytokines that affect other cells. Alveolar macrophages from patients with IPF have been shown to secrete neutrophil chemotactic factor, which does not occur in normal non-activated alveolar macrophages (Hunninghake et al., 1980; 1981). Animal models have shown the presence of inflammation preceding fibrosis and the suppression of the inflammatory response attenuates the progression to fibrosis (Snider, 1986).

More recently, there has been a focus on the fibrotic aspect of the disease as also playing an important role. These theories about the pathogenesis of IPF involve the idea of recurrent, ongoing stimulus and injury to the lung, with abnormal wound healing. The abnormal wound healing is believed to result from a complex interplay of the genetic background of the individual, the predominant inflammatory phenotype (Th1 or Th2), and the environmental triggers. The stimulus that acts as the driving force remains a mystery, as does the mechanism that promotes a pathologic fibrotic response instead of the normal reparative response.

The lung parenchyma regulates its immune and fibrotic processes through cytokine signaling between the cells of the interstitium, including epithelial cells, endothelial cells, fibroblasts, and macrophages, and it is likely that these cells contribute to the pathogenesis of IPF (Martinet et al., 1987; Standiford et al., 1991; Selman et al., 2001). Although the exact sequence of chemokine signaling that leads to the progression of fibrosis is not understood, it has been shown that cytokines are responsible for the cell-to-cell communication and fibroblast activation, proliferation, and collagen production.

One theory is that an imbalance in inflammatory phenotype, shifted towards a Th2 response, is responsible for the fibrotic phenotype. Support for this theory comes from the observation that IL-4 and IL-13, major Th2-type cytokines, have been shown to be major stimuli for fibroblast-derived extracellular matrix deposition (Furuie et al., 1997; Hancock et al., 1998). Interferon-γ, one of the major Th1-type cytokines, has been shown to suppress the production of collagen and fibronectin by fibroblasts (Goldring et al., 1986). The increased presence of eosinophils, which have been associated with Th2 cytokine expression in asthma and parasitic infections, in association with fibrotic changes in IPF is consistent with the concepts of the Th2 paradigm of abnormal healing. Davis et al. (1984) demonstrated that eosinophils represented greater than 5% of the cells in the lavage fluid of 20% of patients with IPF compared with less than 1% of the normal controls. These eosinophils were shown to have collagenase activity and have the capacity to injure lung parenchymal cells.

The pathogenetic mechanisms that lead to the development of pulmonary fibrosis associated with rheumatic diseases have been most extensively studied in systemic sclerosis. The most common theory, which may or may not be applicable to the idiopathic form, is that an initial environmental injury triggers an ongoing, amplified immune response in the lung. TNF-α and TGF-β have both been shown to be upregulated early in the course of the disease (Bolster et al., 1997; Corrin et al., 1994) and there is a cytokine shift from Th1 to Th2 in helper T cells (Bolster et al., 1997). Bronchoalveolar lavage fluid from patients with scleroderma has increased levels of several inflammatory cytokines such as IL-8, TNF-α, and macrophage inflammatory protein (Southcott et al., 1995). The lavage is also believed to have prognostic value in patients with systemic sclerosis. Scleroderma patients with a neutrophil and eosinophil predominant bronchoalveolar lavage have been associated with more extensive pulmonary fibrosis seen on CT and more rapid clinical deterioration (Rossi et al., 1987; Silver et al., 1990; Behr et al., 1996; Friedman et al., 1996; Witt et al., 1999).

The stimulus that leads to the inflammatory response in the lungs of patients with scleroderma is also unknown, but one hypothesis suggests that aspiration of refluxed esophageal and gastric contents may be the cause. The premise behind this theory arises from the fact that many patients with systemic sclerosis have both esophageal dysmotility and pulmonary fibrosis. This association was initially observed in a relatively small cohort of 12 patients (Johnson et al., 1989a). Subsequent studies have found conflicting results when trying to correlate pulmonary function parameters meant to be a surrogate marker of pulmonary fibrosis (such as total lung capacity and diffusion capacity) with esophageal manometry (Troshinsky et al., 1994; Lock et al., 1998). These studies are limited by confounding ele-

ments because both esophageal dysmotility and pulmonary fibrosis may be independent markers of systemic sclerosis disease severity, without having a causal link.

TREATMENT AND OUTCOME

Idiopathic Bronchiolitis Obliterans

The prognosis is poor for patients with idiopathic or rheumatic disease-associated bronchiolitis obliterans, and there are no proven effective medical therapies for these conditions. These patients tend to not respond to corticosteroids, though a 3-month trial of high-dose prednisone (1 mg/kg) is often used. Lung transplantation is sometimes considered for individuals with bronchiolitis obliterans who continue to decline in pulmonary function and are refractory to medical therapy.

The one exception to this rule is in bronchiolitis obliterans secondary to inhalational injury, where corticosteroids are useful in both the acute and chronic phase of the disease (Milne, 1969; Horvath et al., 1978). Steroids should be started as soon as respiratory dysfunction occurs following a toxic inhalation, and be continued for at least 8 weeks to prevent recurrence (Tse and Bockman, 1970).

Given the dramatic effect that macrolides have in diffuse panbronchiolitis—a disorder related to bronchiolitis obliterans characterized by chronic sinobronchial disease and interstitial inflammation of the respiratory bronchioles—some investigators have recommended a trial of macrolides in the treatment of IBO (Ryu et al., 2003). Diffuse panbronchiolitis had a 26% 5-year survival rate in 1984 before the use of macrolides. Since the introduction of macrolides as the mainstay therapy for this disease, it now has a 10-year survival rate of 94% (Kudoh, 1998). Long-term efficacy and safety was demonstrated in 10 patients with diffuse panbronchiolitis who were treated with clarithromycin 200 mg/day for 4 years (Kadota et al., 2003). Most patients will experience a clinical and physiologic improvement within the first month of treatment. Bronchial lavage fluid from these patients has demonstrated a reduction in the number of neutrophils, neutrophil-derived elastolytic activity, IL-8, and other proinflammatory cytokines (Ichikawa et al., 1992; Sakito et al., 1996). Macrolides have not been extensively studied in bronchiolitis obliterans that is idiopathic or associated with rheumatic disease.

Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis usually portends a poor prognosis and is typically poorly responsive to therapy. Early diagnosis and treatment have traditionally been advocated so that therapy can be initiated and, hopefully, prevent irreversible fibrosis. Therefore, the typical medications used to treat IPF work through anti-inflammatory and immunosuppressant mechanisms. However, these traditional approaches have failed to show that the inflammatory or fibrotic process can be altered or reversed. As the understanding of the pathogenesis of the disease improves, newer agents are being developed based on cellular mechanisms to inhibit fibrogenesis. No therapy for IPF has been shown in a prospective, randomized double-blind, placebo controlled trial to improve survival.

Prior studies have suggested that the prognosis may be favorable for patients with pulmonary fibrosis associated with systemic sclerosis (Wells et al., 1994). However, Hubbard and Venn (2002) demonstrated with actuarial data that the mortality rates for patients with pulmonary fibrosis associated with all rheumatic disorders, predominantly RA in their study, are remarkably similar to those that are idiopathic in origin.

Corticosteroids

The most common medications used to treat IPF are corticosteroids, despite the fact that they have not been properly evaluated in a large clinical trial. Early studies showed that 10–30% of patients with IPF appear to improve or survive longer when treated with corticosteroids (Stack et al., 1972; Turner-Warwick et al., 1980). Unfortunately, these studies likely included other forms of idiopathic interstitial pneumonia, such as NSIP or COP, which tend to have a more favorable prognosis than IPF. When histopathologic criteria are used for the diagnosis of IPF, the clinical response (0–16%) and survival rates are much worse than for the other pathologic patterns (Daniil et al., 1999; Ziesche et al., 1999; Nicholson et al., 2000). Despite the lack of evidence for a beneficial role of corticosteroids, many pulmonologists use a 3- to 6-month trial of corticosteroids with close monitoring for radiographic or physiologic improvement. Patients who improve with corticosteroids or who remain stable are then kept on a maintenance dose of 10–20 mg/day prednisone (ATS, 2002). Intravenous pulse corticosteroids for 3–5 days are generally recommended for an acute exacerbation of IPF, as Keogh et al. (1983) have shown that higher doses of corticosteroids can significantly reduce the neutrophil accumulation during an active alveolitis of IPF.

Cytotoxic Agents

Other anti-inflammatory and cytotoxic agents have been investigated for a beneficial role in IPF. Cyclophosphamide, when used in combination with corticosteroids, had promising results towards a survival advantage compared with corticosteroids alone in an early randomized control trial (Johnson et al., 1989b; Baughman and Lower, 1992). Cyclophosphamide has been shown to reduce the neutrophil

component of the active alveolitis after 3 and 6 months of therapy (O'Donnell et al., 1987). However, subsequent studies have shown cyclophosphamide to have limited efficacy and a high frequency of side effects when used to treat patients with IPF who failed to respond to corticosteroid therapy (Zisman et al., 2000). Cyclophosphamide may be particularly effective in patients with pulmonary fibrosis associated with systemic sclerosis, as it was shown to improve pulmonary function testing in this population. Patients with systemic sclerosis and a neutrophil-predominant bronchoalveolar lavage seemed to have the best outcomes, although these studies were limited by either small sample size, lack of a control group, or selection bias from retrospective review (Schnabel et al., 1998; Silver et al., 1990; White et al., 2000). It is currently considered as a second-line drug for patients with progressive IPF. Side effects include leukopenia, thrombocytopenia, and hemorrhagic cystitis.

Azathioprine has been shown to have some efficacy in the treatment for IPF in small prospective case series (Winterbauer et al., 1978; Raghu et al., 1991). Azathioprine exerts cytotoxic effects on lymphocytes and also suppresses the activity of natural killer cells and antibody production. It is also considered a second-line therapy for patients who fail corticosteroids, or may be given in conjunction with corticosteroids. A trial of 3–6 months is recommended for both cyclophosphamide and azathioprine to assess for clinical response.

Antifibrotic Agents

Colchicine has been postulated to have a role in the treatment of IPF because it inhibits collagen formation in vitro and suppresses the release of alveolar macrophage-derived growth factor (Rennard et al., 1988). Colchicine has been suggested in small randomized control trials to be as effective as corticosteroids in treatment of IPF, but has not been followed up with large, controlled studies (Peters et al., 1993; Douglas et al., 1998).

Penicillamine has been noted in several anecdotal case reports to be efficacious in both IPF and rheumatic disease-associated pulmonary fibrosis (Vallance et al., 1995; Selman et al., 1998). However, since there have been no controlled studies and the medication has several significant side effects (stomatitis, nephrotoxicity), penicillamine is not used in the treatment of IPF.

Interferon γ-1b was used by Ziesche et al. (1999) in a one year study of 18 patients with IPF randomized to receive interferon γ-1b and prednisolone, compared to prednisolone alone. Interferon-γ1b has been shown to downregulate the expression of TGF-β1 and inhibit the proliferation of fibroblasts, both of which are considered to have important roles in the pathogenesis of pulmonary fibrosis (Clark et al., 1989; Narayanan et al., 1992). Ziesche's study (Ziesche et al., 1999) suggested a significant improvement in total lung capacity and partial pressure of arterial oxygen; and a decrease in the level of transcription of TGF-α1 and connective-tissue growth factor in the individuals who received interferon-γ1b.

This led to a multicenter, double-blind, placebo controlled trial of interferon-γ1b in patients with IPF who failed to respond to corticosteroids. Interferon-γ1b did not improve any of the main outcomes—progression-free survival, pulmonary function, or quality of life (Raghu et al., 2004). Additionally, there were more adverse side effects in the group that received interferon-γ1b. There was a trend towards increased survival in the group that received interferon-γ1b (16 out of 162 patients in the interferon-γ1b group died compared with 28 out of 168 patients in the placebo group, absolute reduction of 7%). However, given the relatively short follow-up of 1 year, coupled with the fact that this was not a primary end-point of the trial, there are concerns that this trend may be misleading. At this time, interferon-γ1b should not be considered as a proven therapy for IPF, unless used as part of a clinical trial.

Many other novel strategies have been investigated in the treatment of IPF, including cyclosporine, methotrexate, pirfenidone, suramin, and prostaglandin E (King, 2003b). These therapies focus on different aspects of the pathogenesis of the disease, such as antioxidants; inhibitors of cytokines, proteases, and fibroblast growth factors. Unless adequate studies are done to demonstrate efficacy, these agents should not be recommended for general use. The recent joint statement by the American Thoracic Society (ATS) and European Respiratory Society (2002) (ERS) recommended combined therapy with corticosteroids and either azathioprine or cyclophosphamide for a 3-month trial in patients who possess features consistent with a more favorable outcome.

Lung transplantation should be considered for individuals who progress despite optimized medical management. Patients should be referred to a transplant center relatively early in their course since wait times on the list can exceed 2 years due to limited donor availability. Those who receive a successful transplant can experience significant improvement in arterial oxygenation, pulmonary hypertension, and right ventricular dysfunction. Unfortunately, the 5-year survival rates for lung transplant are only about 60%, with death frequently due to graft failure, infection, or bronchiolitis obliterans (Lu and Bhorade, 2004).

CONCLUSIONS

The presumed autoimmune disorders of the lung, notably IBO and IPF, have long been recognized as inflammatory and fibrotic processes that are associated with poor prognosis, whether idiopathic in nature or in association with the

multisystem autoimmune diseases. Both diseases appear to be mediated through repetitive cell-mediated injury, directed at the bronchioles and alveoli/interstitium, respectively, followed by abnormal wound healing. Despite intense efforts, there is currently no approved therapy for either disease. Recent advances in the classification and diagnostic testing for these diseases have improved our ability to study their incidence, prognosis, and response to therapy. While much progress has been made, the search for antigenic stimuli and genetic polymorphisms that are responsible for disease pathogenesis continues. Only through a better understanding of the disease process will we be able to offer new therapies for these devastating diseases.

Acknowledgments

We thank J. Kaplan and N. Mohamed for help in preparing this manuscript. These studies were supported, in part, by M01RR00047 and the Will Rogers Memorial Fund, Los Angeles, CA. BG, is supported, in part, by the Altana/Pfizer CHEST Foundation Clinical Research Trainee Award in COPD.

References

Adams, B.F., Brazelton, T., Berry, G.J., and Morris, R.E. (2000). The role of respiratory epithelium in a rat model of obliterative airway disease. *Transplantation 69,* 661–664.

American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. (2002). This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am. J. Respir. Crit. Care Med. 165,* 277–304.

Arcasoy, S.M. and Kotloff, R.M. (1999). Lung transplantation. *N. Engl. J. Med. 340,* 1081–1091.

Baar, H.S. and Galindo, J. (1966). Bronchiolitis fibrosa obliterans. *Thorax 21,* 209–214.

Basset, F., Ferrans, V.J., Soler, P., Takemura, T., Fukuda, Y., and Crystal, R.G. (1986). Intraluminal fibrosis in interstitial lung disorders. *Am. J. Pathol. 122,* 443–461.

Baughman, R.P. and Lower, E.E. (1992). Use of intermittent, intravenous cyclophosphamide for idiopathic pulmonary fibrosis. *Chest 102,* 1090–1094.

Baumgartner, K.B., Samet, J.M., Coultas, D.B., Stidley, C.A., Hunt, W.C., Colby, T.V., and Waldron, J.A. (2000). Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating Centers. *Am. J. Epidemiol. 152,* 307–315.

Baumgartner, K.B., Samet, J.M., Stidley, C.A., Colby, T.V., and Waldron, J.A. (1997). Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med. 155,* 242–248.

Begin, R., Masse, S., Cantin, A., Menard, H.A., and Bureau, M.A. (1982). Airway disease in a subset of nonsmoking rheumatoid patients. Characterization of the disease and evidence for an autoimmune pathogenesis. *Am. J. Med. 72,* 743–750.

Behr, J., Vogelmeier, C., Beinert, T., Meurer, M., Krombach, F., Konig, G., and Fruhmann, G. (1996). Bronchoalveolar lavage for evaluation and management of scleroderma disease of the lung. *Am. J. Respir. Crit. Care Med. 154,* 400–406.

Bernstein, R.M., Morgan, S.H., Chapman, J., Bunn, C.C., Mathews, M.B., Turner-Warwick, M., and Hughes, G.R. (1984). Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. *BMJ (Clin. Res. Ed.) 289,* 151–152.

Bitterman, P.B., Rennard, S.I., Keogh, B.A., Wewers, M.D., Adelberg, S., and Crystal, R.G. (1986). Familial idiopathic pulmonary fibrosis. Evidence of lung inflammation in unaffected family members. *N. Engl. J. Med. 314,* 1343–1347.

Bolster, M.B., Ludwicka, A., Sutherland, S.E., Strange, C., and Silver, R.M. (1997). Cytokine concentrations in bronchoalveolar lavage fluid of patients with systemic sclerosis. Arthritis Rheum. 40, 743–751.

Boswell, R.T. and McCunney, R.J. (1995). Bronchiolitis obliterans from exposure to incinerator fly ash. *J. Occup. Environ. Med. 37,* 850–855.

Bouros, D., Wells, A.U., Nicholson, A.G., Colby, T.V., Polychronopoulos, V., Pantelidis, P., Haslam, P.L., Vassilakis, D.A., Black, C.M., and du Bois, R.M. (2002). Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am. J. Respir. Crit. Care Med. 165,* 1581–1586.

Burke, C.M., Theodore, J., Dawkins, K.D., Yousem, S.A., Blank, N., Billingham, M.E., Van Kessel, A., Jamieson, S.W., Oyer, P.E., and Baldwin, J.C. (1984). Post-transplant obliterative bronchiolitis and other late lung sequelae in human heart-lung transplantation. *Chest 86,* 824–829.

Carrington, C.B., Gaensler, E.A., Coutu, R.E., FitzGerald, M.X., and Gupta, R.G. (1978). Natural history and treated course of usual and desquamative interstitial pneumonia. *N. Engl. J. Med. 298,* 801–809.

Cervantes-Perez, P., Toro-Perez, A.H., and Rodriguez-Jurado, P. (1980). Pulmonary involvement in rheumatoid arthritis. *JAMA 243,* 1715–1719.

Chan, T.Y., Hansell, D.M., Rubens, M.B., du Bois, R.M., and Wells, A.U. (1997). Cryptogenic fibrosing alveolitis and the fibrosing alveolitis of systemic sclerosis: morphological differences on computed tomographic scans. *Thorax 52,* 265–270.

Chapman, J.R., Charles, P.J., Venables, P.J., Thompson, P.J., Haslam, P.L., Maini, R.N., and Turner Warwick, M.E. (1984). Definition and clinical relevance of antibodies to nuclear ribonucleoprotein and other nuclear antigens in patients with cryptogenic fibrosing alveolitis. *Am. Rev. Respir. Dis. 130,* 439–443.

Cherniack, R.M., Colby, T.V., Flint, A., Thurlbeck, W.M., Waldron, J.A., Jr., Ackerson, L., Schwarz, M.I., and King, T.E., Jr. (1995). Correlation of structure and function in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med. 151,* 1180–1188.

Clark, J.G., Dedon, T.F., Wayner, E.A., and Carter, W.G. (1989). Effects of interferon-gamma on expression of cell surface receptors for collagen and deposition of newly synthesized collagen by cultured human lung fibroblasts. *J. Clin. Invest. 83,* 1505–1511.

Colby, T.V. (1998). Bronchiolitis. Pathologic considerations. *Am. J. Clin. Pathol. 109,* 101–109.

Corrin, B., Butcher, D., McAnulty, B.J., Dubois, R.M., Black, C.M., Laurent, G.J., and Harrison, N.K. (1994). Immunohistochemical localization of transforming growth factor-beta 1 in the lungs of patients with systemic sclerosis, cryptogenic fibrosing alveolitis and other lung disorders. *Histopathology 24,* 145–150.

Coultas, D.B., Zumwalt, R.E., Black, W.C., and Sobonya, R.E. (1994). The epidemiology of interstitial lung diseases. *Am. J. Respir. Crit. Care Med. 150,* 967–972.

Crystal, R.G. (1985). Interstitial lung disease. In J.B. Wyngaarden and L.H. Smith, Jr. (eds), *17th Edition of the Cecil Textbook of Medicine* (pp. 406–419). Philadelphia: W.B. Saunders.

Crystal, R.G., Fulmer, J.D., Roberts, W.C., Moss, M.L., Line, B.R., and Reynolds, H.Y. (1976). Idiopathic pulmonary fibrosis. Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. *Ann. Intern. Med. 85,* 769–788.

Crystal, R.G., Gadek, J.E., Ferrans, V.J., Fulmer, J.D., Line, B.R., and Hunninghake, G.W. (1981). Interstitial lung disease: current concepts of pathogenesis, staging and therapy. *Am. J. Med. 70,* 542–568.

Crystal, R.G., Bitterman, P.B., Rennard, S.I., Hance, A.J., and Keogh, B.A. (1984). Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract. *N. Engl. J. Med. 310,* 235–244.

Daniil, Z.D., Gilchrist, F.C., Nicholson, A.G., Hansell, D.M., Harris, J., Colby, T.V., and du Bois, R.M. (1999). A histologic pattern of nonspecific interstitial pneumonia is associated with a better prognosis than usual interstitial pneumonia in patients with cryptogenic fibrosing alveolitis. *Am. J. Respir. Crit. Care Med. 160,* 899–905.

Davis, W.B., Fells, G.A., Sun, X.H., Gadek, J.E., Venet, A., and Crystal, R.G. (1984). Eosinophil-mediated injury to lung parenchymal cells and interstitial matrix. A possible role for eosinophils in chronic inflammatory disorders of the lower respiratory tract. *J. Clin. Invest. 74,* 269–278.

DiGiovine, B., Lynch, J.P., III, Martinez, F.J., Flint, A., Whyte, R.I., Iannettoni, M.D., Arenberg, D.A., Burdick, M.D., Glass, M.C., Wilke, C.A., Morris, S.B., Kunkel, S.L., and Strieter, R.M. (1996). Bronchoalveolar lavage neutrophilia is associated with obliterative bronchiolitis after lung transplantation: role of IL-8. *J. Immunol. 157,* 4194–4202.

Douglas, W.W., Ryu, J.H., Swensen, S.J., Offord, K.P., Schroeder, D.R., Caron, G.M., and DeRemee, R.A. (1998). Colchicine versus prednisone in the treatment of idiopathic pulmonary fibrosis. A randomized prospective study. Members of the Lung Study Group. *Am. J. Respir. Crit. Care Med. 158,* 220–225.

Douglas, W.W., Tazelaar, H.D., Hartman, T.E., Hartman, R.P., Decker, P.A., Schroeder, D.R., and Ryu, J.H. (2001). Polymyositis-dermatomyositis-associated interstitial lung disease. *Am. J. Respir. Crit. Care Med. 164,* 1182–1185.

Dreisin, R.B., Schwarz, M.I., Theofilopoulos, A.N., and Stanford, R.E. (1978). Circulating immune complexes in the idiopathic interstitial pneumonias. *N. Engl. J. Med. 298,* 353–357.

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,* 37–45.

Epler, G.R., Snider, G.L., Gaensler, E.A., Cathcart, E.S., FitzGerald, M.X., and Carrington, C.B. (1979). Bronchiolitis and bronchitis in connective tissue disease. A possible relationship to the use of penicillamine. *JAMA 242,* 528–532.

Eschenbacher, W.L., Kreiss, K., Lougheed, M.D., Pransky, G.S., Day, B., and Castellan, R.M. (1999). Nylon flock-associated interstitial lung disease. *Am. J. Respir. Crit Care Med. 159,* 2003–2008.

Estenne, M., Maurer, J.R., Boehler, A., Egan, J.J., Frost, A., Hertz, M., Mallory, G.B., Snell, G.I., and Yousem, S. (2002). Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J. Heart Lung Transplant. 21,* 297–310.

Fanning, G.C., Welsh, K.I., Bunn, C., Du, B.R., and Black, C.M. (1998). HLA associations in three mutually exclusive autoantibody subgroups in UK systemic sclerosis patients. *Br. J. Rheumatol. 37,* 201–207.

Flaherty, K.R., Travis, W.D., Colby, T.V., Toews, G.B., Kazerooni, E.A., Gross, B.H., Jain, A., Strawderman, R.L., Flint, A., Lynch, J.P., and Martinez, F.J. (2001). Histopathologic variability in usual and nonspecific interstitial pneumonias. *Am. J. Respir. Crit. Care Med. 164,* 1722–1727.

Fleming, G.M., Chester, E.H., and Montenegro, H.D. (1979). Dysfunction of small airways following pulmonary injury due to nitrogen dioxide. *Chest 75,* 720–721.

Franquet, T. and Stern, E.J. (1999). Bronchiolar inflammatory diseases: high-resolution CT findings with histologic correlation. *Eur. Radiol. 9,* 1290–1303.

Friedman, A.W., Targoff, I.N., and Arnett, F.C. (1996). Interstitial lung disease with autoantibodies against aminoacyl-tRNA synthetases in the absence of clinically apparent myositis. *Semin. Arthritis Rheum. 26,* 459–467.

Fujita, J., Dobashi, N., Ohtsuki, Y., Yamadori, I., Yoshinouchi, T., Kamei, T., Tokuda, M., Hojo, S., Okada, H., and Takahara, J. (1999). Elevation of anti-cytokeratin 19 antibody in sera of the patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with collagen vascular disorders. *Lung 177,* 311–319.

Fulmer, J.D., Roberts, W.C., von Gal, E.R., and Crystal, R.G. (1979). Morphologic-physiologic correlates of the severity of fibrosis and degree of cellularity in idiopathic pulmonary fibrosis. *J. Clin. Invest. 63,* 656–676.

Furuie, H., Yamasaki, H., Suga, M., and Ando, M. (1997). Altered accessory cell function of alveolar macrophages: a possible mechanism for induction of Th2 secretory profile in idiopathic pulmonary fibrosis. *Eur. Respir. J. 10,* 787–794.

Gabbay, E., Tarala, R., Will, R., Carroll, G., Adler, B., Cameron, D., and Lake, F.R. (1997). Interstitial lung disease in recent onset rheumatoid arthritis. *Am. J. Respir. Crit. Care Med. 156,* 528–535.

Gardiner, P. (1993). Primary Sjogren's syndrome. *Baillières Clin. Rheumatol. 7,* 59–77.

Geddes, D.M., Corrin, B., Brewerton, D.A., Davies, R.J., and Turner-Warwick, M. (1977). Progressive airway obliteration in adults and its association with rheumatoid disease. *Q. J. Med. 46,* 427–444.

Goldring, M.B., Sandell, L.J., Stephenson, M.L., and Krane, S.M. (1986). Immune interferon suppresses levels of procollagen mRNA and type II collagen synthesis in cultured human articular and costal chondrocytes. *J. Biol. Chem. 261,* 9049–9055.

Gosink, B.B., Friedman, P.J., and Liebow, A.A. (1973). Bronchiolitis obliterans. Roentgenologic-pathologic correlation. *Am. J. Roentgenol. Radium Ther. Nucl. Med. 117,* 816–832.

Gross, T.J. and Hunninghake, G.W. (2001). Idiopathic pulmonary fibrosis. *N. Engl. J. Med 345,* 517–525.

Hamaguchi, Y., Nishizawa, Y., Yasui, M., Hasegawa, M., Kaburagi, Y., Komura, K., Nagaoka, T., Saito, E., Shimada, Y., Takehara, K., Kadono, T., Steeber, D.A., Tedder, T.F., and Sato, S. (2002). Intercellular adhesion molecule-1 and L-selectin regulate bleomycin-induced lung fibrosis. *Am. J. Pathol. 161,* 1607–1618.

Hamman, L. and Rich, A.R. (1935). Fulminating diffuse interstitial fibrosis of the lungs. *Trans. Am. Clin. Climatol. Assoc. 51,* 154–163.

Hancock, A., Armstrong, L., Gama, R., and Millar, A. (1998). Production of interleukin 13 by alveolar macrophages from normal and fibrotic lung. *Am. J. Respir. Cell Mol. Biol. 18,* 60–65.

Hansell, D.M. (2001). Small airways diseases: detection and insights with computed tomography. *Eur. Respir. J. 17,* 1294–1313.

Harrison, N.K., Myers, A.R., Corrin, B., Soosay, G., Dewar, A., Black, C.M., du Bois, R.M., and Turner-Warwick, M. (1991). Structural features of interstitial lung disease in systemic sclerosis. *Am. Rev. Respir. Dis. 144,* 706–713.

Haslam, P.L., Thompson, B., Mohammed, I., Townsend, P.J., Hodson, M.E., Holborow, E.J., and Turner-Warwick, M. (1979). Circulating immune complexes in patients with cryptogenic fibrosing alveolitis. *Clin. Exp. Immunol. 37,* 381–390.

Hertz, M.I., Jessurun, J., King, M.B., Savik, S.K., and Murray, J.J. (1993). Reproduction of the obliterative bronchiolitis lesion after heterotopic transplantation of mouse airways. *Am. J. Pathol. 142,* 1945–1951.

Herzog, C.A., Miller, R.R., and Hoidal, J.R. (1981). Bronchiolitis and rheumatoid arthritis. *Am. Rev. Respir. Dis. 124,* 636–639.

Hochberg, M.C., Feldman, D., Stevens, M.B., Arnett, F.C., and Reichlin, M. (1984). Antibody to Jo-1 in polymyositis/dermatomyositis: association with interstitial pulmonary disease. *J. Rheumatol. 11,* 663–665.

Hodgson, U., Laitinen, T., and Tukiainen, P. (2002). Nationwide prevalence of sporadic and familial idiopathic pulmonary fibrosis: evidence of founder effect among multiplex families in Finland. *Thorax 57,* 338–342.

Holgate, S.T., Haslam, P., and Turner-Warwick, M. (1983). The significance of antinuclear and DNA antibodies in cryptogenic fibrosing alveolitis. *Thorax 38,* 67–70.

Holmberg, L. and Boman, G. (1981). Pulmonary reactions to nitrofurantoin. 447 cases reported to the Swedish Adverse Drug Reaction Committee 1966–1976. *Eur. J. Respir. Dis. 62,* 180–189.

Horvath, E.P., doPico, G.A., Barbee, R.A., and Dickie, H.A. (1978). Nitrogen dioxide-induced pulmonary disease: five new cases and a review of the leterature. *J. Occup. Med. 20,* 103–110.

Hubbard, R. and Venn, A. (2002). The impact of coexisting connective tissue disease on survival in patients with fibrosing alveolitis. *Rheumatology. (Oxford) 41,* 676–679.

Hubbard, R., Lewis, S., Richards, K., Johnston, I., and Britton, J. (1996). Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. *Lancet 347,* 284–289.

Hubbard, R., Johnston, I., and Britton, J. (1998). Survival in patients with cryptogenic fibrosing alveolitis: a population-based cohort study. *Chest 113,* 396–400.

Hubbs, A.F., Battelli, L.A., Goldsmith, W.T., Porter, D.W., Frazer, D., Friend, S., Schwegler-Berry, D., Mercer, R.R., Reynolds, J.S., Grote, A., Castranova, V., Kullman, G., Fedan, J.S., Dowdy, J., and Jones, W.G. (2002). Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavoring. *Toxicol. Appl. Pharmacol. 185,* 128–135.

Hunninghake, G.W., Gadek, J.E., Kawanami, O., Ferrans, V.J., and Crystal, R.G. (1979). Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am. J. Pathol. 97,* 149–206.

Hunninghake, G.W., Gadek, J.E., Fales, H.M., and Crystal, R.G. (1980). Human alveolar macrophage-derived chemotactic factor for neutrophils: stimuli and partial characterization. *J. Clin. Invest. 66,* 473–483.

Hunninghake, G.W., Gadek, J.E., Lawley, T.J., and Crystal, R.G. (1981). Mechanisms of neutrophil accumulation in the lungs of patients with idiopathic pulmonary fibrosis. *J. Clin. Invest 68,* 259–269.

Hunninghake, G.W., Zimmerman, M.B., Schwartz, D.A., King, T.E., Jr., Lynch, J., Hegele, R., Waldron, J., Colby, T., Muller, N., Lynch, D., Galvin, J., Gross, B., Hogg, J., Toews, G., Helmers, R., Cooper, J.A., Jr., Baughman, R., Strange, C., and Millard, M. (2001). Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med. 164,* 193–196.

Hyland, R.H., Gordon, D.A., Broder, I., Davies, G.M., Russell, M.L., Hutcheon, M.A., Reid, G.D., Cox, D.W., Corey, P.N., and Mintz, S. (1983). A systematic controlled study of pulmonary abnormalities in rheumatoid arthritis. *J. Rheumatol. 10,* 395–405.

Ichikawa, Y., Ninomiya, H., Koga, H., Tanaka, M., Kinoshita, M., Tokunaga, N., Yano, T., and Oizumi, K. (1992). Erythromycin reduces neutrophils and neutrophil-derived elastolytic-like activity in the lower respiratory tract of bronchiolitis patients. *Am. Rev. Respir. Dis. 146,* 196–203.

Irifune, K., Yokoyama, A., Kohno, N., Sakai, K., and Hiwada, K. (2003). T-helper 1 cells induce alveolitis but do not lead to pulmonary fibrosis in mice. Eur. Respir. J. 21, 11–18.

Israel-Biet, D., Labrune, S., and Huchon, G.J. (1991). Drug-induced lung disease: 1990 review. *Eur. Respir. J. 4,* 465–478.

Iwai, K., Mori, T., Yamada, N., Yamaguchi, M., and Hosoda, Y. (1994). Idiopathic pulmonary fibrosis. Epidemiologic approaches to occupational exposure. *Am. J. Respir. Crit. Care Med. 150,* 670–675.

Javaheri, S., Lederer, D.H., Pella, J.A., Mark, G.J., and Levine, B.W. (1980). Idiopathic pulmonary fibrosis in monozygotic twins. The importance of genetic predisposition. *Chest 78,* 591–594.

Johnson, D.A., Drane, W.E., Curran, J., Cattau, E.L., Jr., Ciarleglio, C., Khan, A., Cotelingam, J., and Benjamin, S.B. (1989a). Pulmonary disease in progressive systemic sclerosis. A complication of gastroesophageal reflux and occult aspiration? *Med. Arch. Intern. Med. 149,* 589–593.

Johnson, M.A., Kwan, S., Snell, N.J., Nunn, A.J., Darbyshire, J.H., and Turner-Warwick, M. (1989b). Randomised controlled trial comparing prednisolone alone with cyclophosphamide and low dose prednisolone in combination in cryptogenic fibrosing alveolitis. *Thorax 44,* 280–288.

Kadota, J., Mukae, H., Ishii, H., Nagata, T., Kaida, H., Tomono, K., and Kohno, S. (2003). Long-term efficacy and safety of clarithromycin treatment in patients with diffuse panbronchiolitis. *Respir. Med. 97,* 844–850.

Katzenstein, A.L. and Fiorelli, R.F. (1994). Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. *Am. J. Surg. Pathol. 18,* 136–147.

Kelly, K. and Hertz, M.I. (1997). Obliterative bronchiolitis. *Clin. Chest Med. 18,* 319–338.

Keogh, B.A. and Crystal, R.G. (1980). Clinical significance of pulmonary function tests. Pulmonary function testing in interstitial pulmonary disease. What does it tell us? *Chest 78,* 856–865.

Keogh, B.A., Bernardo, J., Hunninghake, G.W., Line, B.R., Price, D.L., and Crystal, R.G. (1983). Effect of intermittent high dose parenteral corticosteroids on the alveolitis of idiopathic pulmonary fibrosis. *Am. Rev. Respir. Dis. 127,* 18–22.

King, T.E., Jr., Tooze, J.A., Schwarz, M.I., Brown, K.R., and Cherniack, R.M. (2001). Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am. J. Respir. Crit. Care Med. 164,* 1171–1181.

King, T.E., Jr. (2003a). Bronchiolitis. In M.I. Schwarz and T.E. King, Jr. (eds), *Interstitial lung disease* (pp. 787–824). London: Decker.

King, T.E., Jr. (2003b). In M.I. Schwarz and T.E. King, Jr. (eds), *Interstitial lung disease* (pp. 701–786). London: Decker.

Konichezky, S., Schattner, A., Ezri, T., Bokenboim, P., and Geva, D. (1993). Thionyl-chloride-induced lung injury and bronchiolitis obliterans. *Chest 104,* 971–973.

Kraft, M., Mortenson, R.L., Colby, T.V., Newman, L., Waldron, J.A., Jr., and King, T.E., Jr. (1993). Cryptogenic constrictive bronchiolitis. A clinicopathologic study. *Am. Rev. Respir. Dis. Am. Rev. 148,* 1093–1101.

Kravis, T.C., Ahmed, A., Brown, T.E., Fulmer, J.D., and Crystal, R.G. (1976). Pathogenic mechanisms in pulmonary fibrosis: collagen-induced migration inhibition factor production and cytotoxicity mediated by lymphocytes. *J. Clin. Invest. 58,* 1223–1232.

Kreiss, K., Gomaa, A., Kullman, G., Fedan, K., Simoes, E.J., and Enright, P.L. (2002). Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N. Engl. J. Med. 347,* 330–338.

Kudoh, S. (1998). Erythromycin treatment in diffuse panbronchiolitis. *Curr. Opin. Pulm. Med. 4,* 116–121.

Kunkel, S.L. (2004). Cytokine phenotypes and the progression of chronic pulmonary fibrosis. In C. (ed.), *Lenfant Idiopathic pulmonary fibrosis* (pp. 303–320). New York: Maecel Decker.

Lange, W. (1901). Uber eine eigentumliche Erkrankung der kleinen Bronchien obliterans. *Dtsch. Arch. Klin. Med. 70,* 342–364.

Liebow, A.A. and Carrington, D. (1969). The interstitial pneumonias. In M. Simon, E. Potchen, and M. LeMay (eds), *Frontiers of pulmonary radiology* (pp. 102–141). New York: Grune & Stratton.

Line, B.R., Fulmer, J.D., Reynolds, H.Y., Roberts, W.C., Jones, A.E., Harris, E.K., and Crystal, R.G. (1978). Gallium-67 citrate scanning in the staging of idiopathic pulmonary fibrosis: Correlation and physiologic and morphologic features and bronchoalveolar lavage. *Am. Rev. Respir. Dis. 118,* 355–365.

Liu, J.Y., Sime, P.J., Wu, T., Warshamana, G.S., Pociask, D., Tsai, S.Y., and Brody, A.R. (2001). Transforming growth factor-beta(1) overexpression in tumor necrosis factor-alpha receptor knockout mice induces fibroproliferative lung disease. *Am. J. Respir. Cell Mol. Biol. 25,* 3–7.

Lock, G., Pfeifer, M., Straub, R.H., Zeuner, M., Lang, B., Scholmerich, J., and Holstege, A. (1998). Association of esophageal dysfunction and pulmonary function impairment in systemic sclerosis. *Am. J. Gastroenterol. 93,* 341–345.

Lu, B.S. and Bhorade, S.M. (2004). Lung transplantation for interstitial lung disease. *Clin. Chest Med. 25,* 773–782.

Lukacs, N.W., Hogaboam, C., Chensue, S.W., Blease, K., and Kunkel, S.L. (2001). Type 1/type 2 cytokine paradigm and the progression of pulmonary fibrosis. *Chest 120,* 5S–8S.

Mapel, D.W., Hunt, W.C., Utton, R., Baumgartner, K.B., Samet, J.M., and Coultas, D.B. (1998). Idiopathic pulmonary fibrosis: survival in population based and hospital based cohorts. *Thorax 53,* 469–476.

Marguerie, C., Bunn, C.C., Beynon, H.L., Bernstein, R.M., Hughes, J.M., So, A.K., and Walport, M.J. (1990). Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. *Q. J. Med. 77,* 1019–1038.

Markopoulo, K.D., Cool, C.D., Elliot, T.L., Lync, D.A., Newell, J.D., Jr., Hale, V.A., Brown, K.K., Schwarz, M.I., and Tuder, R.M. (2002). Obliterative bronchiolitis: varying presentations and clinicopathological correlation. *Eur. Respir. J. 19,* 20–30.

Marshall, R.P., Puddicombe, A., Cookson, W.O., and Laurent, G.J. (2000). Adult familial cryptogenic fibrosing alveolitis in the United Kingdom. *Thorax 55,* 143–146.

Martin, W.J. and Rosenow, E.C., III (1988). Amiodarone pulmonary toxicity. Recognition and pathogenesis (Part I). *Chest 93,* 1067–1075.

Martinet, Y., Rom, W.N., Grotendorst, G.R., Martin, G.R., and Crystal, R.G. (1987). Exaggerated spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med 317,* 202–209.

McLees, B.D., Adair, N., Moss, J., Fulmer, J.D., Keogh, B., and Crystal, R.G. (1979). Patterns of pulmonary hemodynamic dysfunction: similarities between idiopathic pulmonary fibrosis and panacinar emphysema. *Am. Rev. Respir. Dis. 119,* p. 380.

Meliconi, R., Negri, C., Borzi, R.M., Facchini, A., Sturani, C., Fasano, L., Fabbri, M., and Staldi Ricotti, G.C. (1993). Antibodies to topoisomerase II in idiopathic pulmonary fibrosis. *Clin. Rheumatol. 12,* 311–315.

Milne, J.E. (1969). Nitrogen dioxide inhalation and bronchiolitis obliterans. A review of the literature and report of a case. *J. Occup. Med. 11,* 538–547.

Minai, O.A., Dweik, R.A., and Arroliga, A.C. (1998). Manifestations of scleroderma pulmonary disease. *Clin. Chest Med. 19,* 713–732.

Miyazaki, Y., Araki, K., Vesin, C., Garcia, I., Kapanci, Y., Whitsett, J.A., Piguet, P.F., and Vassalli, P. (1995). Expression of a tumor necrosis factor-alpha transgene in murine lung causes lymphocytic and fibrosing alveolitis. A mouse model of progressive pulmonary fibrosis. *J. Clin. Invest. 96,* 250–259.

Muller, N.L. and Miller, R.R. (1995). Diseases of the bronchioles: CT and histopathologic findings. *Radiology 196,* 3–12.

Myers, J.L. and Colby, T.V. (1993). Pathologic manifestations of bronchiolitis, constrictive bronchiolitis, cryptogenic organizing pneumonia, and diffuse panbronchiolitis. *Clin. Chest Med. 14,* 611–622.

Nakao, A., Fujii, M., Matsumura, R., Kumano, K., Saito, Y., Miyazono, K., and Iwamoto, I. (1999). Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. *J. Clin. Invest. 104,* 5–11.

Nakos, G., Adams, A., and Andriopoulos, N. (1993). Antibodies to collagen in patients with idiopathic pulmonary fibrosis. *Chest 103,* 1051–1058.

Narayanan, A.S., Whithey, J., Souza, A., and Raghu, G. (1992). Effect of gamma-interferon on collagen synthesis by normal and fibrotic human lung fibroblasts. *Chest 101,* 1326–1331.

Nicholson, A.G., Colby, T.V., du Bois, R.M., Hansell, D.M., and Wells, A.U. (2000). The prognostic significance of the histologic pattern of interstitial pneumonia in patients presenting with the clinical entity of cryptogenic fibrosing alveolitis. *Am. J. Respir. Crit. Care Med. 162,* 2213–2217.

Nousari, H.C., Deterding, R., Wojtczack, H., Aho, S., Uitto, J., Hashimoto, T., and Anhalt, G.J. (1999). The mechanism of respiratory failure in paraneoplastic pemphigus. *N. Engl. J. Med. 340,* 1406–1410.

O'Donnell, K., Keogh, B., Cantin, A., and Crystal, R.G. (1987). Pharmacologic suppression of the neutrophil component of the alveolitis in idiopathic pulmonary fibrosis. *Am. Rev. Respir. Dis. 136,* 288–292.

Osler, W. (1892). *The Principles and Practice of Medicine*. New York, D. Appleton & Company p. 1079.

Pantelidis, P., Fanning, G.C., Wells, A.U., Welsh, K.I., and du Bois, R.M. (2001). Analysis of tumor necrosis factor-alpha, lymphotoxin-alpha, tumor necrosis factor receptor II, and interleukin-6 polymorphisms in patients with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med. 163,* 1432–1436.

Paz, H.L., Crilley, P., Topolsky, D.L., Coll, W.X., Patchefsky, A., and Brodsky, I. (1993). Bronchiolitis obliterans after bone marrow transplantation: the effect of preconditioning. *Respiration 60,* 109–114.

Peabody, J.W., Peabody, J.W., Jr., Hayes, E.W., and Hayes, E.W., Jr. (1950). Idiopathic pulmonary fibrosis; its occurrence in identical twin sisters. *Dis. Chest 18,* 330–344.

Penn, C.C. and Liu, C. (1993). Bronchiolitis following infection in adults and children. *Clin. Chest Med. 14,* 645–654.

Peters, S.G., McDougall, J.C., Douglas, W.W., Coles, D.T., and DeRemee, R.A. (1993). Colchicine in the treatment of pulmonary fibrosis. *Chest 103,* 101–104.

Raghu, G., Depaso, W.J., Cain, K., Hammar, S.P., Wetzel, C.E., Dreis, D.F., Hutchinson, J., Pardee, N.E., and Winterbauer, R.H. (1991). Azathioprine combined with prednisone in the treatment of idiopathic pulmonary fibrosis: a prospective double-blind, randomized, placebo-controlled clinical trial. *Am. Rev. Respir. Dis. 144,* 291–296.

Raghu, G., Brown, K.K., Bradford, W.Z., Starko, K., Noble, P.W., Schwartz, D.A., and King, T.E., Jr. (2004). A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med. 350,* 125–133.

Ramirez, J. and Dowell, A.R. (1971). Silo-filler's disease: nitrogen dioxide-induced lung injury. Long-term follow-up and review of the literature. *Ann. Intern. Med. 74,* 569–576.

Ratanatharathorn, V., Ayash, L., Lazarus, H.M., Fu, J., and Uberti, J.P. (2001). Chronic graft-versus-host disease: clinical manifestation and therapy. *Bone Marrow Transplant. 28,* 121–129.

Reichenspurner, H., Girgis, R.E., Robbins, R.C., Yun, K.L., Nitschke, M., Berry, G.J., Morris, R.E., Theodore, J., and Reitz, B.A. (1996). Stanford experience with obliterative bronchiolitis after lung and heart-lung transplantation. *Ann. Thorac. Surg. 62,* 1467–1472.

Remy-Jardin, M., Remy, J., Cortet, B., Mauri, F., and Delcambre, B. (1994). Lung changes in rheumatoid arthritis: CT findings. *Radiology 193,* 375–382.

Rennard, S.I., Bitterman, P.B., Ozaki, T., Rom, W.N., and Crystal, R.G. (1988). Colchicine suppresses the release of fibroblast growth factors from alveolar macrophages in vitro. The basis of a possible therapeutic approach to the fibrotic disorders. *Am. Rev. Respir. Dis. 137,* 181–185.

Reynolds, H.Y., Fulmer, J.D., Kazmierowski, J.A., Roberts, W.C., Frank, M.M., and Crystal, R.G. (1977). Analysis of cellular and protein content of broncho-alveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J. Clin. Invest 59,* 165–175.

Robinson, C., Callow, M., Stevenson, S., Robinson, B.W., and Lake, R.A. (2001). Private specificities can dominate the humoral response to self-antigens in patients with cryptogenic fibrosing alveolitis. *Respir. Res. 2,* 119–124.

Roca, J., Granena, A., Rodriguez-Roisin, R., Alvarez, P., Agusti-Vidal, A., and Rozman, C. (1982). Fatal airway disease in an adult with chronic graft-versus-host disease. *Thorax 37,* 77–78.

Rossi, G.A., Szapiel, S., Ferrans, V.J., and Crystal, R.G. (1987). Susceptibility to experimental interstitial lung disease is modified by immune and non-immune related genes. *Am. Rev. Respir. Dis. 135,* 448–455.

Ryu, J.H., Myers, J.L., and Swensen, S.J. (2003). Bronchiolar disorders. *Am. J. Respir. Crit. Care Med. 168,* 1277–1292.

Sakito, O., Kadota, J., Kohno, S., Abe, K., Shirai, R., and Hara, K. (1996). Interleukin 1 beta, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. *Respiration 63,* 42–48.

Sandoz, E. (1907). Uber zwei Falle von "Fotaler Bronchektasie." *Beitr. Pathol. Anat. 41,* 495–516.

Sauty, A., Rochat, T., Schoch, O.D., Hamacher, J., Kurt, A.M., Dayer, J.M., and Nicod, L.P. (1997). Pulmonary fibrosis with predominant CD8 lymphocytic alveolitis and anti-Jo–1 antibodies. *Eur. Respir. J. 10,* 2907–2912.

Scadding, J.G. (1964). Fibrosing alveolitis. *BMJ 5410,* p. 686.

Schnabel, A., Reuter, M., and Gross, W.L. (1998). Intravenous pulse cyclophosphamide in the treatment of interstitial lung disease due to collagen vascular diseases. *Arthritis Rheum. 41,* 1215–1220.

Schoenberger, C.I. and Crystal, R.G. (1983). Drug induced lung disease. In K.J. Isselbacher, R.D. Adams, E.M.J.B. Braunwald, R.G. Petersdorf, and J.D. Wilson (eds), *Harrison's Principles of Internal Medicine Update IV* (pp. 49–74). New York: McGraw-Hill.

Schoenberger, C.I., Rennard, S.I., Bitterman, P.B., Fukuda, Y., Ferrans, V.J., and Crystal, R.G. (1984). Paraquat-induced pulmonary fibrosis. Role of the alveolitis in modulating the development of fibrosis. *Am. Rev. Respir. Dis. 129,* 168–173.

Schwarz, M.I. (1998). The lung in polymyositis. *Clin. Chest Med. 19,* 701–12.

Selman, M., Carrillo, G., Salas, J., Padilla, R.P., Perez-Chavira, R., Sansores, R., and Chapela, R. (1998). Colchicine, D-penicillamine, and prednisone in the treatment of idiopathic pulmonary fibrosis: a controlled clinical trial. *Chest 114,* 507–512.

Selman, M., King, T.E., and Pardo, A. (2001). Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann. Intern. Med. 134,* 136–151.

Silver, R.M., Miller, K.S., Kinsella, M.B., Smith, E.A., and Schabel, S.I. (1990). Evaluation and management of scleroderma lung disease using bronchoalveolar lavage. *Am. J. Med. 88,* 470–476.

Sleijfer, S. (2001). Bleomycin-induced pneumonitis. *Chest 120,* 617–624.

Snider, G.L. (1986). Interstitial pulmonary fibrosis. *Chest 89,* 115S–121S.

Southcott, A.M., Jones, K.P., Li, D., Majumdar, S., Cambrey, A.D., Pantelidis, P., Black, C.M., Laurent, G.J., Davies, B.H., and Jeffery, P.K. (1995). Interleukin-8. Differential expression in lone fibrosing alveolitis and systemic sclerosis. *Am. J. Respir. Crit. Care Med. 151,* 1604–1612.

Stack, B.H., Choo-Kang, Y.F., and Heard, B.E. (1972). The prognosis of cryptogenic fibrosing alveolitis. *Thorax 27,* 535–542.

Standiford, T.J., Kunkel, S.L., Phan, S.H., Rollins, B.J., and Strieter, R.M. (1991). Alveolar macrophage-derived cytokines induce monocyte chemoattractant protein-1 expression from human pulmonary type II-like epithelial cells. *J. Biol. Chem. 266,* 9912–9918.

Staples, C.A., Muller, N.L., Vedal, S., Abboud, R., Ostrow, D., and Miller, R.R. (1987). Usual interstitial pneumonia: correlation of CT with clinical, functional, and radiologic findings. *Radiology 162,* 377–381.

Stein-Streilein, J., Lipscomb, M.F., Fisch, H., and Whitney, P.L. (1987). Pulmonary interstitial fibrosis induced in hapten-immune hamsters. *Am. Rev. Respir. Dis. 136,* 119–123.

Swyer, P.R. and James, G.C. (1953). A case of unilateral pulmonary emphysema. *Thorax 8,* 133–136.

Targoff, I.N. (1993). Humoral immunity in polymyositis/dermatomyositis. *J. Invest. Dermatol. 100,* 116S–123S.

Tasaka, S., Kanazawa, M., Mori, M., Fujishima, S., Ishizaka, A., Yamasawa, F., and Kawashiro, T. (1995). Long-term course of bronchiectasis and bronchiolitis obliterans as late complication of smoke inhalation. *Respiration 62,* 40–42.

Thomas, A.Q., Lane, K., Phillips, J., III, Prince, M., Markin, C., Speer, M., Schwartz, D.A., Gaddipati, R., Marney, A., Johnson, J., Roberts, R., Haines, J., Stahlman, M., and Loyd, J.E. (2002). Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am. J. Respir. Crit. Care Med. 165,* 1322–1328.

Tisdale, J.E., Follin, S.L., Ordelova, A., and Webb, C.R. (1995). Risk factors for the development of specific noncardiovascular adverse effects associated with amiodarone. *J. Clin. Pharmacol. 35,* 351–356.

Troshinsky, M.B., Kane, G.C., Varga, J., Cater, J.R., Fish, J.E., Jimenez, S.A., and Castell, D.O. (1994). Pulmonary function and gastroesophageal reflux in systemic sclerosis. *Ann. Intern. Med. 121,* 6–10.

Tse, R.L. and Bockman, A.A. (1970). Nitrogen dioxide toxicity. Report of four cases in firemen. *JAMA 212,* 1341–1344.

Turner-Warwick, M. and Doniach, D. (1965). Auto-antibodies studies in interstitial pulmonary fibrosis. *BMJ 5439,* 886–891.

Turner-Warwick, M., Burrows, B., and Johnson, A. (1980). Cryptogenic fibrosing alveolitis: clinical features and their influence on survival. *Thorax 35,* 171–180.

Turton, C.W., Williams, G., and Green, M. (1981). Cryptogenic obliterative bronchiolitis in adults. *Thorax 36,* 805–810.

Vallance, D.K., Lynch, J.P., III, and McCune, W.J. (1995). Immunosuppressive treatment of the pulmonary manifestations of progressive systemic sclerosis. *Curr. Opin. Rheumatol. 7,* 174–182.

Wallace, W.A. and Howie, S.E. (1999). Immunoreactive interleukin 4 and interferon-gamma expression by type II alveolar epithelial cells in interstitial lung disease. *J. Pathol. 187,* 475–480.

Wallace, W.A., Schofield, J.A., Lamb, D., and Howie, S.E. (1994). Localisation of a pulmonary autoantigen in cryptogenic fibrosing alveolitis. *Thorax 49,* 1139–1145.

Wallace, W.A., Ramage, E.A., Lamb, D., and Howie, S.E. (1995). A type 2 (Th2-like) pattern of immune response predominates in the pulmonary interstitium of patients with cryptogenic fibrosing alveolitis (CFA). *Clin. Exp. Immunol. 101,* 436–441.

Weinberger, S.E., Kelman, J.A., Elson, N.A., Young, R.C., Jr., Reynolds, H.Y., Fulmer, J.D., and Crystal, R.G. (1978). Bronchoalveolar lavage in interstitial lung disease. *Ann. Intern. Med. 89,* 459–466.

Wells, A.U., Cullinan, P., Hansell, D.M., Rubens, M.B., Black, C.M., Newman-Taylor, A.J., and du Bois, R.M. (1994). Fibrosing alveolitis associated with systemic sclerosis has a better prognosis than lone cryptogenic fibrosing alveolitis. *Am. J. Respir. Crit. Care Med. 149,* 1583–1590.

White, B., Moore, W.C., Wigley, F.M., Xiao, H.Q., and Wise, R.A. (2000). Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. *Ann. Intern. Med. 132,* 947–954.

Whyte, M., Hubbard, R., Meliconi, R., Whidborne, M., Eaton, V., Bingle, C., Timms, J., Duff, G., Facchini, A., Pacilli, A., Fabbri, M., Hall, I., Britton, J., Johnston, I., and Di Giovine, F. (2000). Increased risk of fibrosing alveolitis associated with interleukin-1 receptor antagonist and tumor necrosis factor-alpha gene polymorphisms. *Am. J. Respir. Crit. Care Med. 162,* 755–758.

Wiedemann, H.P. and Matthay, R.A. (1989). Pulmonary manifestations of the collagen vascular diseases. *Clin. Chest Med. 10,* 677–722.

Winterbauer, R.H., Hammar, S.P., Hallman, K.O., Hays, J.E., Pardee, N.E., Morgan, E.H., Allen, J.D., Moores, K.D., Bush, W., and Walker, J.H. (1978). Diffuse interstitial pneumonitis. Clinicopathologic correlations in 20 patients treated with prednisone/azathioprine. *Am. J. Med. 65,* 661–672.

Witt, C., Borges, A.C., John, M., Fietze, I., Baumann, G., and Krause, A. (1999). Pulmonary involvement in diffuse cutaneous systemic

sclerosis: broncheoalveolar fluid granulocytosis predicts progression of fibrosing alveolitis. *Ann. Rheum. Dis. 58,* 635–640.

Wright, J.L., Cagle, P., Churg, A., Colby, T.V., and Myers, J. (1992). Diseases of the small airways. *Am. Rev. Respir. Dis. 146,* 240–262.

Wusirika, R., Ferri, C., Marin, M., Knight, D.A., Waldman, W.J., Ross, P., Jr., and Magro, C.M. (2003). The assessment of anti-endothelial cell antibodies in scleroderma-associated pulmonary fibrosis. A study of indirect immunofluorescent and western blot analysis in 49 patients with scleroderma. *Am. J. Clin. Pathol. 120,* 596–606.

Xaubet, A., Marin-Arguedas, A., Lario, S., Ancochea, J., Morell, F., Ruiz-Manzano, J., Rodriguez-Becerra, E., Rodriguez-Arias, J.M., Inigo, P., Sanz, S., Campistol, J.M., Mullol, J., and Picado, C. (2003). Transforming growth factor-beta1 gene polymorphisms are associated with disease progression in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med. 168,* 431–435.

Yoshida, S., Akizuki, M., Mimori, T., Yamagata, H., Inada, S., and Homma, M. (1983). The precipitating antibody to an acidic nuclear protein antigen, the Jo-1, in connective tissue diseases. A marker for a subset of polymyositis with interstitial pulmonary fibrosis. *Arthritis Rheum. 26,* 604–611.

Yousem, S.A., Colby, T.V., and Carrington, C.B. (1985). Follicular bronchitis/bronchiolitis. *Hum. Pathol. 16,* 700–706.

Ziesche, R., Hofbauer, E., Wittmann, 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,* 1264–1269.

Zisman, D.A., Lynch, J.P., III, Toews, G.B., Kazerooni, E.A., Flint, A., and Martinez, F.J. (2000). Cyclophosphamide in the treatment of idiopathic pulmonary fibrosis: a prospective study in patients who failed to respond to corticosteroids. *Chest 117,* 1619–1626.

CHAPTER

68

Paraneoplastic Neurologic Diseases: Autoimmune Responses to Tumor Antigens

BRUNO GIOMETTO, MARIKA VIANELLO, AND ROBERTA VITALIANI

Department of Neurosciences, University of Padova, Padova, Italy, and Department of Neurology, Azienda ULSS9, Treviso, Italy

Paraneoplastic neurologic diseases (PND) are a heterogeneous group of disorders associated with systemic malignancies but never related to invasion or compression of, nor metastasis to, the nervous system; in addition, diagnosis should rule out intoxications, infections, and metabolic causes for symptoms (Posner, 1995).

Oppenheim was probably the first to associate cancer and neurologic disease, considering the neurologic symptoms to be a result of toxic processes (Oppenheim, 1888).

Greenfield noted perivascular infiltration and inflammatory nodules in the brain and spinal cord (Greenfield, 1934), and Henson et al. (1965) coined the term "encephalitis with carcinoma," suggesting that the entity was indistinguishable from viral encephalitis because of the severity of inflammatory changes.

In a seminal publication, Henson and Urich (1982) described several clinicopathologic entities in association with cancer (encephalomyelitis, cortical cerebellar degeneration, peripheral neuropathy, muscular and neuromuscular disorders) and discussed the possible etiopathogenesis of PND. After hypothesizing an infectious viral and toxic cause, they considered the immunologic theory to be the most likely, "albeit supported by very slender evidence" (Henson and Urich, 1982)

In 1965, Wilkinson and Zeromski found antibodies in the serum of four individuals suffering from small cell lung cancer and sensory neuropathy. Trotter et al. (1976) detected antibodies reacting with human Purkinje cells in the serum of a 21-year-old woman with Hodgkin lymphoma, while Greenlee and Brashear (1983) described two patients with ovarian cancer and high titers of anti-Purkinje cell antibodies.

In the 1980s, specific autoantibodies (Ab) reacting with both tumors and the central nervous system (CNS) were characterized in the serum and cerebrospinal fluid (CSF) of patients with PND (Posner, 1995) and several antigens were identified. The discovery supports the hypothesis, already advanced by Russell Brain in 1951 (Brain et al., 1951), that most PND could be the result of an immunologic response triggered by a tumor antigen that cross-reacts with a similar protein expressed by the nervous system.

At present, the role of these Ab in the pathogenesis of the various syndromes is still unclear (Posner, 1992). Nevertheless, their detection has become a useful tool both in the diagnosis of neurologic disorders and in directing the search for underlying tumors, since some tumors are preferentially

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

associated with a particular neurological disorder (Posner, 1989, 1995).

CLINICAL AND PATHOLOGIC FEATURES

Paraneoplastic neurologic diseases are rare syndromes that present an estimated incidence of between 1 and 3% of cancer patients. The clinical picture reflects the damage to a single area or multiple regions of the nervous system.

Table 68.1 summarizes the PND according to level of involvement of the nervous system and the presence of Ab.

Paraneoplastic Encephalomyelitis/ Paraneoplastic Sensory Neuropathy (PEM/PSN)

The term encephalomyelitis was first introduced to cancer patients by Henson and Urich in 1965 to describe tumor patients presenting signs of multiple CNS involvement (Henson et al., 1965). Paraneoplastic encephalomyelitis (PEM) is one of the most frequent remote effects of cancer and is pathologically characterized by neuronal loss and inflammatory infiltrates in different areas of the CNS and dorsal root ganglia (DRG) (Henson and Urich, 1982).

Onset of the neurologic syndrome is often subacute with symptoms mostly developing within days or weeks, followed by slower progression and stabilization. Acute onset is seen in 20% and chronic progression of paraneoplastic encephalomyelitis/paraneoplastic sensory neuropathy (PEM/PSN) in 17% of cases (Dalmau et al., 1992b). Patients may present with different clinical pictures, as limbic or brainstem encephalitis, myelitis, cerebellar degeneration, and sensory neuropathy, which may be isolated or present together. Sensory neuropathy is the presenting symptom in 70%, and the predominant picture in 60% of cases, but may also be present as an isolated syndrome.

First described by Denny-Brown in 1948, PSN is characterized by asymmetrical, painful sensory neuropathy, which evolves into complete loss of proprioception.

The neurologic syndrome usually antedates diagnosis of the tumor. Mean time from onset of neurologic symptoms to discovery of a tumor varies from 4 to 12 months with a maximum of 6 years. In a minority of cases, no tumor is found even at autopsy (Lucchinetti et al., 1998). Small cell lung cancer (SCLC) is identified in approximately 80% of patients, usually as a limited disease, but other tumors such as prostate cancer, neuroblastoma, and sarcomas may be detected.

Radiologic studies are usually normal, but abnormalities of the temporal lobe have been observed on computed tomography (CT) or magnetic resonance imaging (MRI) in some case with limbic encephalitis.

Cerebrospinal fluid (CSF) examination may be normal or show mild pleocytosis and increased protein and oligoclonal bands. The majority of patients (85%) with PEM harbors an antineuronal antibody, referred to as anti-Hu, in the serum and CSF; consequently the term "Hu syndrome" is applied alongside the term PEM/PSN (Lucchinetti et al., 1998; Graus et al., 2001).

Limbic Encephalitis

Paraneoplastic limbic encephalitis (PLE) is characterized by acute or subacute onset of an organic brain syndrome, giving rise to severe-to-moderate neurologic deficit (Rankin score >3) in a period of days to weeks. The pathologic features consist of neuronal loss, perivascular inflammatory infiltrate, and gliosis, usually restricted to the limbic system, in particular to the hippocampus and amygdala. The clinical picture is defined by short-term memory loss, confusion,

TABLE 68.1 Paraneoplastic neurological syndromes*

Central nervous system	Peripheral nervous system and dorsal root ganglia	Neuromuscular junction and muscle
Encephalomyelitis	*Sensory neuronopathy*	*Lambert–Eaton myasthenic syndrome*
Limbic encephalitis	–	–
Brain stem encephalitis	–	–
Cerebellar degeneration		
Opsoclonus–Myoclonus	*Autonomic neuronopathy*	*Myasthenia gravis*
Pure cerebellar degeneration	Acute sensorimotor neuropathy	Polymyositis/dermatomyositis
Paraneoplastic visual syndrome	Chronic sensorimotor neuropathy	Acute necrotizing myopathy
Stiff-Person syndrome	Vasculitic neuropathy	Cachetic myopathy
Necrotizing myelopathy	*Neuromyotonia*	Myotonia
Myelitis	–	–
Motor neuron syndrome	–	–
Subacute motor neuronopathy	–	–

**Italics* indicate disorders associated with autoantibodies.

psychiatric symptoms, involvement of the limbic system, and seizures. In the majority of patients, the symptoms antedate the diagnosis of tumor by an average of 3–5 months. Paraneoplastic limbic encephalitis was found to preferentially associate with SCLC (50%), germ cell tumors of the testis (20%), and breast cancer (8%) (Gultekin et al., 2000)

Neuroimaging, electroencephalography (EEG), CSF examination, and antineuronal antibody detection are helpful in the diagnosis of this illness. Alterations on MRI are seen in about 60% of patients. The MRI features of limbic encephalitis are most evident on coronal section and typically consist of abnormal high-signal intensity on T2 sequences in one or both medial temporal lobe(s). On T1 sequences, the temporal-limbic area may be hypointense and atrophic and may sometimes enhance with contrast injection. No alterations are visible in the initial phase of the disease. FDG-PET studies have shown increased tracer activity in the medial temporal lobe, which may be present in the absence of MRI abnormalities and may reflect an acute stage of the inflammatory process (Provenzale et al., 1998).

In 45% of patients, EEG reveals epileptic abnormalities from the temporal lobe, but in the majority of patients it shows unilateral or bilateral temporal slow waves.

Examination of the CSF shows inflammatory signs (e.g., pleocytosis, oligoclonal bands) in about 60% of patients.

Antineuronal Ab may be found in the serum and CSF of approximately 60% of patients with PLE, particularly anti-Hu Ab in patients with SCLC, and anti-Ma2 Ab in patients with testicular and sometimes breast cancer. In PLE, symptoms tend to stabilize in 2–3 months, and the course rarely leads to rapid decline and early death.

Paraneoplastic Cerebellar Degeneration

Paraneoplastic cerebellar degeneration (PCD) is a rare complication of cancer, but the presence of cancer has to be expected in about 50% of middle-aged patients who develop acute or subacute pan-cerebellar disorders. On pathologic examination, the main characteristic is loss of Purkinje cells with little lymphocytic infiltrations.

The onset of disease progresses over a period of 1 day to 16 weeks followed by stabilization. In about one-third of patients, onset may be abrupt with severe ataxia, which may develop overnight. The syndrome is characterized by pan-cerebellar dysfunction, manifesting as truncal and limb ataxia, slurred speech, and nystagmus. Nausea and vertigo are infrequent but may be debilitating. In one-third of patients, bulbar involvement with dysphagia and facial weakness is present (Posner, 1995).

On radiologic studies cerebellar atrophy may be detected in the late stage of the disease. Examination of the CSF may show mild pleocytosis, increased protein, and oligoclonal bands.

All patients with PCD present the same clinical picture, but they can be subdivided into several subgroups by serologic analysis of the associated antineuronal Ab. These subgroups differ in associated cancer, course, and survival (Rojas-Marcos et al., 2003).

An antineuronal antibody directed against Purkinje cell cytoplasm, referred to as anti-Yo, can be detected in the serum of patients with PCD. This syndrome usually develops in women with gynecologic or breast cancer. The cerebellar syndrome is isolated and develops before discovery of the cancer (mean, 15 months). Paraneoplastic cerebellar degeneration often leads to severe handicap, and the average survival is 13 months.

Male patients with a history of Hodgkin's disease may develop PCD. In such cases, the PCD develops 1–54 months after diagnosis of the primary tumor; an antineuronal antibody called anti-Tr, can be detected in the serum of these patients. Survival from time of diagnosis is significantly higher than in PND (mean, >113 months) associated with anti-Yo.

Paraneoplastic cerebellar degeneration may also be detected in patients with SCLC as part of a more widespread encephalomyelitis, as in the Hu syndrome. In this case the clinical course is progressive and mean survival is 7 months (Shams'ili et al., 2003). Anti-VGCC and electrophysiology testing should be performed in patients with SCLC associated with PCD to establish the presence of an associated Lambert–Eaton myasthenic syndrome (LEMS).

Opsoclonus–Myoclonus Syndrome

Opsoclonus is an eye movement disorder characterized by involuntary arrhythmic and multidirectional conjugated saccades. The paraneoplastic variant should be differentiated from paraneoplastic cerebellar degeneration, viral encephalitis, post-viral ataxia, multiple sclerosis, metabolic disorders, and intoxications (Wolpow and Richardson, 1988).

In children, opsoclonus occurs with myoclonus and ataxia; associated signs of encephalopathy (such as impairment in verbal, motor, and intellectual functions) are sometimes present. Opsoclonus–myoclonus syndrome (OMS) in children may develop as a primarily self-limiting disorder following a viral brainstem infection. Approximately 50% of children with OMS harbor a neuroblastoma (Baker et al., 1985), but do have a better prognosis for long-term survival compared with neuroblastoma patients without OMS. An immune mechanism is suggested, though no specific antineuronal antibody has yet been recognized (Antunes et al., 2000; Pranzatelli et al., 2002).

In adults, OMS is recognized as a distinct paraneoplastic syndrome associated in 20% of cases with breast carcinoma (Wirtz et al., 2002), gynecologic cancer, or small cell lung carcinoma (Gennaula and Eidelman, 1995). Recently, other

cancers, such as bladder and neuroendocrine gastric carcinoma, have been reported (Pittok et al., 2003).

Patients usually develop opsoclonus associated with truncal ataxia, dysarthria, myoclonus, and vertigo. Both CT and MRI brain scans are usually normal, and CSF may occasionally show mild pleocytosis or increased proteins (Sutton et al., 2002).

A specific antineuronal antibody called anti-Ri has been identified in association with breast carcinoma (Luque et al., 1991). Paraneoplastic OMS has to be differentiated from idiopathic OMS. The latter is usually described in patients who are younger than patients with the paraneoplastic form, and is characterized by a more benign clinical evolution and response to immunotherapy (Bataller et al., 2001).

Testing sera from patients with several types of OMS (idiopathic, associated with SCLC, and associated with neuroblastoma) has revealed heterogeneous immunity to neuronal antigens, although a single specific antibody marker has not been found. The two main groups of autoantigens were: 1) proteins of the post-synaptic density (considered to be a source of novel autoantigens); and 2) proteins whose function is restricted to neurons (including RNA- or DNA-binding proteins and zinc-finger proteins) (Bataller et al., 2003).

Consequently, there is an extensive variety of antigens recognized by the sera of patients with OMS, suggesting that this syndrome probably derives from different immune mechanisms (Bataller et al., 2003).

Paraneoplastic Stiff-Person Syndrome

Stiff-person syndrome (SPS) is a rare disorder characterized by continuous motor unit activity at rest and painful spasms in response to sound, touch, or electric stimulation. Stiffness spasms later become continuous, precipitated by voluntary movements and preventing normal gait (Folli, 1998). Electrophysiological studies have shown involuntary motor unit firing at rest, while contraction of antagonist muscles fails to induce motor unit relaxation in the leg muscles (Meinck et al., 1995).

Both CT scan and MRI are unremarkable, but CSF examination may reveal an inflammatory profile.

In rare cases, an association of SPS and breast cancer has been reported; these patients harbored anti-amphiphysin Ab in their serum and CSF (Folli et al., 1993).

IMMUNOLOGIC MARKERS IN THE DIAGNOSIS AND AUTOIMMUNE FEATURES OF ANTINEURONAL ANTIBODIES

Different antineuronal Ab are associated with specific PND and can be detected by immunohistochemistry (IHC) on rat or human cerebellum and by western blot using neuronal proteins. The antigen involved in some PND has been identified and the genes responsible have been cloned to produce recombinant proteins, as summarized in Table 68.2.

Anti-Hu Antibody

In 1985, anti-Hu Ab were demonstrated in the serum and CSF of two patients with sensory neuronopathy and SCLC that reacted with the nucleus of all human neurons in the CNS and PNS (Graus et al., 1985). Anti-Hu Ab are polyclonal complement-binding IgG that react with antigens present in the nucleus of both neurons and SCLC cells, sparing the nucleolus and cytoplasm (Graus et al., 1985) (Figure 68.1).

On western blot, using either human or rat neuronal nuclear lysates, a triple band is usually detected in the 38–40 kDa region (Graus et al., 1986).

Hu Antigens

Two highly homologous genes, referred to as HuD (Szabo et al., 1991) and HuC (Sakai et al., 1994) were isolated by screening a human cDNA library with anti-Hu sera. HuD and HuC are members of a family of neuronal RNA-binding proteins homologous to the *Drosophila* embryonic lethal abnormal vision (ELAV) protein, which is involved in the normal development of the fly nervous system (Szabo et al., 1991; King et al., 1994b).

HuD, Hel-N1, and N2 (all similar to ELAV) (King, 1994a; Sekido et al., 1994; King and Dropcho, 1996) are all expressed by SCLC cells (Dalmau et al., 1992a; King and Dropcho, 1996).

The gene for HuD has been mapped to human chromosome 1p34 (Muresu et al., 1994). A recombinant HuD of 43 kDa that reacts with all anti-Hu-positive sera has been produced (Szabo et al., 1991). The use of recombinant proteins by western blotting always increases the sensitivity and specificity of assays to detect anti-Hu Ab compared with IHC alone (Giometto et al., 1997; Dropcho and King, 1994).

Anti-Yo Antibody

In 1983, a circulating autoantibody that reacted with a cytoplasmic antigen of Purkinje cells was described in two patients with PCD and ovarian carcinoma (Greenlee and Brashear, 1983). Serologic Ab reacting with Purkinje cells were later confirmed in those patients and called anti-Yo Ab (Jaeckle et al., 1985).

By using IHC on human or rat cerebellar sections, these polyclonal complement-fixing IgG exhibit coarse granular staining of Purkinje cell cytoplasm and proximal dendrites, sparing the nucleus (Jaeckle et al., 1985; Greenlee et al.,

TABLE 68.2 Classification of paraneoplastic neurologic diseases (PND) according to the specific antibody detected, neurologic syndrome, and the most frequently associated tumor

Antibody	Paraneoplastic neurologic syndrome	Most frequently associated cancers	Immunohistochemistry	Western immunoblot	Antigen
Anti-Hu/ANNA-1	Encephalomyelitis, sensory neuronopathy	SCLC, non-SCLC	Strong staining of all neuronal nuclei and weaker staining of cytoplasm	38–40 kDa reactive triplet bands on extracts of crude or isolated CNS neurons/nuclei and SCLC. Recombinant protein 43 kDa (HuD)	Neuron-specific RNA/DNA binding protein, role in RNA processing of neurons
Anti-Yo/PCA-1	Acute to subacute pan-cerebellar syndrome, dysarthria and upbeat nystagmus	Ovarian, breast, uterus	Purkinje-cell cytoplasm and axons, coarse granular staining	34 and 62 kDa band from Purkinje cell extracts and breast and ovary tumors of patients, reactive with recombinant CDR-34 and CDR-62 patients	DNA binding, gene transcription regulators, carry leucine-zipper and zinc-finger motifs
Anti-Ri/ANNA-2	Opsoclonus–myoclonus	Breast, SCLC	All nuclei of CNS	55 and 80 kDa neuronal protein; 55 kDa recombinant protein	RNA-binding protein, regulates metabolism in a subset of developing neurons
Anti-amphiphysin	Stiff-person syndrome	Breast	Synapses of CNS neurons	128 kDa protein on neuronal extracts	Amphiphysin synaptic vesicle protein, function unknown
anti-Tr	More slowly developing cerebellar syndrome, less frequent dysarthria and nystagmus	Hodgkin's disease	Purkinje cell cytoplasm, fine granular staining in the molecular layer	No reactive protein detected as yet	Not known
Anti-CV2	Subacute cerebellar ataxia, Lambert–Eaton myasthenic syndrome, limbic encephalitis, uveitis	SCLC, thymoma	Cytoplasm of oligodendrocytes	66 kDa protein	CRMP5 (Collapsine response mediator protein 5)
anti-Ta (Me 2)	Limbic encephalitis	Testicular tumors	Nucleus, perykarion	40 kDa	Unknown function

SCLC, small cell lung cancer.

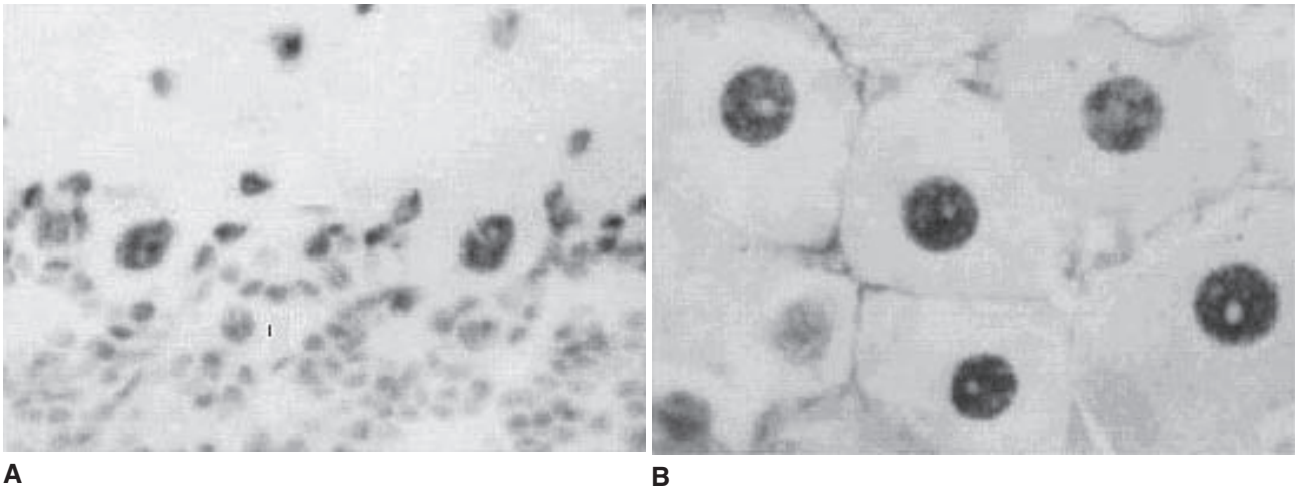


Figure 68.1 Anti-Hu antibody. *A,* rat cerebellar cortex showing homogeneous staining of neuronal nuclei of Purkinje, granular and molecular layer cells (×400). *B,* the autoantibody reacts also with rat dorsal root ganglia (DRG) nuclei (×800).

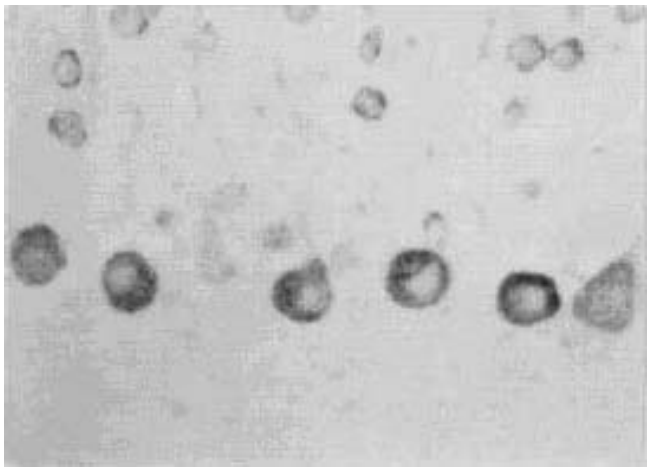


Figure 68.2 Anti-Yo antibody. Coarsely granular staining of the Purkinje and molecular layer cytoplasms sparing the nuclei in Purkinje cells of rat cerebellar cortex (×400).

1988) (Figure 68.2). Though anti-Yo reactivity is widespread in the rat cerebellum, no staining was demonstrated in human cerebral cortical neurons (Greenlee et al., 1988; Altermatt et al., 1991; Dalmau and Posner, 1996).

On western blot with Purkinje cell proteins or cerebellar extracts, anti-Yo Ab react with two bands at 32–34 kDa and 62–64 kDa (Greenlee, 1983; Jaeckle et al., 1985; Cunningham et al., 1986). The antigen is also selectively expressed in tumor tissue from patients with PCD (Furneaux et al., 1990).

The Yo Antigen

Using electron microscopy, it has been shown that the anti-Yo antibody binds to clusters of ribosomes, the Golgi apparatus and the granular endoplasmic reticulum of Purkinje cells (Peterson et al., 1992). The genes for the 34 kDa and 62 kDa Yo antigens have been cloned and the gene products are called cerebellar-degeneration-related proteins CDR1 (CDR-34) and CDR2 (CDR-62) (Fatallah-Shaykh et al., 1991). Two other Yo antigens of 58 kDa (CZF) and 52 kDa (PCD-AA) have been reported (Tanaka et al., 1996). However, anti-Yo Ab binding to 62 kDa, 58 kDa, and 52 kDa antigens tally to a single molecule of 58 kDa when tests are conducted under identical laboratory conditions; this underscores the usefulness of standardizing antibody detection methods to characterize PCD subgroups (Tanaka et al., 1996).

Unlike anti-Hu Ab, which are expressed in all SCLC, the CDR are only expressed in tumors of patients with anti-Yo-positive PCD (Furneaux et al., 1990; Sakai et al., 1992).

The function of these antigens is as yet unknown, although it is thought that they may regulate gene transcription by binding to DNA (Fatallah-Shaykh et al., 1991).

Anti-Ri Antibody

The antineuronal antibody called anti-Ri was identified in association with opsoclonus–myoclonus syndrome in patients with breast carcinoma in 1991 by Luque et al. It reacts on IHC with all neuronal nuclei of the CNS (except the dorsal root ganglia nuclei, thus differentiating it from anti-Hu) (Figure 68.3). On western blot using neuronal extracts, the antibody reacts with two separate bands of 55 and 80 kDa (Luque et al., 1991).

By screening a human cerebellar cDNA library using anti-Ri serum, a gene called NOVA-1 has been cloned. It encodes a 54 kDa protein (Buckanovich et al., 1993). The antigen NOVA-1 is a neuron-specific KH-type RNA-

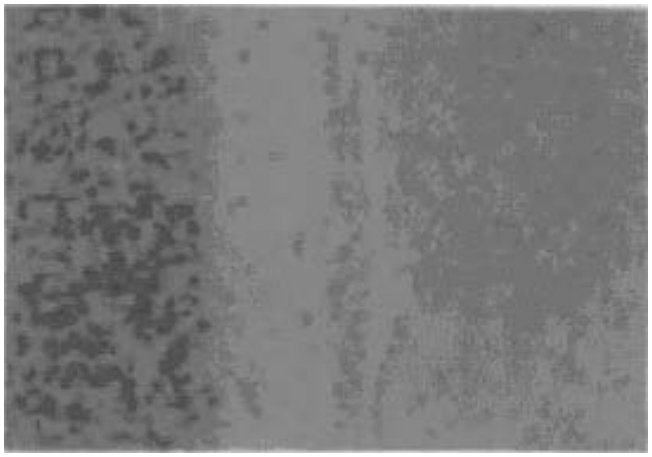


Figure 68.3 Anti-Ri antibody. Pattern of neuronal staining of spinal cord nuclei (bottom) sparing the dorsal root ganglia neurons (left) (×250).

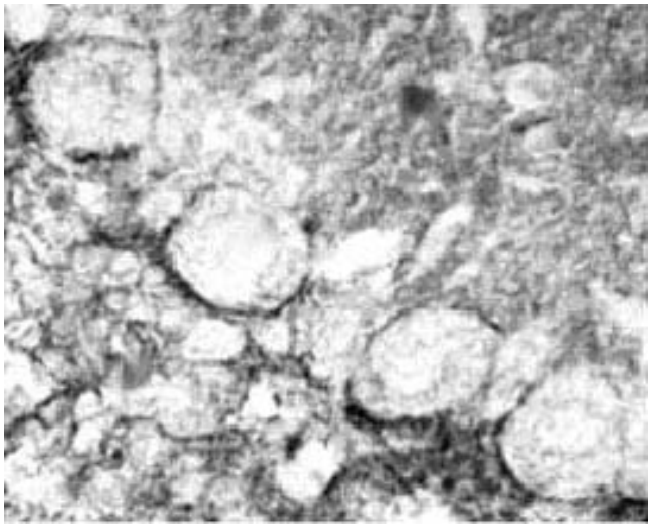


Figure 68.4 Anti-amphiphysin antibody. Staining of the neuropil of rat brain cerebellum in the molecular and granular layers of the Purkinje cell membrane sparing the nuclei and cytoplasms (×800).

binding protein. NOVA-1 transcripts are only present in specific regions of the CNS, as the midbrain and pontine tegmentum, optic tectum, and deep nuclei of the cerebellum (these localizations correlate with clinical symptoms in OMS).

The homology between NOVA-1 and hnRNPK suggests that NOVA-1 regulates metabolism in a specific subset of developing motor neurons (Buckanovich et al., 1993). *In vitro*, anti-Ri Ab can inhibit the RNA-binding activity of the NOVA-1 antigen (Buckanovich et al., 1996).

Anti-Amphiphysin Antibody

Anti-amphiphysin Ab were first described in patients affected by SPS and breast cancer (Folli et al., 1993). On IHC, anti-amphiphysin Ab react with the neuropil of rat brain cerebellum, sparing the neuronal nuclei and cytoplasm (Figure 68.4). On immunoblots of cerebral cortex, sera harboring anti-amphiphysin Ab react with a protein doublet of approximately 128 kDa (De Camilli et al., 1995).

Amphiphysin is a synaptic vesicle-associated protein (De Camilli et al., 1993) whose gene (AMPH) was mapped to the short arm of chromosome 7 (Yamamoto, 1995). Its function remains unclear. It binds to dynamin, involved in the internalization of synaptic vesicle membranes after exocytosis (De Camilli et al., 1995).

Anti-amphiphysin Ab have also been described in patients affected by PEM and SCLC (Dropcho, 1996; Dorresteijn et al., 2002). More recently, the spectrum of PND associated with anti-amphiphysin Ab was further enlarged to encompass limbic encephalitis (Dorresteijn et al., 2002), cerebellar degeneration and opsoclonus (Saiz et al., 1998; Antoine et al., 1999). In addition, anti-amphiphysin Ab are present, at a low frequency, in patients with SCLC without PND (Saiz et al., 1998). In such cases, patients' sera also harbored anti-Hu Ab. Considering the low incidence of the disease, no therapeutic approaches have yet been reported.

Anti-Tr Antibody

Anti-Tr Ab have been described in patients affected by PCD and Hodgkin disease (HD) (Graus et al., 1997). Cerebellar symptoms usually develop in middle-aged males after diagnosis of lymphoma and during remission of the tumor (Hammack et al., 1992).

Anti-Tr Ab label the cytoplasm of Purkinje cells of human and rat cerebellum. The molecular layer of rat cerebellum shows a characteristic dotted pattern suggestive of immunoreactivity by the dendritic terminals of Purkinje cells (Graus et al., 1997) (Figure 68.5). Immunoblots of human Purkinje cells or rat and mouse cerebellum are negative (Graus et al., 1997). Consequently, the corresponding antigen has not yet been identified.

Through laser confocal microscopy and immunoelectronmicroscopy, anti-Tr Ab immunoreactivity was found to be localized in the cytosol and outer surface of the endoplasmic reticulum of the perikarya of neurons of the molecular layer and the cell body and dendrites of Purkinje cells, without a particular concentration in the dendrite spines (Graus et al., 1998).

The antigen recognized by anti-Tr Ab probably appears at an early stage and is widely expressed in the developing rat brain (Graus et al., 1998).

Unlike other antineuronal Ab, anti-Tr Ab can be detected in the CSF but not in the serum and may spontaneously disappear during follow-up (Bernarl et al., 2003) or after improvement of clinical symptoms (Peltola et al., 1998).

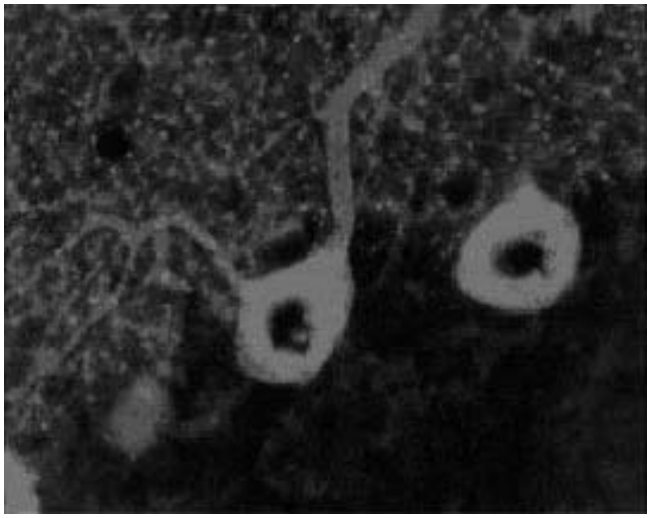


Figure 68.5 Anti-Tr antibody. Dotted staining of the molecular layer and Purkinje cell cytoplasm of rat cerebellum (immunofluorescence) (×800).

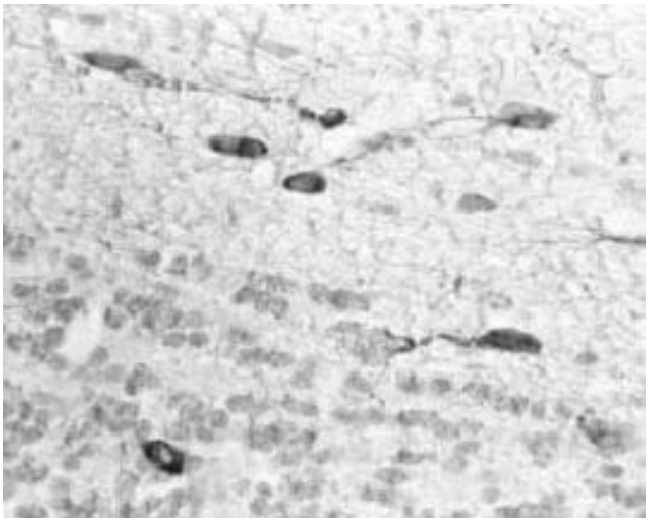


Figure 68.6 Anti-CV2 antibody. Cerebellar white matter (top) showing cytoplasmic labeling of oligodendrocytes (×400).

Anti-Tr Ab immunoreactivity has never been demonstrated in the tumor (Bernarl et al., 2003).

Anti-CV2 Antibody

Anti-CV2 Ab were first reported by Antoine et al. (1995) and Honnorat et al. (1996) in patients affected by subacute ataxia, SCLC, or thymoma. The main neurologic syndrome is a subacute cerebellar disease (66%) although a few cases presented with limbic encephalitis, LEMS, or uveitis (Honnorat et al., 1996). Most patients with anti-CV2 Ab develop SCLC; association with thymoma (Rogemond et al., 2000) and epidermoid carcinoma of the lung (Medrano et al., 2003) is rare.

The paraneoplastic origin of the disease seems to be confirmed by dramatic improvement after tumor excision (De La Sayette et al., 1998). In 20% of patients, anti-Hu Ab are also present. Patients with anti-CV2 Ab presented mixed axonal and demyelinating sensory-motor neuropathy, sometimes superimposed on a subacute sensory neuronopathy when both anti-CV2 and anti-Hu Ab were present (Antoine et al., 2001).

On adult brain tissue sections, anti-CV2 Ab label the cytoplasm of oligodendrocytes (Figure 68.6) and react with a 66 kDa protein of new-born rat brain on western blot (Honnorat et al., 1996).

The antigen (paraneoplastic oligodendrocyte protein of 66 kDa; POP66) is specifically expressed by a subpopulation of oligodendrocytes and not by neurons (Honnorat et al., 1998), in spite of the wide neuronal loss without demyelination pathologically reported in these patients. The involvement of POP66 in neuron survival via neuron/oligodendrocyte interactions has been postulated (Honnorat et al., 1998).

Recently, paraneoplastic chorea, mainly associated with SCLC and thymoma, has been described in patients testing positive for anti-CRMP-5 Ab (Yu et al., 2001; Vernino et al., 2002). CRMP-5 is a neuronal cytoplasmic protein, related to the collapsin response-mediator protein family, which migrates at 66–70 kDa under denaturing gel electrophoresis conditions. CRMP-5-IgG recognizes the neuronal nucleus and cytoplasm, and minor glial population on IHC.

Anti-CV2 Ab are hypothesized to constitute the same entity as CRMP-5-IgG since they react with at least four of the five known Ulip/CRMP (Unc-33-like phosphoprotein/collapsing response mediator protein) (Honnorat et al., 2001), a family of four homologous phosphoproteins considered crucial for brain development. The proteins recognized by anti-CV2 are Ulip3/CRMP1, Ulip4/CRMP3 (Honnorat et al., 1999), Ulip2/CRMP2, and Ulip6/CRMP5 (Ricard et al., 2001).

Anti-CRMP Ab reflect only one aspect of Ulip/CRMP recognition by anti-CV2 Ab (Honnorat et al., 2001).

Anti-Ma/Ta Antibody

Anti-Ta Ab recognize a 40 kDa neuronal protein present in the nucleus and cytoplasm of neurons. By IHC on acetone-fixed, frozen sections of human or rat brain, anti-Ta Ab produce discrete granular reactivity in the nucleus and perikaryon of rat neurons and nuclear staining in the

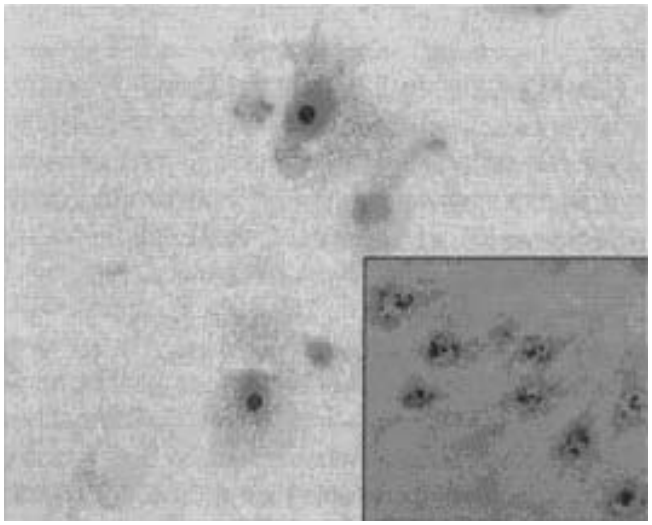


Figure 68.7 Anti-Ta/Ma antibody. Staining of nucleolus in human cortex. Discrete granular reactivity in the nucleus and perikaryon of rat neurons (×400, inset ×800).

Courtesy of Prof. Dalmau.

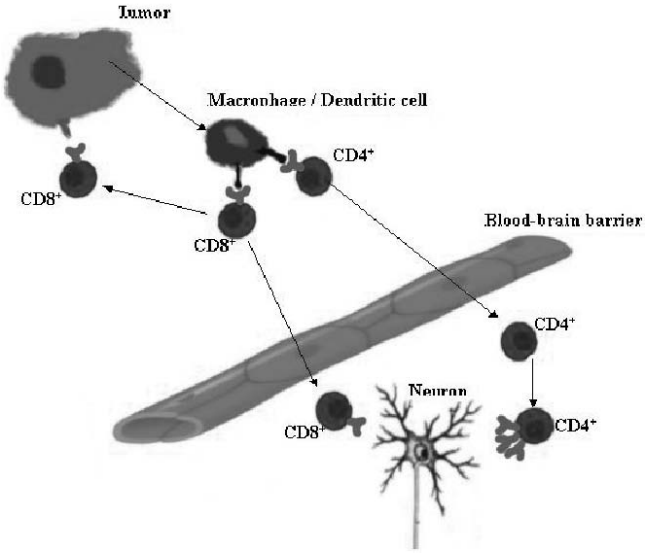


Figure 68.8 Autoimmune responses in paraneoplastic neurological diseases. Onconeuronal antigen expressed within the tumor when the cell dies is taken up by macrophages and presented to both cytotoxic and helper T cells. When these cells migrate through the blood–brain barrier they help B cells to produce specific antibodies. This microenvironment can induce MHC class I expression by neurons thus enabling the cytotoxic T cell to attack the neuron.

human cortex (Figure 68.7). Anti-Ta Ab are restricted to patients with limbic and brainstem encephalitis associated with testicular cancer (Voltz et al., 1998; 1999; Gultekin et al., 2000). Testicular cancer is the second leading neoplasm, after SCLC, to cause paraneoplastic limbic encephalitis.

Anti-Ta Ab has permitted the cloning of a protein called Ma2, which is expressed in the brain and testicular tumors of patients with paraneoplastic limbic encephalitis and anti-Ta Ab. The Ma2 function is unknown but shares homology with Ma1, an antigen expressed in normal brain and testis (Dalmau, 1998).

Antibodies directed against Ma1 are also known as anti-Ma Ab; they are usually harbored in the sera of patients affected by cerebellar and/or brainstem disease associated with breast, parotid, and colon cancer. Anti-Ma Ab react mainly with the nuclei and nucleoli of neurons and, to a lesser degree, with the cytoplasm; in addition, they recognize testicular germ cells. On western blot with neuronal proteins, anti-Ma Ab identified two distinct bands of reactivity at 37 and 40 kDa. As for Ma2, the function of Ma1 protein remains unknown (Dalmau et al., 1999).

Other Antibodies

Recently, other new antineuronal Ab have been characterized: Glutamate receptor (Sillevis-Smitt et al., 2000), and Zic4 (Bataller et al., 2004). However, these reactivities have only been confirmed by one laboratory.

PATHOGENIC MECHANISMS AND ANIMAL MODELS

The pathogenesis of PND is still unknown, but an immune-mediated mechanism is hypothesized. This theory is supported by the detection of antineuronal Ab in PND patients' CSF, which indicates intrathecal endogenous synthesis.

These syndromes seem to be the result of a cross-reaction mechanism in which antigens are expressed by both the tumor and neurons and called "onconeural antigens." The immune system is activated by these antigens, slowing tumor growth and causing the neuronal damage. A possible mechanism of the autoimmune responses is reported in Figure 68.8: onconeural antigens expressed within the tumor are taken up by macrophages and presented to both cytotoxic and helper T cells. When these cells migrate through the blood–brain barrier they help B cells to produce specific Ab. This microenvironment can induce MHC class I expression by neurons, thus enabling the cytotoxic T cell to attack the neuron.

The role of antineuronal Ab in the pathogenesis of PND has only been conclusively demonstrated in neuromuscular junction disorders, such as LEMS. Indeed, inoculation in rabbits of IgG from patients with LEMS was able to reproduce the disease (Lang and Vincent, 1996). This

mechanism occurs, however, irrespective of paraneoplastic etiology.

In the case of other PND, the discovery of specific Ab suggests a similar pathogenesis, although no *in vivo* or *in vitro* studies have been able to demonstrate that antineuronal Ab have a role in inducing the disease. Many onconeural antigens are nuclear or cytoplasmic, and it is, therefore, difficult to understand how Ab might target the neurons. Hu antigens, for example, are expressed in cell nuclei and not on the cell surface; this suggests that they are unavailable as a target for the immune system (Dalmau et al., 1991; 1992a). The only evidence that anti-Hu Ab may have a direct pathogenetic role comes from the finding that anti-Hu Ab destroy rat cerebellar granular cells *in vitro* (Greenlee et al., 1993).

However, anti-Hu-positive sera could be toxic to tumor cell lines even when cells do not express Hu antigens, suggesting that the effect on cells may not be Hu-mediated (Verschuuren et al., 1997).

Passive immunization of experimental animals with anti-Yo Ab has never reproduced the disease, as with anti-Hu Ab (Greenlee et al., 1988; Graus et al., 1991; Sillevis-Smith et al., 1994; Tanaka et al., 1995a; 1995b; René et al., 1996). This suggests involvement of an alternative cellular immune mechanism (for example cytotoxic T lymphocytes or other cytolytic killer cell activity). This hypothesis seems to be confirmed by the discovery of extensive infiltrates in the nervous system of PND patients (Verschuuren et al., 1996) and the detection of activated T-cells in the CSF of patients affected by PCD (Albert et al., 2000). Hence, cytotoxic T lymphocytes have become the candidate for holding the primary pathogenic role in PND. T-cell-receptor analysis in anti-Hu brain tissue revealed oligoclonal expansion of cytotoxic T cells, suggesting a primary immune reaction against onconeural antigens (see Figure 68.8). Very recently (Pellkofer et al., 2004) a model of paraneoplastic disease involving the central nervous system has been reported in which the adoptive transfer of T cells specific for an autologous onconeural antigen caused encephalomyelitis in rats with perivascular infiltrates in the regions affected in human disease.

TREATMENT WITH AUTOANTIBODIES

The rarity of PND has made it impossible to carry out controlled therapeutic studies, and currently available data are derived from limited cases. Correct clinical diagnosis is paramount but may be challenging since the neurologic symptoms sometimes have an acute onset and often antedate the discovery of the tumor. In any event, early cancer detection and treatment represent the gold standard for PND therapy. Indeed, in most cases, treatment of the neoplasm can lead to stabilization and even improvement of the neurologic syndrome, although cancer detection may sometimes be difficult. Moreover, patients may deteriorate in the months preceding diagnosis of the tumor, the neurologic disorder may progress even after achieving complete response to the tumor, or the PND can develop during cancer treatment. For all these reasons, cancer therapy cannot be the only approach to PND management. The use of immunomodulatory therapies, like steroids, intravenous immunoglobulin (Moll et al., 1993, Consuell, 1994; Uchuya et al. 1996), plasma exchange (Graus et al., 1992; Cher et al., 1995), immunosuppressive drugs (Graus and Delattre, 1993; Stark et al., 1995), and combination of these therapies (Keime-Guilbert et al., 2000) has been prompted by increasing evidence that many paraneoplastic syndromes are immunomediated. These therapies seem to be effective for PND with a proven immunomediated mechanism like LEMS (Bain et al., 1996). However, no immunomodulatory approach used to date has been very effective in treating other PND involving the central nervous system. In these cases, clinical symptoms could be caused by massive neuronal death (as in the case of Purkinje cells in PCD) or functional impairment of neuronal networks (as in OMS). In the former group, early treatment is recommended for stabilization of symptoms; in the latter group, complete remission is possible. Complete clinical remission was reported in patients affected by limbic encephalitis (LE) associated with anti-Ma Ab (Rosenfeld et al., 2001); all were treated with antitumor therapy, in some cases in combination with immunosuppression. In these cases a reversible imbalance of the neuronal network seems to be involved, rather than neuronal loss (as in the case of OMS). In addition, LE with anti-Ma Ab is usually associated with radically treated testicular cancer. An argument against the use of immunomodulatory therapy is that it might affect evolution of the neoplasm. There are no data to support this hypothesis; it has, instead, been shown that, in patients with PEM, SCLC outcome was similar in patients who received immunotherapy and those who did not (Keime-Guibert et al., 2000); in addition, long-term outcome of PCD and anti-Yo Ab is also not affected by immunotherapy (Rojas et al., 2000).

CONCLUDING REMARKS

Although categorization of the major PND by 1965 was supported by the detection of antineuronal Ab by 1980, strict diagnostic criteria have only recently been developed (Graus et al. 2004). The diagnosis of PND is still difficult because not all patients with paraneoplastic disorders harbor antineuronal Ab and a neoplasm is not detected in all patients with antineuronal Ab. These criteria divide PND into "definite" and "possible" based upon clinical and immunologic data. We believe that these new criteria will allow classifi-

cation of newly identified paraneoplastic syndromes and should now permit the development of standardized research protocols and multicentric therapeutic trials.

References

Albert, M.L., Austin, L.M., and Darnell, R.B. (2000). Detection and treatment of activated T cells in the cerebrospinal fluid of patients with paraneoplastic cerebellar degeneration. *Ann. Neurol. 47*, 9–17.

Altermatt, H.J., Rodriguez, M., Scheithauer, B.W., and Lennon, V.A. (1991). Paraneoplastic anti-Purkinje and type I anti-neuronal nuclear autoantibodies bind selectively to central, peripheral, and autonomic nervous system cells. *Lab. Invest. 65*, 412–420.

Antoine, J.C., Honnorat, J., Thomas Anterion, C., Aguera, M., Absi, L., Fournel, P., and Michel, D. (1995). Limbic encephalitis and immunological perturbations in two patients with thymoma. *J. Neurol. Neurosurg. Psychiatr. 58*, 706–710.

Antoine, J.C., Absi, L., Honnorat, J., Boulesteix, J.M., De Brouker, T., Vial, C., Butler, M., De Camilli, P., and Michel, D. (1999). Antiamphiphysin antibodies are associated with various paraneoplastic neurological syndromes and tumours. *Arch. Neurol. 56*, 172–177.

Antoine, J.C., Honnorat, J., Camdessanchè, J.P., Magistris, M., Absi, L., Mosnier, J.F., Petiot, P., Kopp, N., and Michel, D. (2001). Paraneoplastic anti-CV2 antibodies react with peripheral nerve and are associated with a mixed axonal and demyelinating peripheral neuropathy. *Ann. Neurol. 49*, 214–221.

Antunes, N.L., Khakoo, Y., Matthay, K.K., Seeger, R.C., Stram, D.O., Gerstner, E., Abrey, L.E., and Dalmau, J. (2000). Antineuronal antibodies in patients with neuroblastoma and paraneoplastic opsoclonus-myoclonus. *J. Pediatr. Hematol. Oncol. 22*(4), 315–320.

Bain, P.G., Motomura, M., Newson-Davies, J., Misbah, S.A., Chapel, H.M., Lee, M.L., Vincent, A., and Lang, B. (1996). Effects of intravenous immunoglobulin on muscle weakness and calcium-channel autoantibodies in the Lambert–Eaton myasthenic syndrome. *Neurology 47*, 678–683.

Baker, M.E., Kirbs, D.R., Korobkia, M., Bowie, J.D., and Filston, H.C. (1985). The association of neuroblastoma and myoclonic encephalopathy: an imaging approach. *Pediatr. Radiol. 15*, 185–189.

Bataller, L., Graus, F., Saiz, A., and Vilchez, J.J. (2001). Clinical outcome in adult onset idiopathic or paraneoplastic opsoclonus-myoclonus. *Brain 124*, 437–443.

Bataller, L., Rosenfeld, M.R., Graus, F., Vilchez, J.J., Cheung, N.-K.V., and Dalmau, J. (2003). Autoantigen diversity in the opsoclonus-myoclonus syndrome. *Ann. Neurol. 53*, 347–353.

Bataller, L., Wade, D.F., Graus, F., Stacey, H.D., Rosenfeld, M.R., and Dalmau, J. (2004). Antibodies to Zic4 in paraneoplastic neurologic disorders and small-cell lung cancer. *Neurology 62*, 778–82.

Bernarl, F., Shams'ili, S., Rojas, I., Sanchez-Valle, R., Saiz, A., Dalmau, J., Honnorat, J., Sillevis Smitt, P., and Graus, F. (2003). Anti-Tr antibodies as marker of paraneoplastic cerebellar degeneration and Hodgkin's disease. *Neurology 60*, 230–234.

Buckanovich, R.J., Posner, J.B., and Darnell, R.B. (1993). Nova, the paraneoplastic Ri antigen, is homologous to an RNA-binding protein and is specifically expressed in the developing motor system. *Neuron 11*, 657–672.

Buckanovich, R.J., Yang, Y.Y.Z., and Darnell, R.B. (1996). The onconeural antigen Nova-1 is a neuron-specific RNA binding protein, the activity of which is inhibited by paraneoplastic antibodies. *J. Neuroscience 16*, 1114–1122.

Cher, L.M., Hochberg, F.H., Teruya, J., Nitschke, M., Valenzuela, R.F., Schmahmann, J.D., Herbert, M., Rosas, H.D., and Stowel, C. (1995). Therapy for paraneoplastic neurologic syndromes in six patients with protein A column immunoadsorption. *Cancer 75*, 1678–1683.

Consuell, C.E., McLeod, M., and Grant, R. (1994). Reversal of subacute paraneoplastic cerebellar syndrome with intravenous immunoglobulin. *Neurology 44*, 1184–1185.

Cuuningham, J., Graus, F., Anderson, N., and Posner, J.B. (1986). Partial characterization of the Purkinje cell antigens in paraneoplastic cerebellar degeneration. *Neurology 36*, 1163–1168.

Dalmau, J. (1998). Paraneoplastic neurological disorders. Clinical immunological correlation. *J. Neurol. 245*, 339–340.

Dalmau, J.O. and Posner, J.B. (1996). Purkinje cell autoantibodies, type 1 (Yo). In J.B. Peter and Y. Shoenfeld (eds), *Autoantibodies*, (pp. 655–659). Amsterdam: Elsevier.

Dalmau, J., Graus, F., Rosenblum, M.K., and Posner, J.B. (1991). Detection of the anti-Hu antibody in specific regions of the nervous system and tumor from patients with paraneoplastic encephalomyelitis/sensory neuropathy. *Neurology 41*, 1757–1764.

Dalmau, J., Furneaux, H.M., Cordon-Cardo, C., and Posner, J.B. (1992a). The expression of the Hu (paraneoplastic encephalomyelitis/sensory neuronopathy) antigen in normal and tumor tissues. *Am. J. Pathol. 141*, 881–886.

Dalmau, J., Graus, F., Rosenblum, M.K., and Posner, J.B. (1992b). Anti-Hu-associated paraneoplastic encephalomyelitis/sensory neuronopathy. A clinical study of 71 patients. *Medicine 71*, 59–72.

Dalmau, J., Gultekin, S.H., Voltz, R., Hoard, R., DesChamps, T., Balmaceda, C., Batchelor, T., Gerstner, E., Eichen, J., Frennier, J., Posner, J.B., and Rosenfeld, M.R. (1999). Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. *Brain 122*, 27–39.

De Camilli, P., Thomas, A., Cofiell, R., Folli, F., Lichte, B., Piccolo, G., Menick, H.M., Austoni, M., Fassetta, G., Bottazzo, G.F., and Solimena, M. (1993). The synaptic vesicle-associated protein amphiphysin is the 128 kDa autoantigen of stiff-man syndrome with breast cancer. *J. Exp. Med. 178*, 2219–2223.

De Camilli, P., Takei, K., and McPherson, P.S. (1995). The function of dynamin in endocytosis. *Curr. Opin. Neurobiol. 5*(5), 559–565.

De La Sayette, V., Bertran, F., Honnorat, J., Schaeffer, S., Iglesias, S., and Defer, G. (1998). Paraneoplastic cerebellar syndrome and optic neuritis with anti-CV2 antibodies: clinical response to excision of the primary tumor. *Arch. Neurol. 55*, 405–408.

Denni-Brown, D. (1948) Primary sensory neuropathy with muscular changes associated with carcinoma. *J. Neurol. Neurosurg. Psychiatry. 11*, 73–87.

Dorresteijn, L.D., Kappelle, A.C., Renier, W.O., and Gijtenbeek, J.M. (2002). Anti-amphiphysin associated limbic encephalitis: a paraneoplastic presentation of small-cell lung carcinoma. *J. Neurol. 249*, 1307–1308.

Dropcho, E.J. (1996). Antiamphiphysin antibodies with small-cell lung carcinoma and paraneoplastic encephalomyelitis. *Ann. Neurol. 39*, 659–667.

Dropcho, E.J. and King, P.H. (1994). Autoantibodies against the Hel-N1 RNA-binding protein among patients with lung carcinoma: an association with type I anti-neuronal nuclear antibodies. *Ann. Neurol. 36*, 200–205.

Fatallah-Shaykh, H., Wolf, S., Wong, E., Posner, J.B., and Furneaux, H.M. (1991). Cloning of a leucine-zipper protein recognized by the sera of patients with antibody-associated paraneoplastic cerebellar degeneration. *Proc. Natl. Acad. Sci. 88*, 3451–3545.

Folli, F. (1998). Stiff-man syndrome, 40 years later. *J. Neurol. Neurosurg. Psychiatry 65*(5), 618.

Folli, F., Solimena, M., Cofiell, R., Austoni, M., Tallini, G., Fassetta, G., Bates, D., Carlidge, N., Bottazzo, G.F., Piccolo, G., and De Camilli, P. (1993). Autoantibodies to a 128-kD synaptic protein in three women

with the stiff-man syndrome and breast cancer. *N. Engl. J. Med. 328*, 546–551.

Furneaux, H.M., Rosenblum, M.K., Dalmau, J., Wong, E., Woodruff, P., Graus, F., and Posner, J.B. (1990). Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. *N. Engl. J. Med. 227*, 1844–1851.

Gennaula, C.P. and Eidelman, B.H. (1995). Anti-Ri paraneoplastic syndrome in association with primary lung carcinoma. *Neurology 45*, 1417.

Giometto, B., Marchiori, G.C., Nicolao, P., Scaravilli, T., Lion, A., Bardin, P.G., and Tavolato, B. (1997). Subacute cerebellar degeneration with anti-Yo autoantibodies: immunohistochemical analysis of the immune reaction in the central nervous system. Neuropathol. *Appl. Neurobiol. 23*, 468–474.

Graus, F., Vega, F., Delattre, J.-Y., Bonaventura, I., Rene, R., Arbaiza, D., and Tolosa, E. (1992). Effect of plasmapheresis and antineoplastic treatment in CNS paraneoplastic syndromes with antineuronal autoantibodies. *Neurology 42*, 536–540.

Graus, F., Cordon-Cardo, C., and Posner, J.B. (1985). Neuronal antinuclear antibody in sensory neuronopathy from lung cancer. *Neurology 35*, 538–543.

Graus, F., Elkon, K.B., Cordon-Cardo, C., and Posner, J.B. (1986). Sensory neuronopathy and small cell lung cancer. Antineuronal antibody that also reacts with the tumor. *Am. J. Med. 80*, 45–52.

Graus, F., Illa, I., Agusti, M., Ribalta, T., Cruz-Sanchez, F., and Juarez, C. (1991). Effect of intraventricular injection of an anti-Purkinje cell antibody (anti-Yo). in a guinea pig model. *J. Neurol. Sci. 106*, 82–87.

Graus, F. and Delattre, J.Y. (1993). Immune modulation of paraneoplastic neurological disorders. *Clin. Neurol. Neurosurg. 97*,112–116.

Graus, F., Dalmau, J., Valldeoriola, F., Ferrer, I., Rene, R., Marin, C., Vecht, C.J., Arbizu, T., Targa, C., and Moll, J.W. (1997). Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *J. Neuroimmunol. 74*, 55–61.

Graus, F., Gultekin, S.H., Ferrer, I., Reiriz, J., Alberch, J., and Dalmau, J. (1998). Localization of the neuronal antigen recognized by anti-Tr antibodies from patients with paraneoplastic cerebellar degeneration and Hodgkin's disease in the rat nervous system. *Acta Neuropathol. (Berl.) 96*, 1–7.

Graus, F., Keime-Guilbert, F., René, R., Benyahia, B., Ribalta, T., Ascaso, C., Escaramis, G., and Delattre, Y. (2001). Anti-Hu associated paraneoplastic encephalomyelitis: analysis of 200 patients. *Brain 124*, 1138–1148.

Graus, F., Delattre, J.Y., Antoine, J.C., Dalmau, J., Giometto, B., Grisold, W., Honnorat, J., Smitt, P.S., Vedeler, C.H., Verschuuren, J.J., Vincent, A., and Voltz, R. (2004). Recommended diagnostic criteria for paraneoplastic neurological syndromes. *J. Neurol. Neurosurg. Psychiatry 75*(8),1135–1140.

Greenfield, J.G. (1934). Subacute spinocerebellar degeneration occurring in elderly patients. *Brain 57*, 161–176.

Greenlee, J.E. and Brashear, H.R. (1983). Antibodies to cerebellar Purkinje cells in patients with paraneoplastic cerebellar degeneration and ovarian carcinoma. *Ann. Neurol. 14*, 609–613.

Greenlee, J.E., Brashear, H.R., and Herndon, R.M. (1988). Immunoperoxidase labelling of rat brain section with sera from patients with paraneoplastic cerebellar degeneration and systemic neoplasia. *J. Neuropathol. Exp. Neurol. 47*, 561–571.

Greenlee, J.E., Parks, T.N., and Jaeckle, K.A. (1993). Type Ia ("anti-Hu"). antineuronal antibodies produce destruction of rat cerebellar granule neurons *in vitro*. *Neurology 43*, 2049–2054.

Gultekin, S.H., Rosenfeld, M.R., Voltz, R., Eichen, J., Posner, J.B., and Dalmau, J. (2000). Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumor association in 50 patients. *Brain 123*, 1481–1494.

Hammack, J., Kotanides, H., Rosenblum, M.K., and Posner, J.B. (1992). Paraneoplastic cerebellar degeneration. II. Clinical and immunologic finding in 21 patients with Hodgkin's disease. *Neurology 42*, 1938–1943.

Henson, R.A. and Urich, H. (1982). Encephalomyelitis with carcinoma. In R.A. Henson and H. Urich (eds) *Cancer and Nervous System,* (pp. 314–345). Oxford: Oxford Blackwell Scientific.

Henson, R.A., Hoffman, H.L., and Urich, H. (1965). Encephalomyelitis with carcinoma. *Brain 88*, 449–464.

Honnorat, J., Antoine, J.C., Derrington, E., Aguera, M., and Belin, M.F. (1996). Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. J. Neurol. Neurosurg. *Psychiatr. 61*, 270–278.

Honnorat, J., Aguera, M., Zalc, B., Goujet, C., Quach, T., Antoine, J.C., and Belin, M.F. (1998). POP66, a paraneoplastic encephalomyelitis-related antigen, is a marker of adult oligodendrocytes. *J. Neuropathol. Exp. Neurol. 57*, 311–322.

Honnorat, J., Byk, T., Kusters, I., Aguera, M., Ricard, D., Rogemond, V., Quach, T., Aunis, D., Sobel, A., Mattei, M.G., Kolattukudy, P., Belin, M.F., Antoine, J.C. (1999). Ulip/CRMP proteins are recognized by autoantibodies in paraneoplastic neurological syndromes. *Eur J Neurosci 11*, 4226–4232.

Honnorat, J., Antoine, J.C., and Belin, M.F. (2001). Are the "newly discovered" paraneoplastic anti-collapsin response-mediator protein-5 antibodies simply anti-CV2 antibodies? *Ann. Neurol. 49*, 214–221.

Jaeckle, K.A., Graus, F., Houghton, A., Cordon-Cardo, C., Nielsen, S.L., and Posner, J.B. (1985). Autoimmune response of patients with paraneoplastic cerebellar degeneration to a Purkinje cell cytoplasmic protein antigen. *Ann. Neurol. 18*, 592–600.

Keime-Guilbert, F., Graus, F., Fleury, A., René, R., Honnorat, J., Broet, P., and Delattre, J.Y. (2000). Treatment of paraneoplastic neurological syndromes with antineuronal antibodies (anti-Hu, anti-Yo) with combination of immunoglobulins, cyclophosphamide, and methylprednisolone. *J. Neurol. Neurosurg. Psychiatry 68*: 479–482.

King, P.H. (1994a). Hel-N2: a novel isoform of Hel-N1 which is conserved in rat neural tissue and produced in early embryogenesis. *Gene 151*, 261–265.

King, P.H. and Dropcho, E.J. (1996). Expression of Hel-N1 and Hel-N2 in small-cell lung carcinoma. *Ann. Neurol. 39*, 679–681.

King, P.H., Levine, T.D., Fremeau, R.T. Jr., and Keene, J.D. (1994b). Mammalian homologs of Drosophila ELAV localized to a neuronal subset can bind *in vitro* to the 3′ UTR of mRNA encoding the Id transcriptional repressor. *J. Neurosci. 14*, 1943–1952.

Lang, B. and Vincent, A. (1996). Autoimmunity to ion-channel and other proteins in paraneoplastic disorders. *Curr. Opin. Immunol. 8*, 865–871.

Lucchinetti, C.F., Kimmel, D.W., and Lennon, V.A. (1998). Paraneoplastic and oncologic profile of patients seropositive for type 1 antineuronal nuclear autoantibodies. *Neurology 50*, 652–657.

Luque, F.A., Furneaux, H.M., Ferziger, R., Rosenblum, M., Wray, S.H., Clifford Schold, S., Glantz, M.J., Jaeckle, K.A., Biran, H., Lesser, M., Paulsen, W.A., River, M.E., and Posner, J.B. (1991). Anti-Ri: an antibody associated with paraneoplastic opsoclonus and breast cancer. *Ann. Neurol. 29*, 241–251.

Medrano, V., Sempere, A.P., and Gonzales, E. (2003). Paraneoplastic ataxia associated to anti-CV2 antibodies. *Rev. Neurol. 36*, 131–133.

Meinck, H.M., Ricker, K., Hulser, P.J., and Solimena, M. (1995). Stiff-man syndrome: neurophysiological findings in eight patients. *J. Neurol. 242*, 134–142.

Moll, J.W.B., Henzen-Logmans, S.C., van der Meche, F.G.A., and Vecht, C.J. (1993). Early diagnosis and intravenous immune globulin therapy in paraneoplastic cerebellar degeneration. *J. Neurol. Neurosurg. Psychiatry 56*, 112–115.

Muresu, R., Baldini, A., Gress, T., Posner, J.B., Furneaux, H.M., and Siniscalco, M. (1994). Mapping of the gene coding for a paraneoplastic encephalomyelitis antigen (HuD). to human chromosome site 1p34. Cytogenet. *Cell Genet. 65*, 177–178.

Oppenheim, H. (1888). Uber Hirnsymptome bei carcinomatose ohne nachweisbatre. Veraanderungen im Gehim. *Charité-Annalen (Berlin) 13*, 335–344.

Pellkofer, H., Schubart, A.S., Höftberg, R., Schutze, N., Pagany, M., Schuller, M., Lassmann, H., Holfeld, R., Voltz, R., and Linington, C. (2004) Modeling paraneoplastic CNS disease: T-cell specific for the onconeuronal antigen PNMA-1 mediate autoimmune encephalomyelitis in the rat. *Brain 127*, 1822–30.

Peltola, J., Hietaharju, A., Rantala, I., Lehtinen, T., and Haapasalo, H. (1998). A reversible neuronal antibody (anti-Tr). associated paraneoplastic cerebellar degeneration in Hodgkin's disease. *Acta Neurol. Scand. 98*, 360–363.

Peterson, K., Rosenblum, M.K., Kotanides, H., and Posner, J.B. (1992). Paraneoplastic cerebellar degeneration. A clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology 42*, 1931–1937.

Pittock, S.J., Lucchinetti, C.F., and Lennon, V.A. (2003). Anti-neuronal nuclear autoantibody type 2: paraneoplastic accompaniments. *Ann. Neurol. 53*, 580–587.

Posner, J.B. (1989). Paraneoplastic syndromes involving the nervous system. In Aminoff, M.J. (ed.) *Neurology and General Medicine*, (pp. 341–364). Churchill Livingstone, New York.

Posner, J.B. (1992). Pathogenesis of central nervous system paraneoplastic syndromes. *Rev. Neurol. 148*, 502–513.

Posner, J.B. (1995). Paraneoplastic syndromes. In J.B. Posner (ed.), *Neurologic Complication of Cancer. Contemporary Neurology series 45,* (pp 353–383). Philadelphia: F.A. Davis Company.

Pranzatelli, M.R., Tate, E.D., Wheeler, A., Bass, N., Gold, A.P., Griebel, M.L., Gumbinas, M., Heydemann, P.T., Holt, P.J., Jacob, P., Kotagal, S., Minarcik, C.J., and Schub, H.S. (2002). Screening for autoantibodies in children with opsoclonus-myoclonus-ataxia. *Pediatr. Neurol. 27*(5), 384–387.

Provenzale, J.M., Barboriak, D.P., and Coleman, R.E. (1998). Limbic encephalitis: comparison of FDG PET and MRI findings. *Am. J. Roentgenol. 18*, 1659–1660.

René, R., Ferrer, I., and Graus, F. (1996). Clinical and immunohistochemical comparison of *in vivo* injected anti-Hu and control IgG in the nervous system of the mouse. *Eur. J. Neurol. 3*, 319–323.

Ricard, D., Rogemond, V., Charrier, E., Aguera, M., Bagnard, D., Belin, M.F., Thomasset, N., and Honnorat, J. (2001). Isolation and expression pattern of human Unc-33-like phosphoprotein 6/collapsing response mediator protein 5 (Ulip6/CRMP5).: coexistence with Ulip2/CRMP2 in Sema3A-sensitive oligodendrocytes. *J. Neurosci. 21*, 7203–7214.

Rogemond, V. and Honnorat, J. (2000). Anti-Cv2 autoantibodies and paraneoplastic neurological syndromes. *Clin. Rev. Allergy. Immunol. 19*, 51–59.

Rojas, I., Graus, F., and Keime-Guilbert, F. (2000). Long-term clinical outcome of paraneoplastic cerebellar degeneration and anti-Yo antibodies. *Neurology 55*, 713–715.

Rojas-Marcos, I., Rousseau, A., Keime-Guilbert, F., Rene, R., Cartalat-Carel, S., Delattre, J.Y., and Graus, F. (2003). Spectrum of paraneoplastic neurologic disorders in women with breast and gynecologic cancer. *Medicine 82*(3), 216–223.

Rosenfeld, M.R., Eichen, J.G., and Wade, D.F. (2001). Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. *Ann. Neurol. 50*, 339–348.

Russel Brain, W., Daniel, P.M., and Greenfield, J.G. (1951). Subacute cortical cerebellar degeneration and its relation to carcinoma. *J. Neurol. Neurosurg. Psychiatr. 14*, 59–75.

Saiz, A., Dalmau, J., Butler, M., Chen, Q., Delattre, J.Y., De Camilli, P., and Graus, F. (1998). Anti-amphiphysin antibodies in patients with paraneoplastic neurologic disorders (PND) and small-cell lung cancer (SCLC). *J. Neurol. Neurosurg. Psychiatry 66*(2), 214–217.

Sakai, K., Negami, T., Yoshioka, A., and Hirose, G. (1992). The expression of a cerebellar degeneration-associated neural antigen in human tumor line cells. *Neurology 42*, 361–366.

Sakai, K., Gofuku, M., Kitagawa, Y., Ogasawara, T., Hirose, G., Yamazaki, M., Koh, C.S., Yanagisawa, N., and Steinman, L. (1994). A hippocampal protein associated with paraneoplastic neurologic syndrome and small cell lung carcinoma. *Biochem. Biophys. Res. Commun. 199*, 1200–1208.

Sekido, Y., Bader, S.A., Carbone, D.P., Johnson, B.E., and Minna, J.D. (1994). Molecular analysis of the HuD gene encoding a paraneoplastic encephalomyelitis antigen in human lung cancer cell lines. *Cancer Res. 54*, 4988–4992.

Shams'ili, S., Grefkens, J., De Leeuw, B., Van Den Bent, M., Hooijkaas, H., Van Der Holt, B., Vecht, C., and Sillevis Smitt, P. (2003). Paraneoplastic cerebellar degeneration associated with anti-neuronal antibodies: analysis of 50 patients. *Brain 126*, 1409–1418.

Sillevis-Smitt, P.A.E., Manley, G.T., and Posner, J.B. (1994). High titer antibodies but no disease in mice immunized with the paraneoplastic antigen HuD. *Neurology 43*, 2049–2054.

Sillevis-Smitt, P., Kinoshita, A., DeLeeuw, B, Moll, W., Coesmans, M., Jaarsma, D., Henzer-Longmans, S., Vecht, C., De Zeeuw, C., Sekiyama, N., Nakanishi, S., Shigemoto, R. (2000). Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. *N. Engl. J. Med. 342*, 21–27.

Stark, E., Wurster, U., Patzold, U., Sailer, M., and Haas, J. (1995). Immunological and clinical response to immunosuppressive treatment in paraneoplastic cerebellar degeneration. *Arch. Neurol. 52*, 814–818.

Sutton, I.J., Barnett, M.H., Watson, J.D.G., Ell, J.J., and Dalmau, J. (2002). Paraneoplastic brainstem encephalitis and anti-Ri antibodies. *J. Neurol. 249*, 1597–1598.

Szabo, A., Dalmau, J., Manley, G., Rosenfeld, M., Wong, E., Henson, J., Posner, J.B., and Furneaux, H.M. (1991). HuD a paraneoplastic encephalomyelitis antigen, contain RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell 67*, 325–333.

Tanaka, M., Tanaka, K., Onodera, O., and Tsuji, S. (1995a). Trial to establish an animal model of paraneoplastic cerebellar degeneration with anti-Yo antibody. Mouse strains bearing different MHC molecules produce antibodies on immunization with recombinant Yo protein, but do not cause Purkinje cell loss. *Clin. Neurol. Neurosurg. 97*, 95–100.

Tanaka, K., Tanaka, K., Igarashi, S., Onodera, O., Miyatake, T., and Tsuji, S. (1995b). Trial to establish an animal model of paraneoplastic cerebellar degeneration with anti-Yo antibody. Passive transfer of murine mononuclear cells activated with recombinant Yo protein to paraneoplastic cerebellar degeneration lymphocytes in severe combined immunodeficiency mice. *Clin. Neurol. Neurosurg. 97*, 101–105.

Tanaka, K., Tanaka, M., Tsukamoto, T., Uehara, T., and Tsuji, S. (1996). Anti-Yo antibody binding to 62-, 52-, or 58-kDa protein recognizes a single molecule. *Neurology 46*, 1777–1778.

Trotter, J.L., Hendin, B.A., and Osterland, C.K. (1976). Cerebellar degeneration with Hodgkin disease. An immunological study. *Arch. Neurol. 33*, 660–661.

Uchuya, M., Graus, F., Vega, F., Rene, R., and Delattre, J.-Y. (1996). Intravenous immunoglobulin treatment in paraneoplastic neurological syndromes with antineuronal autoantibodies. *J. Neurol. Neurosurg. Psychiatry 60*, 388–392.

Vernino, S., Tuite, P., Adler, C.H., Meschia, J.F., Boevee, B.F., Boasberg, P., Parisi, J.E., and Lennon, V. (2002). Paraneoplastic chorea associated with CRMP-5 neuronal antibody and lung carcinoma. *Ann. Neurol. 51*, 625–630.

Verschuuren, J.J., Chuang, L., Rosenblum, M.K., Lieberman, F., Pryor, A., Posner, J.B., and Dalmau, J. (1996). Inflammatory infiltrates and com-

plete absence of Purkinje cells in anti-Yo-associated paraneoplastic cerebellar degeneration. *Acta Neuropathol. (Berl.) 91*, 519–525.

Verschuuren, J.J., Dalmau, J., Hoard, R., and Posner, J.B. (1997). Paraneoplastic anti-Hu serum, studies on human tumor cell lines. *J. Neuroimmunol. 79*, 202–205.

Voltz, R., Gultekin, S.H., Posner, J.B., Rosenfeld, M.R., and Dalmau, J. (1998). Sera of patients with testicular cancer and paraneoplastic limbic/brainstem encephalitis harbour an antineuronal antibody (anti-Ta) that recognizes a novel neuronal protein [abstract]. *J. Neurol. 245*, 379.

Voltz, R., Gultekin, S.H., Rosenfeld, M.R., Gerstner, E., Eichen, J., Posner, J.B., and Dalmau, J. (1999). A serologic marker of paraneoplastic limbic/brainstem encephalitis in patients with testicular cancer. *N. Engl. J. Med. 340*, 1788–1795.

Yamamoto, R., Li, X., Winter, S., Francke, U., and Kilimann, M.W. (1995). Primary structure of human amphiphysin, the dominant autoantigen of paraneoplastic stiff-man syndrome, and mapping of its gene (AMPH). to chromosome 7p13-p14. *Hum. Mol. Genet. 4*(2), 265–268.

Yu, Z., Kryzer, T.J., Griesmann, G.E., Kim, K., Benarroch, E.E., and Lennon, V.A. (2001). CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. *Ann. Neurol. 49*, 146–154.

Wilkison, P.C. and Zeromski, J. (1965). Immunofluorescent detection of antibodies against neurones in sensory carcinomatous neuropathy. *Brain 88*, 529–538.

Wirtz, P.W., Sillevis Smith, P.A., Hoff, J.I., De Leeuw, B., Lammers, G.J., Van Diunen, S.G., and Verschuuren, J.J. (2002). Anti-Ri antibody positive opsoclonus-myoclonus in a male patient with brest carcinoma. *J. Neurol. 249*, 1710–1712.

Wolpow, E.R. and Richardson, E.D. (1988). Case records of the Massachusetts General Hospital. Case 9–1988. A 57-year-old woman with worsening opsoclonus. *N. Engl. J. Med. 318*, 563–570.

CHAPTER

69

Lymphoma and Autoimmune Disease

KATHERINE MORLAND HAMMITT*
**Sjögren Syndrome Foundation, Bethesda, Maryland*

ELAINE L. ALEXANDER†
†Aeolus Pharmaceuticals, Inc., San Diego, California

Lymphoid malignancies occur with increased frequency in autoimmune disease *vis-á-vis* the general population. Lymphomas have long been linked with chronic stimulation of the immune system (Armstrong et al., 1970), so their occurrence in an autoimmune disease setting is not unexpected. The actual development of lymphoma in autoimmune disease, however, is complex and multifactorial. Autoimmune disease per se, with its inherent chronic inflammation and stimulation of the immune system, contributes to the development of lymphoma, and, in addition, genetic and environmental factors influence the transition.

Lymphomas and autoimmune diseases demonstrate a close correlation from several aspects. Thus, lymphomas, especially B-cell non-Hodgkin lymphomas (NHLs), occur with increased frequency among autoimmune diseases, and features of autoimmune diseases occur in lymphoma even without any evidence of autoimmunity. Environmental provocation, including infections (mainly viral) and chemical exposures, is coming under increasing scrutiny in the development of lymphoma in autoimmune disease. Also, information is expanding as sequencing of the human genome progresses, and genetic profiling is providing opportunities in diagnosis, prognosis, improved classification, and treatment of lymphoma.

The increased prevalence of lymphoma in autoimmune diseases is clearly established in some disorders, while it appears less clearly established and is based primarily on case studies or animal models in others. Our understanding of both non-Hodgkin B-cell lymphoma and autoimmune disease has increased substantially in the last couple of decades. Formerly categorized solely by grade as *low, intermediate,* and *high*, new classifications for non-Hodgkin B-cell lymphoma were devised starting in 1994 with the "Revised European American Classification of Lymphoid Neoplasms" and published by the International Lymphoma Study Group, and most recently modified in 1999. These discussions led to designations based on genetic features, morphology, immunophenotype, and clinical features (Harris et al., 1999; Jaffe et al., 1999).

Increased knowledge about pathogenesis will translate into better avenues for research and therapies. The clinical setting already is rapidly changing with newer knowledge of risk factors, diagnostic procedures, and regimens of treatment. With these opportunities, though, come the disappointments as some new biologic agents, while improving symptoms in many autoimmune diseases, can be accompanied by induction of lymphoma in the autoimmune diseases that these agents were meant to treat. However, the advances in such areas as cytokine treatments (including antagonists of tumor necrosis factor [TNF] and B-cell activating

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

factors), apoptosis, immunogenetics, and environmental provocations hold exceptional promise in moving forward the autoimmune-lymphoma field connection.

OCCURRENCE OF LYMPHOMA IN AUTOIMMUNE DISEASES

The occurrence of lymphoma in autoimmune disease is illustrated most clearly in Sjögren syndrome, autoimmune thyroiditis, and autoimmune hemolytic anemia (Mackay and Rose, 2001). A connection with lymphoma also has been proposed in rheumatoid arthritis, systemic lupus erythematosus (SLE), celiac disease, and Henoch–Schönlein disease, and has been suggested in a broad range of additional autoimmune disorders.

Autoimmune Diseases Having the Clearest Association with Lymphoma

Certain autoimmune diseases have been historically recognized as associated with lymphoproliferation, which can remain benign or transform into a frank lymphoma. The term "lymphoproliferative disease" was used as early as 1959 by Dameshek to describe this association (Dameshek and Schwartz, 1959). Diseases in this category include autoimmune hemolytic anemia, Sjögren syndrome, and autoimmune thyroiditis, described in Chapters 41, 31 and 35, with a prominent histologic feature in the latter two being ectopic lymphoid hyperplasia in the affected tissue and prominent germinal centers. Additional autoimmune diseases having a clear association with lymphoma are rheumatoid arthritis, SLE, and celiac disease.

Sjögren Syndrome

The link between Sjögren syndrome and lymphoma was the first to be identified among autoimmune diseases (Talal and Bunim, 1964). Sjögren syndrome, among all autoimmune diseases, carries the greatest risk for lymphoma development, the highest incidence of transformation, and clearest progression from autoimmunity to lymphoproliferation to malignancy. In 2001, an International Workshop on Autoimmunity and Lymphoma concluded that Sjögren syndrome best illustrated the transformation from a benign autoimmune process to malignancy (Mackay and Rose, 2001). The risk generally has been stated at 5% of those with Sjögren syndrome developing lymphoma over their lifetime, or a 44-fold increased risk compared with the general population, but some studies place the risk as high as 10%, and a realistic figure would be in a range from 6–10% (Ramos-Casals et al., 2000). In those individuals with a disease onset before age 45 years, the risk is estimated at a 60-fold increase over that of the general population (Kassan et al., 1978).

The most common type of lymphoma occurring in Sjögren syndrome is a B-cell NHL of the mucosa-associated lymphoid tissue (MALT) type. The salivary glands are the most common initiating site, but lymphomas also develop in the cervical nodes, lungs, and elsewhere, including the gastrointestinal tract.

Autoimmune Thyroid Disease

Hashimoto's thyroiditis is a lymphoproliferative autoimmune disease that often coexists with Sjögren syndrome, rheumatoid arthritis, SLE, or other autoimmune disorders and confers an increased risk of lymphoma. Thyroid lymphomas in a Denmark population cohort showed an incidence of 2 cases in 1 million per year, with 66% of those having pre-existing Hashimoto's thyroiditis (Pedersen and Pederson, 1996), and a risk of 40–80 times that of the general population. Numbers derived from a Swedish Cancer Register concur with a clear correlation and also note a relatively low overall risk of developing thyroid lymphoma for those with Hashimoto's thyroiditis, at less than 1% (Holm et al., 1985). While thyroid lymphoma is an unusual neoplasm, it most often is seen in patients with Hashimoto's thyroiditis (Hyjek and Isaacson, 1988). Similar to the lymphomas found more frequently in Sjögren syndrome, those with autoimmune thyroid disease are identified as B cell and of the MALT type. They also share a histologic feature in which centrocyte-like (CCL) B cells surround reactive follicles forming lymphoepithelial lesions (Hyjek et al., 1988; Isaacson, 1992; Mackay and Rose, 2001).

Autoimmune Hemolytic Anemia

A clear association with B-cell lymphomas is also evident in autoimmune hemolytic anemia (Sokol et al., 1981). Autoimmune hemolytic anemia (AIHA) may be marked in some patients by diffuse and extensive lymphadenopathy, a risk factor for development of lymphoma. In one cohort of AIHA patients, 18% developed malignant lymphoproliferative disorders, and development of serum monoclonal IgM protein was noted as a significant predictive factor (Sallah et al., 2001).

Rheumatoid Arthritis

Patients with rheumatoid arthritis (RA) face an increase in relative risk for lymphoma of 1.5–8.7 that of the general population (Prior, 1985; Ehrenfeld et al., 2001). The risk is cited as much higher for men than women. An hypothesis recently put forward on this disparity suggests that a lack of genetic protection against development of autoimmune

disease in such men might contribute to their susceptibility to develop lymphoproliferation and malignancy (Whelan and Scadden, 2004). However, gathering data on the prevalence of lymphoma in RA patients has become increasingly complicated because of use of treatments such as TNF inhibitors and immunosuppressive drugs that may themselves increase the risk of lymphoma.

Rheumatoid arthritis is associated with a range of hematologic and other malignancies, including NHL, Hodgkin's disease, and lung cancer, but conversely has a negative association with colorectal cancer (Mellemkjaer et al., 1996). Rheumatoid arthritis is notably correlated with development of diffuse large B-cell lymphoma (DLBCL) (Baecklund et al., 2003), and with acute nonlymphoblastic leukemia and chronic myelogenous leukemia (Gridley et al., 1993). A large database from Sweden disclosed that, while its patients with RA (76,527) did have an increased risk of lymphoma, this overall risk was not evident in first degree relatives (70,290), giving little indication of notably co-inherited risk factors or of environmental effects. This lymphoma was seen as the direct consequence of the inflammation or the treatment of RA (Ekström et al., 2003).

Systemic Lupus Erythematosus

Systemic lupus erythematosus shares with other autoimmune disorders some pathogenic mechanisms that can induce lymphoma development. In addition, lymphadenopathy is a common feature in lupus, further increasing the risk. The risk of developing NHL in SLE has been commonly cited at 5.2% (Mellemkjaer et al., 1997). Non-Hodgkin lymphoma is the most prevalent malignancy in SLE (Abu-Shakra et al., 1996). In patients with SLE, the occurrence of all types of malignancy lies within a broad range of 2.5–13.8% of affected individuals (Abu-Shakra et al., 2002).

Celiac Disease

Patients with celiac disease experience a higher incidence of malignant diseases, 8.4%, and this is even higher, 22.2%, in older patients (Freeman, 2004). Most of the malignancies are NHLs (Catassi et al., 2002). However, these are dissimilar from the B-cell lymphomas that most commonly occur in other autoimmune diseases, since the most prevalent malignancy is a small bowel T-cell lymphoma (Green and Jabri, 2003; Smedby et al., 2005). This enteropathy-associated T-cell lymphoma (EATL) has several notable features (Catassi et al., 2005). It is a substantial clinical problem, as the outcome is poor, although the relative risk for intestinal lymphoma conferred by celiac disease (<5.0) is less than hitherto thought. It accounts for some 35% of all small bowel lymphomas and occurs after many years of latency, and hence mostly in older adults. The immunophenotype of EATL is $CD3^+$, $CD4^-$, $CD8^-$ and $CD103^+$; immunophenotyping suggests a clonal proliferation of intraepithelial lymphocytes derived from $\alpha\beta$ rather than $\gamma\delta$ T cells. A gluten-free diet seems protective. Thus, as for autoimmune-based B-cell lymphomas, chronic antigenic stimulation (of T cells) is presumably provocative. A genetic anomaly, germline or somatic, has not been implicated.

Other Autoimmune or Immune-Mediated Diseases Associated with Lymphoma

A high frequency of hematologic malignancies has been reported in patients diagnosed with Henoch–Schönlein purpura after the age of 40 years. In one study, more than one-third of such patients developed a hematologic malignancy, and 10% of those were lymphomas, either of the non-Hodgkin or Hodgkin type (Pertuiset et al., 2000).

Non-Hodgkin lymphoma is reported in association with other autoimmune diseases, but large-scale epidemiological studies and further mechanistic investigations are necessary to sustain such associations. Those cited in coincident case reports or small-scale epidemiology studies but that remain inconclusive include Crohn's disease (Mellemkjaer et al., 2000; Raderer et al., 2004); Wegener's granulomatosus (Cohen et al., 2004); eczema and psoriasis (Zhang et al., 2004); polymyositis and dermatomyositis (Leandro and Isenberg, 2001; Varoczy et al., 2002); and Addison's disease and autoimmune hepatitis (Varoczy et al., 2002). Hematopoietic cancers, and especially myeloid leukemia, are increased by 20% in patients with inflammatory bowel disease, with a moderately increased risk of lymphoma found in Crohn's disease (Askling et al., 2005). Cancer in general, including NHL, is reported to occur more frequently in systemic sclerosis (scleroderma) (Rosenthal et al., 1995; Leandro and Isenberg, 2001; Varoczy et al., 2002; Pearson and Silman, 2003). Interestingly, in a recent review of 421 patients with NHL, as many as 7.6% had evidence of an autoimmune disease, most frequently Sjögren syndrome, and the autoimmune disease preceded the diagnosis of lymphoma in 70% of the associated cases (Varoczy et al., 2002).

Types of Lymphoma in Autoimmune Diseases

It is the non-Hodgkin B-cell type of lymphoma that is most closely linked with autoimmune disease, and MALT-type lymphomas are especially correlated with Sjögren syndrome and Hashimoto's thyroiditis. However, Hodgkin disease also appears in the context of autoimmune disease, and additional hematologic malignancies have been cited as occurring in an autoimmune disease setting, including multiple myeloma, B-cell chronic lymphocytic leukemia

(B-CLL), and Waldenstrom macroglobulinemia. T-cell lymphoma is unusual among autoimmune diseases except for celiac disease.

An interesting comparison was made by Bende et al. (2005) of amino-acid sequences of the IgV_H-CDR3 of B-cell lymphomas with sequences in GenBank. The repertoire expressed by lymphomas aligned with that of normal B cells except for salivary and gastric MALT lymphomas wherein there was strong CDR3 homology to rheumatoid factors, indicative of selection for reactivity to autologous Fc of IgG; this did not pertain however for MALT lymphomas with the (11;18) translocation. This supports the idea of lymphomagenic effects of chronic antigenic stimulation (Mackay and Rose, 2001) in the above examples, by Fc of IgG in complexes of IgG-ANA (salivary glands), or of IgG-*Helicobacter pylori* (stomach).

PATHOGENIC MECHANISMS

Lymphoma can develop in the context of chronic antigenic stimulation initiated or prolonged by autoimmune disease and/or infection. Viral or bacterial influences have a suspected etiologic role, and genetic aberrations also are involved.

Autoimmunity and Chronic Inflammation

A basic characteristic of autoimmune disease is long-term stimulation of the immune system and chronic inflammation, which augment the potential for development of lymphoma.

Viral and Bacterial Associations

Viruses and bacteria are linked with the development of numerous autoimmune, rheumatic, and related diseases and are also linked with the development of lymphoma. Therefore, infection has become a prime target in searching for an autoimmune-lymphoma link. Particularly in the past 15 years, investigators have recognized and legitimized the role that viruses and bacteria might play in either autoimmune disease or lymphoma (Parsonnet et al., 2004). Viruses and bacteria with a noted link with lymphoma cross a broad range of organisms.

The herpes-type Epstein–Barr virus (EBV) was the first human virus to be associated with malignancy, specifically Burkitt's lymphoma, and later with Hodgkin disease and primary central nervous system lymphoma. Epstein–Barr virus has also been investigated in the etiology of autoimmune diseases, but no direct link has been proven yet, either with autoimmunity itself or with lymphoma in autoimmune disease. Links with other herpes viruses have been established, such as human herpes virus (HHV8), which has been linked with lymphoma and lymphoid hyperplasia in autoimmune disease.

Hepatitis C virus (HCV) is a single-stranded RNA virus that has demonstrated a clear correlation with both autoimmune features and malignancy. In fact, identification of HCV in autoimmunity and lymphoma underlines the triple association between autoimmunity, infection, and cancer (Ramos-Casals et al., 2004). This association is clearest in specific geographic areas in which HCV is prevalent. Hepatitis C virus is most closely associated with B-cell lymphoproliferative disorders, and specifically with B-cell NHL, and also with type II mixed cryoglobulinemia (Ramos-Casals et al., 2004; 2005b).

Simian virus 40 has been linked to non-Hodgkin's lymphoma (Vilchez et al., 2004). *H. pylori* is another microorganism, a bacterium, that is clearly established in the etiology of malignancy, specifically gastric MALT lymphoma (Wotherspoon et al., 1991). *H. pylori* can lead to B-cell lymphocytic infiltration of the epithelium, a characteristic of MALT. The bacterium *Borrelia burgdorferi* causes Lyme disease and is also associated with MALT lymphoma.

In addition to specific infection being linked with immune dysfunction, persistent infection leads to chronic immune stimulation, which influences the development of lymphoma. Chronic stimulation of B cells, such as rheumatoid factor synthesizing B cells, most likely launches the process of lymphomagenesis in autoimmune disease as well as chronic infection (Marietta, 2001).

Potential Role of Apoptosis and the Tumor Necrosis Factor Family

Current research into how the immune system malfunctions and promotes development of autoimmune disease and malignancy centers on apoptosis, or programmed cell death, and the impact on apoptosis of the TNF family. The TNF-associated ligands and receptors for those ligands play a powerful role in maintaining or disrupting immune homeostasis.

The autoimmune lymphoproliferative syndrome (ALPS) is a classic example of how dysregulated apoptosis can lead to autoimmune disease and potentially to malignancy (see Chapter 70). In ALPS, gene mutations result in defective apoptosis, leading to abnormal lymphocyte accumulation and survival of lymphocytes that would normally be eliminated, including autoreactive lymphocytes and those that might transform into malignant cells. Lymphocytic accumulation in ALPS causes lymphadenopathy and splenomegaly, and supervening complications include autoimmune hemolytic anemia, thrombocytopenia, and neutropenia, and in some cases lymphoma. The etiopathogenesis in ALPS depends on mutations of CD95 (Fas) and CD178 (Fas ligand) genes, the specific involvement of

heterozygous mutations in CD95, and survival of an increased number of double-negative αβ $CD3^+CD4^-CD8^-$ T cells (Straus et al., 1999; 2001; Bleesing, 2003; Bleesing et al., 2001).

Defective apoptosis may likewise participate in other autoimmune diseases and in the development of lymphoma, and, in these cases, TNF family molecules are critical to dysregulation. The B-cell activating factor (BAFF; also referred to as B-lymphocyte-stimulator or BLyS) is one such member of the TNF family and promotes the survival and maturation of B cells, and, as such, is vital to normal B-cell development. However, excess BAFF results in impaired apoptosis and allows too many of the wrong kinds of B cells to survive, including those that are autoreactive and those predisposed to neoplasia. The critical checkpoint for BAFF activity most likely occurs during B-cell maturation in the spleen (Groom et al., 2002; Batten et al., 2004). Besides allowing potentially malignant B cells to survive at this vital point at which they should be eliminated, excess BAFF can be produced by malignant cells (Mackay and Tangye, 2004) and so would continue to promote the development of both B-CLL and NHL. The level of BAFF increases as malignancy becomes more aggressive (Novak et al., 2004).

Elevated serum levels of BAFF are evident in B-cell non-Hodgkin lymphoma (Briones et al., 2002; Batten et al., 2004). Similar high levels are seen in some autoimmune diseases, particularly those in which autoantibodies and lymphoproliferation are key features. Particular examples include Sjögren syndrome, SLE and RA (Groom et al., 2002; Mariette et al., 2003; Batten et al., 2004; Szodoray et al., 2004; Mackay et al., 2005). In contrast, normal levels of BAFF were found in primary biliary cirrhosis and autoimmune adult-onset type 1 diabetes, diseases in which B-cell proliferation is less evident (Mackay et al., 2002). B-cell-activating factor is implicated in the polyclonal activation of B cells, a characteristic of Sjögren syndrome (Lavie et al., 2004), and, not surprisingly, BAFF levels in Sjögren syndrome increase according to the severity of disease and the degree of systemic involvement, with levels linked to hypergammaglobulinemia (Szodoray et al., 2004), lymphoproliferation, and higher levels of autoantibodies (Mariette et al., 2003).

Another TNF family member related to BAFF is an apoptosis-inducing ligand known as APRIL. This member shares receptors with BAFF, of which three have been identified to date (Jelinek and Darce, 2005), but BAFF and APRIL maintain different functions. APRIL is involved in T-cell survival and T-independent antigen responses, has been discovered in some tumor cell lines and tissue, and can induce proliferation of non-lymphoid cells (Mackay and Ambrose, 2003; Mackay et al., 2003). Both BAFF and APRIL promote the growth and survival of some B-cell malignancies (Jelinek and Darce, 2005). In some non-Hodgkin lymphomas, BAFF and APRIL further contribute to the survival of malignant cells by upregulating c-Myc, which promotes cell proliferation by downregulating p53, which is an inhibitor of cell proliferation, and increasing Bcl-6 which arrests B-cell differentiation (He et al., 2004). Our knowledge and opportunities in research will certainly expand greatly as additional members of the TNF family are identified and their roles established in immune tolerance, autoimmunity, and malignancy (Mackay and Kalled, 2002).

In RA, BAFF is found not only in the serum but in the synovial fluid, and, in Sjögren syndrome, excess BAFF is also upregulated in the affected salivary glands and is expressed in T-cells at the site of salivary gland damage (Lavie et al., 2004). The appearance of BAFF in sites where autoimmune damage occurs in these diseases suggests a potential role for BAFF in their pathogenesis.

Marginal zone (MZ) B-lymphocytes also appear to have a role in autoimmunity, inflammation, and tissue damage (Mackay et al., 2005) (see Chapter 11). They form a separate spleen compartment, which most likely becomes a reservoir for autoreactive B cells (Groom et al., 2002). In addition to the spleen, MZ B cells also have been detected in the lymph nodes, blood, and salivary glands of BAFF-transgenic (TG) mice (Batten et al., 2004), with MZ-like infiltrates present in the salivary glands of this mouse model (Groom et al., 2002). Similar cells have also been detected among B-cell infiltrates in the thyroid gland of patients with Graves' disease (Segundo et al., 2001).

It is becoming increasingly apparent that the balance of many elements is critical to immune homeostasis. Tumor necrosis factor of itself provides a protective element in preventing B-cell proliferation, while TNF family members BAFF and APRIL, in usual amounts, are important for normal apoptosis. However, when BAFF and APRIL are produced in excess, they cause B-cell proliferation. Interestingly, in a recent study, TNF-deficient BAFF-TG mice developed autoimmune disease in spite of the lack of TNF, and B-cell lymphoma increased in those mice by 35% (Batten et al., 2004). These findings demonstrate that TNF might not necessarily be involved in the mediation of autoimmune disease, but it certainly does have a crucial role in preventing malignancy. Further study of the TNF family molecules and interactions among these members holds great potential for developing new therapeutics and understanding the complications of others.

Genetics

Genes and their products can affect the way the immune system functions and reacts to environmental triggers, so it is not surprising that genetic factors contribute to the susceptibility of developing autoimmune disease as well as cancer. Key research areas in disease development involve the complex interaction of susceptibility genes and defects

in the regulation of those genes. New frontiers include the potential role of genetic aberrations to cause dysregulation of apoptosis and so influence oncogenic events, such as p53 mutations and Bcl-2 chromosomal translocations, and the runt-related family (RUNX) of genes associated with autoimmunity.

Genetic Aberrations of p53 and Bcl-2

Genetic irregularities are linked with defective apoptosis, which has been shown to determine malignancy and autoimmunity. These aberrations appear to promote apoptosis via the cell-surface receptor for CD95 (Fas) (Hale et al., 1996) and include inactivation of tumor suppressor gene p53 and overexpression of Bcl-2.

Mutations of the p53 gene can induce abnormal cell growth and increase cell survival and can promote progression of certain B-cell lymphomas. Such mutations are frequently found in connection with human tumors, and anti-p53 antibodies appear in sera of patients with a variety of malignancies, occurring in about half of all human cancers (Ichikawa, 2000). In one study, p53 dysfunction was evident in 22% of cases of aggressive B-cell lymphoma (Ichikawa et al., 1997). MALT lymphoma and its progression from low-grade to high-grade have also been linked to p53 mutations (Du et al., 1995). Interestingly, cancer patients can develop features of autoimmune diseases with production of autoantibodies targeted to autoantigens involved in oncogenesis such as p53 (Abu-Shakra et al., 2001).

Further evidence of the interplay between genetics, malignancy, and autoimmunity is seen in p53 recognition of DNA damage (Herkel et al., 2000). Patients with SLE may produce antibodies to p53, and, together with anti-DNA antibodies often found in SLE, can block p53 function, thereby affecting apoptosis in those patients (Herket et al., 2001). Finally, additional research into the impact of p53 is expanding to other p53 family members and target genes of p53, which are also involved in regulation of apoptosis (Fridman and Lowe, 2003; de Stanchina et al., 2004; Rosenblum et al., 2004).

Bcl-2 is another genetic protein involved in apoptosis and malignancy and contributes to development of NHL when overexpressed. It is specifically correlated with B-cell NHL and later stage disease, with evidence of Bcl-2 activation in 51% of cases of B-cell NHL, compared with 17% of T-cell NHL, and with evidence that 44% of the patients had stage III-IV disease (Hermine et al., 1996).

Aberrations in Chromosomal Translocations

A clear correlation between MALT lymphoma and three chromosomal translocations has been established, providing additional evidence that genetic aberrations can permit lymphoproliferation by allowing malignant cells to evade apoptosis. Those translocations include t(11;18)(q21;q21); t(1;14)(p22;q32); and t(14;18)(q32;q21). The occurrence of each translocation appears to be mutually exclusive and identified with specific primary sites, t(11;18)(q21;q21) being connected with those lymphomas originating in gastric and pulmonary sites, and t(14;18)(q32;q21) with those appearing in ocular adnexa, salivary glands, and the skin (Streubel et al., 2004c). When the t(11;18)(q21;q21) translocation is associated with gastric MALT lymphoma, it is also closely correlated with *H. pylori* infection of the stomach; in fact, the translocation aberrations are influenced by the disease or infection that precedes transformation to malignancy (Ye et al., 2003).

Development of lymphoma most likely involves a complex interplay between the chromosome translocations, MALT1, nuclear factor κB (NFκB), and BCL10, and may also involve p53 regulation. The t(11;18)(q21;q21) chromosomal abnormality leads to fusion between AP12 and MALT1 genes, which is known to activate NFκB and in turn inhibit p53, thereby establishing a link between NFκB signaling pathways and inhibition of p53-mediated apoptosis (Stoffel and Levine, 2004; Stoffel et al., 2004). The role of NFκB in apoptosis is clearly an emerging area for study in this intricate interaction. Another MALT lymphoma-associated molecule, BCL10, may be deregulated by t(1;14)(p22;q32) (Isaacson, 2005) and t(14;18)(q32;q21) (Ye et al., 2005). The MALT1 gene is an important regulator of BCL10 (Ruland et al., 2003), which is essential for NFκB activation and, when overexpressed, interferes with normal apoptosis (Zhang et al., 1999).

Finally, microvascular endothelial cells in B-cell lymphomas may have a genetically close relationship with the lymphoma and display tumor-related characteristics. The endothelial cells in such lymphomas often display lymphoma-specific chromosomal translocations, although the precise mechanisms for this are not clear (Streubel et al., 2004a).

A Role for the Runt-Related Proteins

The runt-related proteins (RUNX) have been linked with both autoimmune disease and cancer. These proteins are transcription factors that regulate genes and include the RUNX1, RUNX2, and RUNX3 members that are each linked with malignancy (Ito, 2004). RUNX1 is associated with acute myelogenic leukemia (Yamada et al., 2004) and RUNX3 with gastric cancer. It is likely, however, that more than a single RUNX member may be involved in etiology. For example, two RUNX members in addition to RUNX3 have recently been linked with gastric cancer (Sakakura et al., 2005), demonstrating a role for multiple RUNX members in malignancy and an even more complex and widespread involvement of RUNX members than previ-

ously thought. Additional evidence demonstrates that the interaction of one or another RUNX members can impact others, creating an additional mechanism for influencing disease development (Spender et al., 2005).

It is of particular interest that significant associations have been found between RUNX1 and development of at least three autoimmune diseases: psoriasis, SLE, and RA (Alarcón-Riquelme, 2004). RUNX1 is correlated with defective regulation of genes linked to these autoimmune disorders. In psoriasis, strong suspicion has been cast on RUNX1 as a cause of defective regulation of two genes, SLC9A3R1 and NAT9, which are linked to this disease (Helms et al., 2003). Similarly, dysregulation by RUNX1 of a gene associated with RA, SLC22A4, likely contributes to the development of that disease (Tokuhiro et al., 2003; Yamada et al., 2004). Systemic lupus erythematosus is associated with RUNX1 and the programmed cell death 1 gene (PDCD1) (Prokunina et al., 2002); PDCD1 has also been associated with development of RA (Prokunina et al., 2004).

How does RUNX cause dysregulation? Regulatory single nucleotide polymorphisms (rSNPs) are believed to affect the binding site for RUNX and thereby regulate the expression of genes (Alarcón-Riquelme, 2003). These rSNPs are involved in loss of the binding site, which influences the autoimmune disease-linked genes and leads to disease progression. In the case of psoriasis, five rSNPs have been identified as likely candidates for affecting RUNX binding sites, whereas in RA, specific rSNPs have not yet been identified, although the RUNX1 link is clear. Much remains to be learned, but the role of RUNX in autoimmunity and malignancy provides numerous potentially fruitful avenues for research.

THE CLINICAL SETTING

Risk Factors

Immune impairment of itself creates a risk for malignancy. Therefore, individuals with any autoimmune or rheumatic disease, or even with rheumatoid factor, or undergoing immunosuppressive therapy, should be assessed for additional risk factors and actual development of lymphoma. Age, disease duration and severity, and environmental influences confer a higher risk. In addition, particular symptoms or laboratory findings, medications used, and genetic profiling can predict susceptibility to lymphoma.

The risk of lymphoid malignancy increases with age (Mackay and Rose, 2001; Sallah et al., 2001). The younger the individual at the onset of autoimmune disease, the higher will be the risk (Kassan et al., 1978; Ramos-Casals et al., 1998). The propensity to develop lymphoid malignancy also increases with autoimmune disease duration. This link is well documented in Sjögren syndrome (Ioannidis et al., 2002), rheumatoid arthritis (Gridley et al., 1993; Mellemkjaer et al., 1996), and in ALPS (attributed to Elaine Jaffey, Bethesda, MD, by Mackay and Rose 2001). The diagnosis of the associated autoimmune disease usually precedes that of NHL, implying that the sustained antigen drive associated with autoimmune disease predisposes to malignancy. Severity of disease generally appears to correlate with a risk for lymphoma, but the link between severity and lymphoproliferation in the case of SLE was not established (Ehrenfeld et al., 2001).

Serologic results found in autoimmune disease can also be a feature of malignancy. Thus antinuclear antibody (ANA) can indicate a risk for non-Hodgkin lymphoma. Also, ANA may be increased in patients with NHL following treatment, and, more importantly, they are found in a substantial number of patients with NHL prior to treatment for malignancy and hence are independent of treatment factors. Among NHL patients, 19% had evidence of ANA before any treatment, with the highest incidence in marginal zone MALT lymphomas (Guyomard et al., 2003). Laboratory tests often performed in autoimmune disease and correlating with risk of development of NHL include positive results for cryoglobulins, hypergammaglobulinemia, monoclonal gammopathy, Coombs' antiglobulin test, antibodies to neutrophils and platelets, and complement deficiency.

Cryoglobulinemia is correlated with palpable purpura, often secondary to cutaneous vasculitis and a risk factor for NHL. In addition, when evidence of HCV, of itself linked with malignancy, is coupled with cryoglobulinemia, it can herald transformation from benign lymphoproliferation to a malignant process. When records of patients with cryoglobulinemia were examined in one study, 5% developed a hematologic malignancy; of those, HCV was evident in 52% and both HCV and autoimmune disease in 48% (Trejo et al., 2003). While monoclonal immunoglobulins can occur in the absence of lymphoma, they are linked with development of lymphoma. More patients with Sjögren syndrome, compared with those with SLE or RA, have evidence of mixed monoclonal IgM immunoglobulin (Tzioufas et al, 1986, 1996). Monoclonal gammopathy is increased in 25% of those with autoimmune hemolytic anemia, which increases their risk of lymphoma as well as affecting survival (Sallah et al., 2000).

Non-standard laboratory tests also can forecast risk of malignancy. Such correlations with B-cell lymphoma include increased levels of Blys/BAFF in serum or synovial fluid (Cheema et al., 2001), and this is also indicative of reduced survival (Novak, 2004); increased levels of serum β2-microglobulin or Bcl-2 (Hermine et al., 1996); or presence of anti-p53 antibodies (Maxwell and Roth, 1994; Ichikawa, 2000). Genetic profiling will become increasingly used in the future, in helping with diagnosis, risk assessment, and prognosis. It is already known, for example, that

the chromosomal translocations t(11;18)(q21;q21) lead to fusion between AP12 and MALT1 genes, and these factors, in addition to evidence of t(1;14)(p22;q32) and t(14;18)(q32;q21), can be of prognostic value in MALT lymphoma (Okabe et al., 2003; Isaacson, 2005).

Comorbid factors linked with development of lymphoma should prompt close monitoring and scrutiny of treatment protocols. These factors include evidence of specific viral or bacterial infections, as mentioned in the section on pathology, such as HCV or *H. pylori*; exposure to ultraviolet radiation (Fisher and Fisher, 2004); and use of immunosuppressive drugs (Ehrenfeld et al., 2001; Zhang et al., 2004).

Specific symptoms can identify patients at increased risk of developing lymphoma. Evidence of lymphoproliferation, lymphadenopathy, splenomegaly, vasculitis, and palpable purpura in autoimmune disease are correlated with risk for lymphoma. In Sjögren syndrome, an additional risk factor is persistent parotid enlargement; a recent study found that 84% of patients with Sjögren syndrome who developed lymphoma had bilateral parotid gland swelling (Jonsson et al., 2001). The presence of anti-SSA (or Ro) autoantibodies is a serologic marker for Sjögren syndrome (and also SLE) and increases the likelihood of developing those symptoms that are predictive of lymphoma, such as peripheral neuropathy (Voulgarelis et al., 1999). In Sjögren syndrome, lymphoproliferative disease (LPD) and the likelihood of progression to lymphoma are significantly higher in those patients with palpable purpura and low C4 levels early in the disease (Ioannidis et al., 2002); in fact, all patients in the Ioannidis study who developed lymphoma had palpable purpura and low C4 during their first clinic visit and others also report that low complement levels in Sjögren syndrome, including low C3, C4 and/or CH50, are linked with a higher probability of systemic disease and lymphoma (Ramos-Casals et al., 2005a).

Treatment

The long-term goal is to prevent the development of lymphoma in the first place. The first line then is to control the autoimmune disease and reduce the hyperactivity of autoreactive B cells (Mariette, 2001). The newest biologic response modifiers used to treat autoimmune disease include inhibitors of TNF, a class of drugs that includes etanercept and infliximab. Anti-TNF has proven successful in some autoimmune disorders and specifically approved for use in rheumatoid arthritis and Crohn's disease (Feldmann and Maini, 2001; Maini et al., 2002). It may also be beneficial in a broader range of autoimmune disorders, since successful trials have already been reported for ankylosing spondylitis, psoriasis, and psoriatic arthritis (Feldmann et al., 2004; Taylor et al., 2004). Sometimes, TNF inhibitors are combined with more conventional therapies for autoimmune disease to obtain better results (Lipsky et al., 2000). When an environmental agent is implicated in NHL development, its eradication may result in resolution of the malignancy, e.g., resolution of MALT lymphoma when *H. pylori* is eliminated (Arima and Tsudo, 2003). *H. pylori,* however, is less amenable to treatment in the setting of gastric autoimmune disease (Raderer et al., 2001; 2003), likely because chromosomal aberrations as in MALT lymphoma have occurred (Streubel et al., 2004b).

Therapy for NHL most recently has entailed a combination of chemotherapeutic drugs, with optimal combinations, amounts, and schedule of delivery still being sought. Chemotherapy with ACVBP (dose-intensified doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone) has been found to be more effective than CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) in a study of 747 patients with localized aggressive B-cell lymphomas (Reyes et al., 2005). Current breakthroughs in genetically engineered human-mouse chimeric monoclonal antibodies (MAbs) and radioimmunotherapy offer additional options and may be combined with one another and with chemotherapy. The first MAb to become available, rituximab (Rituxan), was approved by the U.S. Food and Drug Administration (FDA) in 1997, and seven MAbs have quickly followed. Rituximab, an anti-CD20 MAb, rapidly depletes B cells and downregulates Bcl-2 expression, rendering malignant cells more sensitive to chemotherapy (Alas et al., 2002; Bosly et al., 2002; Chanan-Khan et al., 2004). Bcl-2 regulation is especially critical in view of the connection between Bcl-2 overexpression and an aggressive clinical course, resistance to therapy, and ultimately a poor disease outcome (Chanan-Khan et al., 2004). Combinations of MAbs and chemotherapy may be even more effective than either alone, as is the case with CHOP plus rituximab in diffuse large B-cell lymphomas (Czuczman et al., 1999; 2005; Vose et al., 2001; Coiffier et al., 2002; 2004; Feugier et al., 2005).

The use of rituximab and other MAbs in treating autoimmune diseases (see Chapter 76) may not only control the disease but also prevent the lymphoproliferative overlay and complications that predispose to lymphoma. While rituximab has proven effective in many autoimmune disorders (Specks et al., 2001; Gorman et al., 2003; Gottenberg et al., 2004; Rastetter et al., 2004), costs and adverse effects (immunosuppression) could preclude its routine use.

One of the latest therapeutic approaches combines a MAb with radioimmunotherapy, which allows the targeting of radiosensitivity of malignant B cells (Connors, 2005; Kaminski et al., 2005). The first radioimmunotherapy, and first conjugated antibody accepted for NHL treatment, is Yttrium-90 (^{90}Y) ibritumomab tiuxetan (Zevalin), approved by the FDA in 2002 (Grillo-Lopez, 2002) and in Europe in 2004. It is primarily used for low-grade or follicular NHL and in relapsed B-cell NHL (Grillo-Lopez, 2002; Witzig,

2004). Additional therapeutic developments have followed, including tositumomab (Bexxar), a chimeric anti-CD20 antibody that has been coupled with a radionucleotide and is selective for follicular lymphoma (Kaminski et al., 2005), and the radiolabeled monoclonal ^{90}Y-epratuzumab or ^{90}Y-DOTA humanized anti-CD22 IgG, also promising for NHL treatment (Sharkey et al., 2003). These therapies are clearly a work in progress with much remaining to be learned about each, the effectiveness of various combinations, and the additional interplay of knowledge brought by new therapies that will be developed and new concepts and understanding of NHL brought by changing concepts of staging in NHL (Armitage, 2005).

LYMPHOMA AS A COMPLICATION OF BIOLOGIC TREATMENT OF AUTOIMMUNE DISEASE

Treatment of autoimmune disease may be implicated in causing or modulating the development of lymphoma. Therefore, benefits and risks must be weighed according to the specific autoimmune disease, patient, and proposed therapy. It has been long suspected that use of immunosuppressive drugs can increase the risk of lymphoma (Ehrenfeld et al., 2001), so a relation between cumulative exposure to immunosuppressive agents and malignancy is not surprising (Asten et al., 1999). Such publicized links have been reported recently with two ointments approved for eczema and used in other autoimmune skin disorders. In 2005, the U.S. FDA issued warnings for pimecrolimus (Elidel) and tacrolimus (Protopic), linking their use to lymphoma and skin cancer. Both are immunosuppressants, and the FDA states that the incidence of malignancy likely increases with the amount of medication given (FDA, 2005).

Patients with RA treated with nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, are at greater risk of developing NHL than patients not treated with those drugs. One study reported the highest increased risk with use of NSAIDs excluding aspirin but still found a significant risk in those taking aspirin alone (Cerhan et al., 2003). Comparatively, osteoarthritis patients had no evidence of increased risk of lymphoma in those taking NSAIDs or aspirin.

Concern has arisen over development of malignancy following the use of cytotoxic (anticancer) drugs, methotrexate, and anticytokine therapies such as TNF and interleukin 1 (IL-1) inhibitors. A 10-fold increase in RA patients taking the cytotoxic drug azathioprine has been reported, compared with a 5-fold increase in RA patients not taking the drug (Silman et al., 1988). Suspicion centers on the possibility that the drug might induce chromosomal abnormalities or reactivate latent viral infections (Ehrenfeld et al., 2001). The disease modifying antirheumatic therapy (DMARD), methotrexate, is tentatively linked with lymphoproliferative disease and lymphoma, although a link is not certain (Salloum et al., 1996). Some reports demonstrate no clear connection between immunosuppression and lymphoma, with the exception of a small group of patients with RA or polymyositis who developed EBV-linked NHL while taking methotrexate (Mariette et al., 2002; Feng et al., 2004). Overall, however, little support exists for an increased risk of malignancy with use of methotrexate (Baecklund et al., 2004; Wolfe and Michaud, 2004).

Similar questions have been raised about TNF inhibitors and lymphoma. Patients with RA were reported as having a 4.9% increased risk of lymphoma when taking anti-TNF agents compared with those not taking the drug in one study, although the investigators cited a need for additional studies (Geborek et al., 2005), and no increased risk was found in another study (Askling et al., 2005b). The TNF blockers infliximab (Remicade), etanercept (Enbrel), and adlimumab (Humira), as well as the IL-1 inhibitor anakinra (Kineret), have been linked to lymphoma and include this risk in prescribing warning labels (Fleischmann et al., 2004).

While an increased risk of lymphoma appears evident with these medications, a clear and definite relationship remains to be proven. Patients with autoimmune disease already are predisposed to malignancy, use of immunosuppressive agents increase this predisposition, and the anti-TNF and anti-IL-1 agents bring additional immunosuppression into the equation. It is difficult to determine cause when natural predilection, medication history, and use of new biologics are involved. Only additional and larger studies will elucidate the possible association between particular therapies and the subsequent development of lymphoma.

A LOOK TO THE FUTURE

We are on the verge of an exciting and new generation of therapeutics for lymphoma. The future for treatment of non-Hodgkin lymphomas will build on what we are just beginning to learn about specific molecular targets and methods of delivery. In addition to creating new anticytokine agents and monoclonal antibodies and improving radioimmunotherapy, other areas of current and future interest will continue to evolve. These include STAT3 pathway inhibitors that might lead to a new class of targeted therapeutics (Alas and Bonavida, 2001; 2003; Alas et al., 2001) and the continuous search for different mechanisms to inhibit TNF and suppress overexpression of Bcl-2. Vaccines provide another potential treatment strategy to prevent relapses in those who have developed lymphoma. Vaccines can use the patient's own immune system against malignancy and provide a less toxic therapy (Hsu et al., 1997; Bendandi et al., 1999; Dar and Kwak, 2003).

Ultimately, the goal for the future is to tailor the therapy directly to the disease and the patient. As we learn more about pathogenesis of autoimmune disease and lymphoma, we can better understand how autoimmunity transforms from a benign to a malignant state so that this transformation might be prevented. Gene microarrays may lead to our ability to better predict susceptibility to lymphoma in autoimmune disease. New methods of examination providing a precise molecular diagnosis will allow us to recognize the differences among lymphomas that appear similar in histopathology but are indeed quite different (Cheson, 2004). These advances will lead to better classification of lymphoma and enable us to determine those therapies that have the greatest chance of success.

Gene expression profiling will be used increasingly in the future to help predict outcome and determine treatment. Already, the identification of ZAP70 in diffuse large B-cell lymphoma has provided a prognostic marker for that disease and can establish who might benefit from a specific chemotherapy treatment and who might not (Rosenwald, 2003; Rosenwald and Staudt, 2003; Rosenwald et al., 2002). As we continue to develop and better understand genetic microarrays and how specific genetic profiles might affect response to different treatments and overall prognosis, we can modify treatment protocols to fit the individual patient. The future should bring greater understanding of the pathogenesis of autoimmune disease and lymphoma, the impact of genetic profiling, and the promise of new biologics or other agents in treating these diseases.

References

Abu-Shakra, M., Gladman, D.D., and Urowitz, M.B. (1996). Malignancy in systemic lupus erythematosus. *Arthritis Rheum. 39*(6), 1050–1054.

Abu-Shakra, M., Buskila, D., Ehrenfeld, M., and Shoenfeld, Y. (2001). Cancer and autoimmunity: autoimmune and rheumatic features in patients with malignancies. *Ann. Rheum. Dis. 60*(5), 433–441.

Abu-Shakra, M., Ehrenfeld, M., and Shoenfeld, Y. (2002). Systemic lupus erythematosus and cancer: associated or not? *Lupus 11*(3), 137–144.

Alarcón-Riquelme, M. (2003). A RUNX trio with a taste for autoimmunity. *Nat. Genet*. *4*, 299–300.

Alarcón-Riquelme, M. (2004). Role of RUNX in autoimmune diseases linking rheumatoid arthritis, psoriasis and lupus. *Arthritis Res. Ther. 6*(4), 169–173.

Alas, S. and Bonavida, B. (2001). Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl–2 and sensitization to cytotoxic drugs. *Cancer Res. 61*(13), 5137–5144.

Alas, S. and Bonavida, B. (2003). Inhibition of constitutive STAT3 activity sensitizes resistant non-Hodgkin's lymphoma and multiple myeloma to chemotherapeutic drug-mediated apoptosis. *Clin. Cancer Res. 9*(1), 316–326.

Alas, S., Emmanouilides, C., and Bonavida, B. (2001). Inhibition of interleukin 10 by rituximab results in down-regulation of bcl-2 and sensitization of B-cell non-Hodgkin's lymphoma to apoptosis. *Clin. Cancer Res. 7*(3), 709–23.

Alas, S., Ng, C.P., and Bonavida, B. (2002). Rituximab modifies the cisplatin-mitochondrial signaling pathway, resulting in apoptosis in cisplatin-resistant non-Hodgkin's lymphoma. *Clin. Cancer Res. 8*(3), 836–45.

Arima, N. and Tsudo, M. (2003). Arima extragastric mucosa-associated lymphoid tissue lymphoma showing the regression by *Helicobacter pylori* eradication therapy. *Br. J. Haematol. 120*(5), 790–792.

Armitage, J.O. (2005). Defining the stages of aggressive non-Hodgkin's lymphoma—a work in progress. *N. Engl. J. Med. 352*(12), 1250–1252.

Armstrong, M.Y.K., Gleichmann, E., Gleichmann, H., Beldotti, L., Andre-Schwartz, J., and Schwartz R. (1970). Chronic Allogeneic Disease: II. Development of Lymphomas. *J. Exp. Med. 132,* 417–439.

Askling, J., Brandt, L., Lapidus, A., Karlen, P., Bjorkholm, M., Lofberg, R., and Ekbom, A. (2005a). Risk of haematopoietic cancer in patients with inflammatory bowel disease. *Gut 54*(5), 617–622.

Askling, J., Fored, M., Baecklund, E., Brandt, L., Backlin, C., Ekbom, A., Sundstrom, C., Bertilsson, L., Coster, L., Geborek, P., Jacobsson, L., Lindblad, S., Lysholm, J., Rantapaa-Dahlqvist, S., Saxne, T., Klareskog, L., and Feltelius, N. (2005b). Haematopoietic malignancies in rheumatoid arthritis. Lymphoma risk and characteristics after exposure to tumour necrosis factor antagonists. *Ann. Rheum. Dis.* Oct; *64*(10):1414–20.

Asten, P., Barrett, J., and Symmons, D. (1999). Risk of developing certain malignancies is related to duration of immunosuppressive drug exposure in patients with rheumatic diseases. *J. Rheumatol. 26*(8), 1705–1714.

Baecklund, E., Sundstrom, C., Ekbom, A., Catrina, A.I., Biberfeld, P., Feltelius, N., and Klareskog, L. (2003). Lymphoma subtypes in patients with rheumatoid arthritis: increased proportion of diffuse large B cell lymphoma. *Arthritis Rheum. 48*(6), 1543–1550.

Baecklund, E., Askling, J., Rosenquist, R., Ekbom, A., and Klareskog, L. (2004). Rheumatoid arthritis and malignant lymphomas. *Curr. Opin. Rheumatol. 16*(3), 254–261.

Batten, M., Fletcher, C., Ng, L.G., Grrom, J., Wheway, J., Laâbi, Y., Xin, X., Scheider, P., Tschopp, J., Mackay, C.R., and Mackay, F. (2004). TNF deficiency fails to protect BAFF transgenic mice against autoimmunity and reveals a predisposition to B cell lymphoma. *J. Immunol. 172,* 812–822.

Bendandi, M., Gocke, C.D., Kobrin, C.B., Benko, F.A., Sternas, L.A., Pennington, R., Watson, T.M., Reynolds, C.W., Gause, B.L., Duffey, P.L., Jaffe, E.S., Creekmore, S.P., Longo, D.L., and Kwak, L.W. (1999). Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. *Nat. Med. 5*(10), 1171–1177.

Bende, R.J., Aarts, W.M., Riedl, R.G., de Jong, D., Pals, S.T., and van Noesel, J.M. (2005). Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. *J. Exp. Med. 201,* 1229–1241.

Bleesing, J.J. (2003). Autoimmune lymphoproliferative syndrome (ALPS). *Curr. Pharm. Des. 9*(3), 265–278.

Bleesing, J.J., Brown, M.R., Straus, S.E., Dale, J.K., Siegel, R.M., Johnson, M., Lenardo, M.J., Puck, J.M., and Fleisher, T.A. (2001). Immunophenotypic profiles in families with autoimmune lymphoproliferative syndrome. *Blood 98*(8), 2466–2473.

Bosly, A., Keating, M.J., Stasi, R., and Bradstock, K. (2002). Rituximab in B-cell disorders other than non-Hodgkin's lymphoma. *Anticancer Drugs 13*(Suppl 2), S25–33.

Briones, J., Timmerman, J.M., Hilbert, D.M., and Levy, R. (2002). BLyS and BLyS receptor expression in non-Hodgkin's lymphoma. *Exp. Hematol. 30*(2), 135–141.

Catassi, C., Fabiani, E., Corrao, G., Barbato, M., De Renzo, A., Carella, A.M., Gabrielli, A., Leoni, P., Carroccio, A., Baldassarre, M., Bertolani,

P., Caramaschi, P., Sozzi, M., Guariso, G., Volta, U., and Corazza, G.R. (2002). Italian Working Group on Coeliac Disease and Non-Hodgkin's-Lymphoma. Risk of non-Hodgkin lymphoma in celiac disease. *The Journal of the American Medical Association JAMA 287*(11), 1413–1419.

Catassi, C., Bearzi, I., and Holmes, G.K. (2005). Association of celiac disease and intestinal lymphomas and other cancers. *Gastroenterology 128*(4 Suppl 1), S79–86.

Cerhan, J.R., Anderson, K.E., Janney, C.A., Vachon, C.M., Witzig, T.E., and Habermann, T.M. (2003). Association of aspirin and other non-steroidal anti-inflammatory drug use with incidence of non-Hodgkin lymphoma. *Int. J. Cancer 106*(5), 784–788.

Chanan-Khan, A. and Czuczman, M.S. (2004). Bcl-2 antisense therapy in B-cell malignant proliferative disorders. Curr. Treat. *Options Oncol. 5*(4), 261–267.

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*(6), 1313–1319.

Cheson, B. (2004). What is new in lymphoma? *CA Cancer J. Clin. 54*(5), 260–272.

Cohen, Y., Amir, G., Schibi, G., Amariglio, N., and Polliack, A. (2004). Rapidly progressive diffuse large B-cell lymphoma with initial clinical presentation mimicking seronegative Wegener's granulomatosis. *Eur. J. Haematol. 73*(2), 134–138.

Coiffier, B. (2004). Effective immunochemotherapy for aggressive non-Hodgkin's lymphoma. *Semin. Oncol. 31*(1 Suppl 2), 7–11.

Coiffier, B., Lepage, E., Briere, J., Herbrecht, R., Tilly, H., Bouabdallah, R., Morel, P, Van Den Neste, E., Salles, G., Gaulard, P., Reyes, F., Lederlin, P., and Gisselbrecht, C. (2002). CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N. Engl. J. Med. 346*(4), 235–242.

Connors, J.M. (2005). Radioimmunotherapy—hot new treatment for lymphoma. *N. Engl. J. Med. 352,* 496–498.

Czuczman, M.S., Grillo-Lopez, A.J., White, C.A., Saleh, M., Gordon, L., LoBuglio, A.F., Jonas, C., Klippenstein, D., Dallaire, B., and Varns, C. (1999). Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J. Clin. Oncol. 17*(1), 268–276.

Czuczman, M.S., Koryzna, A., Mohr, A., Stewart, C., Donohue, K., Blumenson, L., Bernstein, Z.P., McCarthy, P., Alam, A., Hernandez-Ilizaliturri, F., Skipper, M., Brown, K., Chanan-Khan, A., Klippenstein, D., Loud, P., Rock, M.K., Benyunes, M., Grillo-Lopez, A., and Bernstein, S.H. (2005). Rituximab in combination with fludarabine chemotherapy in low-grade or follicular lymphoma. *J. Clin. Oncol. 23*(4), 694–704.

Dameshek, W. and Schwartz, R.S. (1959). Leukemia and auto-immunization—some possible relationships. *Blood. 14,* 1151–1158.

Dar, M.M. and Kwak, L.W. (2003). Vaccination strategies for lymphomas. *Curr. Oncol. Rep. 5*(5), 380–386.

de Stanchina, E., Querido, E., Narita, M., Davuluri, R.V., Pandolfi, P.P., Ferbeyre, G., and Lowe, S.W. (2004). PML is a direct p53 target that modulates p53 effector functions. *Mol. Cell. 13*(4), 523–535.

Du, M., Peng, H., Singh, N., Isaacson, P.G., and Pan, L. (1995). The accumulation of p53 abnormalities is associated with progression of mucosa-associated lymphoid tissue lymphoma. *Blood 86,* 4587.

Ehrenfeld, M., Abu-Shakra, M., Buskila, D., and Shoenfeld, Y. (2001). The dual association between lymphoma and autoimmunity. *Blood Cell. Mole. Dis. 27*(4), 750–756.

Ekström, K., Hjalgrim, H., Brandt, L., Baecklund, E., Klareskog, L., Ekbom, A., and Askling, J. (2003). Risk of malignant lymphomas in patients with rheumatoid arthritis and in their first-degree relatives. *Arthritis Rheum. 48*(4), 963–970.

FDA Press Release and Consumer Alert (2005). Issues Public Health Advisory Informing Health Care Providers of Safety Concerns Associated with the Use of Two Eczema Drugs, Elidel and Protopic. Mar 10; http://www.fda.gov.

Feldmann, M., Brennan, F.M., Paleolog, E., Cope, A., Taylor, P., Williams, R., Woody, J., and Maini, R.N. (2004). Anti-TNFalpha therapy of rheumatoid arthritis: what can we learn about chronic disease? *Novartis Found. Symp. 256,* 53–69; discussion 69–73, 106–11, 266–9.

Feldmann, M. and Maini, R.N. (2001). Anti-TNF-alpha therapy of rheumatoid arthritis: what have we learned? *Ann. Rev. Immunol. 19,* 163.

Feng, W.H., Cohen, J.I., Fischer, S., Li, L., Sneller, M., Goldbach-Mansky, R., Raab-Traub, N., Delecluse, H.J., and Kenney, S.C. (2004). Reactivation of latent Epstein–Barr virus by methotrexate: a potential contributor to methotrexate-associated lymphomas. *J. Natl. Cancer Inst. 96*(22), 1691–702.

Feugier, P., Van Hoof, A., Sebban, C., Solal-Celigny, P., Bouabdallah, R., Ferme, C., Christian, B., Lepage, E., Tilly, H., Morschhauser, F., Gaulard, P., Salles, G., Bosly, A., Gisselbrecht, C., Reyes, F., and Coiffier, B. (2005). Long-Term Results of the R-CHOP Study in the Treatment of Elderly Patients With Diffuse Large B-Cell Lymphoma: A Study by the Groupe d'Etude des Lymphomes de l'Adulte. *J. Clin. Oncol. 23*(18), 4117–4126.

Fisher, S.G. and Fisher, R.I. (2004). The epidemiology of non-Hodgkin's lymphoma. *Oncogene 23*(38), 6524–6534.

Fleischmann, R.M., Iqbal, I., and Stern, R.L. (2004). Considerations with the use of biological therapy in the treatment of rheumatoid arthritis. *Expert Opin. Drug Safety 3*(5), 391–403.

Freeman, H.J. (2004). Lymphoproliferative and intestinal malignancies in 214 patients with biopsy-defined celiac disease. *J. Clin. Gastroenterol. 38*(5), 429–434.

Fridman, J.S. and Lowe, S.W. (2003). Control of apoptosis by p53. *Oncogene 22*(56), 9030–9040.

Geborek, P., Bladstrom, A., Turesson, C., Gulfe, A., Petersson, I., Saxne, T., Olsson, H., and Jacobsson, L.T. (2005). TNF blockers do not increase overall tumour risk in patients with rheumatoid arthritis, but may be associated with increased risk of lymphomas. *Ann. Rheum. Dis. 64*(5), 699–703.

Gorman, C., Leandro, M., and Isenberg, D. (2003). B cell depletion in autoimmune disease. *Arthritis Res. Ther. 5*(Suppl 4), S17–S21.

Gottenberg, J., Guillevin, L., Lambotte, O., Combe, B., Allanore, Y., Cantagrel, A., Larroche, C., Soubrier, M., Bouillet, L., Dougados, M., Fain, O., Farge, D., Kyndt, X., Lortholary, O., Masson, C., Moura, B., Remy, P., Thomas, T., Wendling, D., Anaya, J., Sibilia, J., and Mariette, X. (2004). Tolerance and short-term efficacy of rituximab in 43 patients with systemic autoimmune diseases. *Ann. Rheum. Dis. 64*(6):913–920.

Green, P.H. and Jabri, B. (2003). Coeliac disease. *Lancet. 2003 Aug 2;362*(9381), 383–91.

Gridley, G., McLaughlin, J.K., Ekbom, A., Klareskog, L., Adami, H.O., Hacker, D.G., Hoover, R., and Fraumeni, J.F. Jr (1993). Incidence of cancer among patients with rheumatoid arthritis. *J. Natl. Cancer Inst. 85*(4), 307–311.

Grillo-Lopez, A.J. (2002). Zevalin: the first radioimmunotherapy approved for the treatment of lymphoma. *Expert Rev. Anticancer Ther. 2*(5), 485–493.

Groom, J., Kalled, S.L., Cutler, A.H., Olson, C., Woodcock, S.A., Schneider, P., Tschopp, J., Cachero, T.G., Batten, M., Wheway, J., Mauri, D., Cavill, D., Gordon, T.P., Mackay, C.R., and Mackay, F. (2002). Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. *J. Clin. Invest. 109*(1), 59–68.

Guyomard, S., Salles, G., Coudurier, M., Rousset, H., Coiffier, B., Bienvenu, J., and Fabien, N. (2003). Prevalence and pattern of antinuclear autoantibodies in 347 patients with non-Hodgkin's lymphoma. *Br. J. Haematol. 123*(1), 90–99.

Hale, A.J., Smith, C.A., Sutherland, L.C., Stoneman, V.E., Longthorne, V.L., Culhane, A.C., and Williams, G.T. (1996). Apoptosis: molecular regulation of cell death. *Eur. J. Biochem. 236*(1), 1–26.

Harris, N.L., Jaffe, E.S., Diebold, J., Flandrin, G., Muller-Hermelink, H.K., Vardiman, J., Lister, T.A., and Bloomfield, C.D. (1999). World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. Report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November, 1997. *Ann. Oncol. 10*(12), 1419–1432.

He, B., Chadburn, A., Jou, E., Schattner, E.J., Knowles, D.M., and Cerutti, A. (2004). Lymphoma B cells evade apoptosis through the TNF family members BAFF/BLyS and APRIL. *J. Immunol. 172*(5), 3268–3279.

Helms, C., Cao, L., Krueger, J.G., Wijsman, E.M., Chamian, F., Gordon, D., Heffernan, M., Daw, J.A., Robarge, J., Ott, J., Kwok, P.Y., Menter, A., and Bowcock, A.M. (2003). Putative RUNX1 binding site variant between SLC9A3R1 and NAT9 is associated with susceptibility to psoriasis. *Nat. Genet. 35*(4), 349–356.

Herkel, J., Erez-Alon, N., Mimran, A., Wolkowicz, R., Harmelin, A., Ruiz, P., Rotter, V., and Cohen, I.R. (2000). Systemic lupus erythematosus in mice, spontaneous and induced, is associated with autoimmunity to the C-terminal domain of p53 that recognizes damaged DNA. *Eur. J. Immunol. 30*(4), 977–984.

Herkel, J., Mimran, A., Erez, N., Kam, N., Lohse, A.W., Marker-Hermann, E., Rotter, V., and Cohen, I.R. (2001). Autoimmunity to the p53 protein is a feature of systemic lupus erythematosus (SLE) related to anti-DNA antibodies. *J. Autoimmun. 17*(1), 63–69.

Hermine, O., Haioun, C., Lepage, E., d'Agay, M.F., Briere, J., Lavignac, C., Fillet, G., Salles, G., Marolleau, J.P., Diebold, J., Reyas, F., and Gaulard, P. (1996). Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Groupe d'Etude des Lymphomes de l'Adulte (GELA). *Blood. 87*(1), 265–272.

Holm, L.E., Blomgren, H., and Lowhagen, T. (1985). Cancer risks in patients with chronic lymphocytic thyroiditis. *N. Engl. J. Med. 312*(10), 601–604.

Hsu, F.J., Caspar, C.B., Czerwinski, D., Kwak, L.W., Liles, T.M., Syrengelas, A., Taidi-Laskowski, B., and Levy, R. (1997). Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma—long-term results of a clinical trial. *Blood 89*(9), 3129–3135.

Hyjek, E. and Isaacson, P.G. (1988). Primary B cell lymphoma of the thyroid and its relationship to Hashimoto's thyroiditis. *Hum. Pathol. 19*(11), 1315–1326.

Ichikawa, A. (2000). Prognostic and predictive significance of p53 mutation in aggressive B-cell lymphoma. *Int. J. Hematol. 71*(3), 211–220.

Ichikawa, A., Kinoshita, T., Watanabe, T., Kato, H., Nagai, H., Tsushita, K., Saito, H., and Hotta, T. (1997). Mutations of the p53 gene as a prognostic factor in aggressive B-cell lymphoma. *N. Engl. J. Med. 337*(8), 529–534.

Ioannidis, J.P.A., Vassilou, V.A., and Moutsopoulos, H.M. (2002). Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjögren syndrome. *Arthritis Rheum. 46*(3), 741–747.

Isaacson, P.G. (2005). Update on MALT lymphomas. *Best Pract. Res. Clin. Haematol. 18*(1), 57–68.

Isaacson, P.G. (1992). Extranodal lymphomas: the MALT concept. *Verh. Dtsch. Ges. Pathol. 76,* 14–23.

Ito, Y. (2004). Oncogenic potential of the RUNX gene family: "overview". *Oncogene. 23*(24), 4198–4208.

Jaffe, E.S., Harris, N.L., Diebold, J., and Muller-Hermelink, H.K. (1999). Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. A progress report. *Am. J. Clin. Pathol. 111*(1 Suppl 1), S8–12.

Jelinek, D.F. and Darce, J.R. (2005). Human B lymphocyte malignancies, exploitation of BLyS and APRIL and their receptors. *Curr. Dir. Autoimmun. 8,* 266–288.

Jonsson, R., Bowman, S., and Gordon, T.P. (2004). Sjögren syndrome. In W.J. Koopman (ed.), *Arthritis and Allied Conditions: A textbook of rheumatology* 15th ed. Philadelphia: Lippincott, Williams, and Wilkins, pp 1681–1705.

Kaminski, M.S., Tuck, M., Estes, J., Kolstad, A., Ross, C.W., Zasadny, K., Regan, D., Kison, P., Fisher, S., Kroll, S., and Wahl, R.L. (2005). ^{131}I-tositumomab therapy as initial treatment for follicular lymphoma. *N. Engl. J. Med. 352*(5), 441–449.

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*(6), 888–892.

Lavie, F., Miceli-Richard, C., Quillard, J., Roux, S., Leclere, P., and Mariette, X. (2004). Expression of BAFF (BLyS) in T cells infiltrating labial salivary glands from patients with Sjögren syndrome. *J. Pathol. 202*(4), 496–502.

Leandro, M.J. and Isenberg, D.A. (2001). Rheumatic diseases and malignancy—is there an association? *Scand. J. Rheumatol. 30*(4), 185–188.

Lipsky, P.E., van der Heijde, D.M., St Clair, E.W., Furst, D.E., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Weisman, M., Emery, P., Feldmann, M., Harriman, G.R., and Maini, R.N. (2000). Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N. Engl. J. Med. 343*(22), 1594–1602.

Mackay, F. and Ambrose, C. (2003). The TNF family members BAFF and APRIL: the growing complexity. *Cytokine Growth Factor Rev. 14*(3–4), 311–324.

Mackay, F. and Kalled, S.L. (2002). TNF ligands and receptors in autoimmunity: an update. *Curr. Opin. Immunol. 14*(6), 783–790.

Mackay, F. and Tangye, S.F. (2004). The role of the BAFF/APRIL system in B cell homeostasis and lymphoid cancers. *Curr Opin Pharmacol. 4*(4), 347–354.

Mackay, F., Schneider, P., Rennert, P., and Browning, J. (2003). BAFF AND APRIL: a tutorial on B cell survival. *Ann. Rev. Immunol. 21*, 231–264.

Mackay, F., Sierro, F., Grey, S.T., and Gordon, T.P. (2005). The BAFF/APRIL system: an important player in systemic rheumatic diseases. *Curr. Dir. Autoimmun. 8,* 243–265.

Mackay, I.R. and Rose, N.R. (2001). Autoimmunity and lymphoma: tribulations of B cells. *Nat. Immunol. 2*(9), 793–795.

Mackay, I.R., Groom, J., and Mackay, C.R. (2002). *Autoimmunity. 35*(8), 551–553.

Maini, R.N. and Feldmann, M. (2002). How does infliximab work in rheumatoid arthritis? *Arthritis Res. 4*(Suppl 2), S22–28.

Marietta, X. (2001). Lymphomas complicating Sjögren syndrome and hepatitis C virus infection may share a common pathogenesis: chronic stimulation of rheumatoid factor B cells. *Ann. Rheum. Dis. 60*(11), 1007–1010.

Mariette, X., Cazals-Hatem, D., Warszawki, J., Liote, F., Balandraud, N., Sibilia, J., and Investigators of the Club Rhumatismes et Inflammation (2002). Lymphomas in rheumatoid arthritis patients treated with methotrexate: a 3-year prospective study in France. *Blood 99*(11), 3909–3915.

Mariette, X., Roux, S., Zhang, J., Bengoufa, D., Lavie, F., Zhou, T., and Kimberly, R. (2003). The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren syndrome. *Ann. Rheum. Dis. 62*(2), 168–171.

Maxwell, S.A. and Roth, J.A. (1994). Posttranslational regulation of p53 tumor suppressor protein function. *Crit. Rev. Oncog. 5*(1), 23–57.

Mellemkjaer, L., Linet, M.S., Gridley, G., Frisch, M., Moller, H., and Olsen, J.H. (1996). Rheumatoid arthritis and cancer risk. *Eur. J. Cancer 32A*(10), 1753–1757.

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*(4), 761–768.

Mellemkjaer, L., Johansen, C., Gridley, G., Linet, M.S., Kjaer, S.K., and Olsen, J.H. (2000). Crohn's disease and cancer risk (Denmark). *Cancer Causes Control. 11*(2), 145–150.

Novak, A.J., Grote, D.M., Stenson, M., Ziesmer, S.C., Witzig, T.E., Habermann, T.M., Harder, B., Ristow, K.M., Bram, R.J., Jelinek, D.F., Gross, J.A., and Ansell, S.M. (2004). Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. *Blood 104*(8), 2247–2253.

Okabe, M., Inagaki, H., Ohshima, K., Yoshino, T., Li, C., Eimoto, T., Ueda, R., and Nakamura, S. (2003). API2-MALT1 fusion defines a distinctive clinicopathologic subtype in pulmonary extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. *Am. J. Pathol. 162*(4), 1113–1122.

Parsonnet, J. and Isaacson, P.G. (2004). Bacterial infection and MALT lymphoma. *N. Engl. J. Med 350*(3), 213–215.

Pearson, J.E. and Silman, A.J. (2003). Risk of cancer in patients with scleroderma. *Ann. Rheum. Dis. 62*, 697–699.

Pedersen, R.K. and Pedersen, N.T. (1996). Primary non-Hodgkin's lymphoma of the thyroid gland: a population based study. *Histopathology 28*(1), 25–32.

Pertuiset, E., Liote, F., Launay-Russ, E., Kemiche, F., Cerf-Payrastre, I., and Chesneau, A.M. (2000). Adult Henoch-Schönlein purpura associated with malignancy. *Semin. Arthritis Rheum. 29*(6), 360–367.

Prior, P. (1985). Cancer and rheumatoid arthritis: epidemiologic considerations. *Am. J. Med. 78*(1A), 15–21.

Prokunina, L., Castillejo-Lopez, C., Oberg, F., Gunnarsson, I., Berg, L., Magnusson, V., Brookes, A.J., Tentler, D., Kristjansdottir, H., Grondal, G., Bolstad, A.I., Svenungsson, E., Lundberg, I., Sturfelt, G., Jonssen, A., Truedsson, L., Lima, G., Alcocer-Varela, J., Jonsson, R., Gyllensten, U.B., Harley, J.B., Alarcon-Segovia, D., Steinsson, K., and Alarcon-Riquelme, M.E. (2002). A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat. Genet. 32*(4), 666–669.

Prokunina, L., Padyukov, L., Bennet, A., de Faire, U., Wiman, B., Prince, J., Alfredsson, L., Klareskog, L., and Alarcon-Riquelme, M. (2004). Association of the PD–1.3A allele of the PDCD1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope. *Arthritis Rheum. 50*(6), 1770–1773.

Raderer, M., Osterreicher, C., Machold, K., Formanek, M., Fiebiger, W., Penz, M., Dragosics, B., and Chott, A. (2001). Impaired response of gastric MALT-lymphoma to *Helicobacter pylori* eradication in patients with autoimmune disease. *Ann. Oncol. 12*(7), 937–939.

Raderer, M., Puspok, A., Stummvoll, G., Langle, F., and Chott, A. (2003). Early cancer of the stomach arising after successful treatment of gastric MALT lymphoma in patients with autoimmune disease. *Scand. J. Gastroenterol. 38*(3), 294–297.

Raderer, M., Puspok, A., Birkner, T., Streubel, B., and Chott, A. (2004). Primary gastric mantle cell lymphoma in a patient with long standing history of Crohn's disease. *Leuk. Lymphoma 45*(7), 1459–1462.

Ramos-Casals, M., Cervera, R., Font, J., Garcia-Carrasco, M., Espinosa, G., Reino, S., Pallares, L., and Ingelmo, M. (1998). Young onset of primary Sjögren syndrome: clinical and immunological characteristics. *Lupus 7*(3), 202–206.

Ramos-Casals, M., Garcia-Carrasco, M., Font, J., and Cervera, R. (2000). Sjögren syndrome and lymphoproliferative diseases. In Y. Schoenfeld and M.E. Gershwin (eds), *Cancer and autoimmunity*. Amsterdam: Elsevier Science, *55*: 55–80.

Ramos-Casals, M., Trejo, O., Garcia-Carrasco, M., Cervera, R., De La Red, G., Gil, V., Lopez-Guillermo, A., Ingelmo, M., and Font, J. (2004). Triple association between hepatitis C virus infection, systemic autoimmune diseases, and B cell lymphoma. *J. Rheumatol. 31*(3), 495–499.

Ramos-Casals, M., Brito-Zeron, P., Yague, J., Akasbi, M., Bautista, R., Ruano, M., Claver, G., Gil, V., and Font, J. (2005a). Hypocomplementaemia as an immunological marker of morbidity and mortality in patients with primary Sjögren syndrome. *Rheumatology (Oxford). 44*(1), 89–94.

Ramos-Casals, M., De Vita, S., and Tzioufas, A.G. (2005b). Hepatitis C virus, Sjögren syndrome and B-cell lymphoma: linking infection, autoimmunity and cancer. *Autoimmun. Rev. 4*(1), 8–15.

Rastetter, W., Molina, A., and White, C.A. (2004). Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Ann. Rev. Med. 55,* 477–503.

Reyes, F., Lepage, E., Ganem, G., Molina, T.J., Brice, P., Coiffier, B., Morel, P., Ferme, C., Bosly, A., Lederlin, P., Laurent, G., Tilly, H., and Groupe d'Etude des Lymphomes de l'Adulte (GELA) (2005). ACVBP versus CHOP plus radiotherapy for localized aggressive lymphoma. *N. Engl. J. Med. 352*(12), 1197–1205.

Rosenblum, M.D., Olasz, E., Woodliff, J.E., Johnson, B.D., Konkol, M.C., Gerber, K.A., Orentas, R.J., Sandford, G., and Truitt, R.L. (2004). CD200 is a novel p53-target gene involved in apoptosis-associated immune tolerance. *Blood 103*(7), 2691–2698.

Rosenthal, A.K., McLaughlin, J.K., Gridley, G., and Nyren, O. (1995). Incidence of cancer among patients with systemic sclerosis. *Cancer 76*(5), 910–914.

Rosenwald, A. (2003). DNA microarrays in lymphoid malignancies. *Oncology (Huntingt). 17*(12), 1743–1748; discussion 1750, 1755, 1758–1759.

Rosenwald, A. and Staudt, L.M. (2003). Gene expression profiling of diffuse large B-cell lymphoma. *Leuk. Lymphoma 44*(Suppl 3), S41–47.

Rosenwald, A., Wright, G., Chan, W.C., Connors, J.M., Campo, E., Fisher, R.I., Gascoyne, R.D., Muller-Hermelink, H.K., Smeland, E.B., Giltnane, J.M., Hurt, E.M., Zhao, H., Averett, L., Yang, L., Wilson, W.H., Jaffe, E.S., Simon, R., Klausner, R.D., Powell, J., Duffey, P.L., Longo, D.L., Greiner, T.C., Weisenburger, D.D., Sanger, W.G., Dave, B.J., Lynch, J.C., Vose, J., Armitage, J.O., Montserrat, E., Lopez-Guillermo, A., Grogan, T.M., Miller, T.P., LeBlanc, M., Ott, G., Kvaloy, S., Delabie, J., Holte, H., Krajci, P., Stokke, T., Staudt, L.M., and Lymphoma/Leukemia Molecular Profiling Project (2002). The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N. Engl. J Med. 346*(25), 1937–1947.

Ruland, J., Duncan, G.S., Wakeham, A., and Mak, T.W. (2003). Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity 19*(5), 749–758.

Sakakura, C., Hagiwara, A., Miyagawa, K., Nakashima, S., Yoshikawa, T., Kin, S., Nakase, Y., Ito, K., Yamagishi, H., Yazumi, S., Chiba, T., and Ito, Y. (2005). Frequent downregulation of the runt domain transcription factors RUNX1, RUNX3 and their cofactor CBFB in gastric cancer. *Int. J. Cancer 113*(2), 221–228.

Sallah, S., Sigounas, G., Vos, P., Wan, J.Y., and Nguyen, N.P. (2000). Autoimmune hemolytic anemia in patients with non-Hodgkin's lymphoma: characteristics and significance. *Ann. Oncol. 11*(12), 1571–1577.

Sallah, S., Wan, J.Y., and Hanrahan, L.R. (2001). Future development of lymphoproliferative disorders in patients with autoimmune hemolytic anemia. *Clin. Cancer Res. 7*(4), 791–794.

Salloum, E., Cooper, D.L., Howe, G., Lacy, J., Tallini, G., Crouch, J., Schultz, M., and Murren, J. (1996). Spontaneous regression of lymphoproliferative disorders in patients treated with methotrexate for rheumatoid arthritis and other rheumatic diseases. *J. Clin. Oncol. 14*(6), 1943–1949.

Segundo, C., Rodriquez, C., Garcia-Poley, A., Aguilar, M., Gavilan, I., Bellas, C., and Brieva, J.A. (2001). Thyroid-infiltrating B lymphocytes in Graves' disease are related to marginal zone and memory B cell compartments. *Thyroid 11,* 525.

Sharkey, R.M., Brenner, A., Burton, J., Hajjar, G., Toder, S.P., Alavi, A., Matthies, A., Tsai, D.E., Schuster, S.J., Stadtmauer, E.A., Czuczman, M.S., Lamonica, D., Kraeber-Bodere, F., Mahe, B., Chatal, J.F., Rogatko, A., Mardirrosian, G., and Goldenberg, D.M. (2003). Radioimmunotherapy of non-Hodgkin's lymphoma with 90Y-DOTA humanized anti-CD22 IgG (90Y-Epratuzumab): do tumor targeting and dosimetry predict therapeutic response? *J. Nucl. Med. 44*(12), 2000–2018.

Silman, A.J., Petrie, J., Hazelman, B., and Evans, S.J. (1988). Lymphoproliferative cancer and other malignancy in patients with rheumatoid arthritis treated with azathioprine: A 20 year follow up study. *Ann. Rheum. Dis. 47,* 988–992.

Smedby, K.E., Akerman, M., Hildebrand, H., Glimelius, B., Ekbom, A., and Askling, J. (2005). Malignant lymphomas in coeliac disease: evidence of increased risks for lymphoma types other than enteropathy-type T cell lymphoma. *Gut 54*(1), 54–59.

Sokol, R.J., Hewitt, S., and Stamps, B.K. (1981). Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. *BMJ (Clin. Res. Ed.) 282*(6281), 2023–2027.

Specks, U., Fervenza, F.C., McDonald, T.J., and Hogan, M.C.E. (2001). Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy. *Arthritis Rheum. 44*(12), 2836–2840.

Spender, L.C., Whiteman, H.J., Karstegl, C.E., and Farrell, P.J. (2005). Transcriptional cross-regulation of RUNX1 by RUNX3 in human B cells. *Oncogene 24*(11), 1873–1881.

Stoffel, A. and Levine, A.J. (2004). Actvation of NF-kappaB by the API2/MALT1 fusions inhibits p53 dependent but not FAS induced apoptosis: a directional link between NF-kappaB and p53. *Cell Cycle 3*(8), 1017–1020.

Stoffel, A., Chaurushiya, M., Singh, B., and Levine, A.J. (2004). Activation of NF-kappaB and inhibition of p53-mediated apoptosis by API2/mucosa-associated lymphoid tissue 1 fusions promote oncogenesis. *Proc. Natl. Acad. Sci. U.S.A. 101*(24), 9079–9084.

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*(7), 591–601.

Straus, S.E., Jaffe, E.S., Puck, J.M., Dale, J.K., Elkon, K.B., Rosen-Wolff, A., Peters, A.M., Sneller, M.C., Hallahan, C.W., Wang, J., Fischer, F.E., Jackson, C.M., Lin, A.Y., Baumler, C., Siegert, E., Marx, A., Vaishnaw, A.K., Grodzicky, T., Fleisher, T.A., and Lenardo, M.J. (2001). The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. *Blood 98*(1), 194–200.

Streubel, B., Chott, A., Huber, D., Exner, M., Jager, U., Wagner, O., and Schwarzinger, I. (2004a). Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas. *N. Engl. J. Med. 351*(3), 250–259.

Streubel, B., Huber, D., Wohrer, S., Chott, A., and Raderer, M. (2004b). Frequency of chromosomal aberrations involving MALT1 in mucosa-associated lymphoid tissue lymphoma in patients with Sjögren syndrome. *Clin. Cancer Res. 10*(2), 476–480.

Streubel, B., Simonitsch-Klupp, I., Mullauer, L., Lamprecht, A., Huber, D., Siebert, R., Stolte, M., Trautinger, F., Lukas, J., Puspok, A., Formanek, M., Assanasen, T., Muller-Hermelink, H.K., Cerroni, L., Raderer, M., and Chott, A. (2004c). Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. *Leukemia 18*(10), 1722–1726.

Szodoray, P., Jellestad, S., Alex, P., Zhou, T., Wilson, P.C., Centola, M., Brun, J.G., and Jonsson, R. (2004). Programmed cell death of peripheral blood B cells determined by laser scanning cytometry in Sjögren syndrome with a special emphasis on BAFF. *J. Clin. Immunol. 24*(6), 600–611.

Talal, N. and Bunim, J.J. (1964). Development of malignant lymphoma in the course of Sjögren syndrome. *Am. J. Med. 37*, 529–540.

Taylor, P.C., Williams, R.O., and Feldmann, M. (2004). Tumour necrosis factor alpha as a therapeutic target for immune-mediated inflammatory diseases. *Curr. Opin. Biotechnol. 15*(6), 557–563.

Tokuhiro, S., Yamada, R., Chang, X., Suzuki, A., Kochi, Y., Sawada, T., Suzuki, M., Nagasaki, M., Ohtsuki, M., Ono, M., Furukawa, H., Nagashima, M., Yoshino, S., Mabuchi, A., Sekine, A., Saito, S., Takahashi, A., Tsunoda, T., Nakamura, Y., and Yamamoto, K. (2003). An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat. Genet. 35*(4), 341–348.

Trejo, O., Ramos-Casals, M., Lopez-Guillermo, A., Garcia-Carrasco, M., Yague, J., Cervera, R., Font, J., and Ingelmo, M. (2003). Hematologic malignancies in patients with cryoglobulinemia: association with autoimmune and chronic viral diseases. *Semin. Arthritis Rheum. 33*(1), 19–28.

Tzioufas, A.G., Manoussakis, M.N., Costello, R., Silis, M., Papadopoulos, N.M., and Moutsopoulos, H.M. (1986). Cryoglobulinemia in autoimmune rheumatic diseases. Evidence of circulating monoclonal cryoglobulins in patients with primary Sjögren syndrome. *Arthritis Rheum. 29*(9), 1098–1104.

Tzioufas, A.G., Boumba, D.S., Skopouli, F.N., and Moutsopoulos, H.M. (1996). Mixed monoclonal cryoblobulinemia and monoclonal rheumatoid factor cross-reactive idiotype as predictive factors for the development of lymphoma in primary Sjögren syndrome. *Arthritis Rheum. 39*, 767–772.

Varoczy, L., Gergely, L., Zeher, M., Szegedi, G., and Illes, A. (2002). Malignant lymphoma-associated autoimmune diseases—a descriptive epidemiological study. *Rheumatol. Int. 22*(6), 233–237.

Vilchez, R.A. and Butel, J.S. (2004). Emergent human pathogen simian virus 40 and its role in cancer. *Clin. Microbiol. Rev. 17*(3), 495–508.

Vose, J.M., Link, B.K., Grossbard, M.L., Czuczman, M., Grillo-Lopez, A., Gilman, P., Lowe, A., Kunkel, L.A., and Fisher, R.I. (2001). Phase II study of rituximab in combination with chop chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. *J. Clin. Oncol. 19*(2), 389–397.

Voulgarelis, M., Dafni, U.G., Isenberg, K.A., and Moutsopoulos, H.M. (1999). Malignant lymphoma in primary Sjögren syndrome—A multicenter, retrospective, clinical study by the European concerted action on Sjögren syndrome. *Arthritis Rheum. 8*, 1765–1772.

Whelan, J.P. and Scadden, D.T. (2004). Lymphoma and abnormalities of the immune system. In P.M. Mauch, J.O. Armitage, B. Coiffier, R. Dalla-Favera, and N.L. Harris (eds), *Non-Hodgkin's Lymphomas,* Philadelphia: Lippincott, Williams, and Wilkins, pp 729–748.

Witzig, T.E. (2004). Yttrium-90-ibritumomab tiuxetan radioimmunotherapy: a new treatment approach for B-cell non-Hodgkin's lymphoma. *Drugs Today (Barc). 40*(2), 111–119.

Wolfe, F. and Michaud, K. (2004). Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. *Arthritis Rheum. 50*(6), 1740–1751.

Wotherspoon, A.C., Ortiz-Hidalgo, C., Falzon, M.R., and Isaacson, P.G. (1991). *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet 338*(8776), 1175–1176.

Yamada, R., Tokuhiro, S., Chang, X., and Yamamoto, K. (2004). SLC22A4 and RUNX1: identification of RA susceptible genes. *J. Mol. Med. 82*(9), 558–564.

Ye, H., Liu, H., Attygalle, A., Wotherspoon, A.C., Nicholson, A.G., Charlotte, F., Leblond, V., Speight, P., Goodlad, J, Lavergne-Slove, A., Martin-Subero, J.I., Siebert, R., Dogan, A., Isaacson, P.G., and Du, M.Q. (2003). Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of *H. pylori* in gastric MALT lymphoma. *Blood 102*(3), 1012–1018.

Ye, H., Gong, L., Liu, H., Hamoudi, R.A., Shirali, S., Ho, L., Chott, A., Streubel, B., Siebert, R., Gesk, S., Martin-Subero, J.I., Radford, J.A.,

Banerjee, S., Nicholson, A.G., Ranaldi, R., Remstein, E.D., Gao, Z., Zheng, J., Isaacson, P.G., Dogan, A., and Du, M.Q. (2005). MALT lymphoma with t(14;18)(q32;q21)/IGH-MALT1 is characterized by strong cytoplasmic MALT1 and BCL10 expression. *J. Pathol. 205*(3), 293–301.

Zhang, Y., Holford, T.R., Leaderer, B., Zahm, S.H., Boyle, P., Morton, L.M., Zhang, B., Zou, K., Flynn, S., Tallini, G., Owens, P.H., and Zheng, T. (2004). Prior medical conditions and medications use and risk of non-Hodgkin lymphoma in Connecticut United States women. *Cancer Causes Control 15*(4), 419–428.

Zhang, Q., Siebert, R., Yan, M., Hinzmann, B., Cui, X., Xue, L., Rakestraw, K.M., Naeve, C.W., Beckmann, G., Weisenburger, D.D., Sanger, W.G., Nowotny, H., Vesely, M., Callet-Bauchu, E., Salles, G., Dixit, V.M., Rosenthal, A., Schlegelberger, B., and Morris, S.W. (1999). Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). *Nat. Genet. 22*(1), 63–68.

CHAPTER

70

Apoptosis and Autoimmunity: Lymphoproliferative Syndromes

DAVID L. VAUX

The Walter and Eliza Hall Institute, 1G Royal Parade, Parkville, Victoria, Australia

Autoimmune lymphoproliferative syndrome (ALPS), also known as Canale–Smith syndrome, is a genetically determined disease characterized by accumulation of lymphoid cells, increased numbers of $CD4^{-}CD8^{-}$ (double negative) $CD45R^{+}$ T cells, hypergammaglobulinemia, and autoimmunity. It is caused by mutations in genes encoding components of the cell death pathway that is activated by the ligation of Fas (CD95/APO1/TNFRSF6), a member of the tumor necrosis factor (TNF) receptor family. Although ALPS is very rare, study of this disease and its murine counterpart has revealed much about the roles and mechanisms of cell death in the immune system.

HISTORIC BACKGROUND

Canale–Smith syndrome was described in 1967 by Virgina Canale and Carl Smith who examined five children with a benign generalized lymphadenopathy and autoimmune disease (Canale and Smith, 1967). During the following years, the syndrome has also been called ALPS, chronic lymphadenopathy simulating malignant lymphoma, chronic pseudomalignant immunoproliferation, benign immunoproliferative syndrome, and lymphoproliferative syndrome with autoimmunity.

A similar disease was observed in the MLR strain of mice bearing the *lpr* (lymphoproliferative) mutation in 1978 (Andrews et al., 1978), and mice with the *gld* (generalized lymphoproliferative disease) mutation in 1984 (Roths et al., 1984). Unaware of the earlier report of Canale–Smith syndrome, Sneller et al. described two patients with ALPS, and recognized the similarity to the murine *lpr* and *gld* diseases (Sneller et al., 1992).

In 1992, Nagata and coworkers (Watanabe-Fukunaga et al., 1992) showed that *lpr* disease of mice was due to mutations in Fas (CD95), a member of the TNF receptor family that gives a pro-apoptotic signal when ligated (Yonehara et al., 1989). Two years later, they cloned the gene for the *gld* mutation, and showed it encoded the ligand for Fas (Takahashi et al., 1994).

Mutations of components of the Fas pathway in humans with ALPS were observed by Rieux-Laucat et al. in France in 1995 (Rieux-Laucat et al., 1995), and a short time later by Fisher et al. in the United States (Fisher et al., 1995). The formal demonstration that ALPS, *lpr* disease, *gld* disease and Canale–Smith syndrome were all manifestations of loss-of-function mutations in the same pathway came with the identification of mutations in the Fas gene of patients with Canale–Smith syndrome by Drappa et al. in 1996 (Drappa et al., 1996).

Because it has become the most frequently used term, this chapter will use the acronym ALPS, even though it was predated by the term Canale–Smith syndrome, and this disease is associated with accumulation of lymphocytes, rather than their proliferation.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

CLASSICAL AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME—CLINICAL AND AUTOIMMUNE FEATURES

Autoimmune lymphoproliferative syndrome is a very rare syndrome, with an approximate total of 100 cases described to date worldwide. There are four classical features: a defect in the Fas-induced apoptotic pathway; a polyclonal, non-malignant accumulation of unusual $CD4^{-}CD8^{-}CD45R^{+}$ T-lymphocytes; hypergammaglobulinemia; and autoimmunity. Similar features are seen in mice homozygous for loss-of-function mutations in Fas (i.e., the *lpr* mutant mice) or FasL (i.e., *gld* mutant mice).

Autoimmune lymphoproliferative syndrome is usually seen in young children (0–5 years), although some cases have only been diagnosed in adulthood (Deutsch et al., 2004). Affected individuals usually present with afebrile lymphadenopathy and splenomegaly. The majority also exhibit signs of autoimmunity, most commonly autoimmune hemolytic anemia, thrombocytopenia, or neutropenia, but involvement of other organs such as the kidneys and liver has also been observed (Sneller et al., 1997; Rieux-Laucat et al., 1999). Levels of IgG and IgA in the blood are increased, often with autoantibodies to red blood cells, platelets, nuclear antigens, and IgG (rheumatoid factor), whereas antibodies to DNA are not seen, unlike the mouse *lpr* disease.

Although the lymphadenopathy and splenomegaly resemble those of malignant lymphoma, ALPS is not a neoplastic disease. Elevated numbers (more than 1% of circulating lymphocytes) of a peculiar subset of T-lymphocytes can be found in the blood. These T-receptor-positive cells lack both of the T-cell markers CD4 and CD8, but express the CD45R (B220) antigen, which is usually only seen on B-lineage cells. The origin of these cells, which also accumulate in the mouse form of the syndrome, remains enigmatic. Presumably in normal individuals these cells are removed by Fas-signaled apoptosis, but persist and accumulate when Fas pathways are interrupted.

The disease can be confirmed *in vitro* by demonstrating insensitivity of peripheral blood lymphocytes to Fas-induced apoptosis. This can be carried out either by activating the cells with antibodies to the T-cell receptor-associated antigen CD3, and measuring the amount of activation-induced cell death, which is dependent on Fas–FasL interactions (Wu et al., 1996), or by incubating the lymphocytes with antibodies that cross-link Fas, and determining the amount of apoptosis (Sneller et al., 1997).

GENETIC INHERITANCE

Autoimmune lymphoproliferative syndrome is caused by a failure of the Fas pathway. This can result from recessive inheritance of two nonfunctional genes for a component of the pathway, or due to a dominant interfering mutation, which would lead to a dominant inheritance pattern.

In the mouse *gld* disease (due to mutation to the genes for Fas ligand) and *lpr* disease (due to low or absent expression of Fas) are recessively inherited. While three cases of homozygous null mutations to Fas have been reported in humans (ALPS 0), the great majority of human ALPS, about 100 cases, exhibit a partially penetrant dominant inheritance pattern (ALPS Ia), and are heterozygous for mutations to Fas. It is thought that the mutant Fas proteins act in a dominant negative manner by interfering with the ability of the normal molecules to form trimers that are capable of efficiently activating the death-inducing signaling complex (Kischkel et al., 1995) comprised of FADD, caspase 8, and caspase 10.

Genotyping of family members of ALPS patients for Fas mutations usually reveals a dominant pattern of inheritance, but relatives that share the mutation often have no history of lymphadenopathy or autoimmune disease, even though their lymphocytes are resistant to apoptosis following ligation of Fas (Sneller et al., 1997). This illustrates the great variation in the penetrance of the disease.

FAS ACTIVATED SIGNALING PATHWAYS

Fas (CD95/APO1/TNFRSF6) is a member of the TNF receptor family that exists as a homotrimer in the plasma membrane of several cell types, including T- and B-lineage cells. The extracellular aminoterminal half of Fas can be bound by Fas ligand (FasL), leading to formation of higher order multimers (Holler et al., 2003) and activation of signaling (Kischkel et al., 1995). The intracellular carboxy-terminal half of Fas bears a protein interaction motif termed a death domain (DD) that allows it to interact with the DD in the adaptor protein FADD (Chinnaiyan et al., 1996, Boldin et al., 1996). The cytoplasmic adaptor molecule FADD can, in turn, bind to latent forms of caspase 8 and caspase 10, leading to activation of these proteases (Boldin et al., 1996; Muzio et al., 1996). Once active, these caspases can cleave proteins within the cell, and activate other caspases, resulting in death of the cell by apoptosis.

By interacting with other DD-bearing adaptor proteins such as RIP (Ting et al., 1996), in some cells Fas can activate other signal transduction pathways including the NFκB and Jun transcription factor pathways (Duckett, 2002). Modulation of Fas signaling by another adaptor molecule, termed FLIP, can determine whether ligation of Fas leads to apoptosis or the activation of other biochemical pathways (Irmler et al., 1997; Lens et al., 2002).

Binding of Fas by FasL can, therefore, set in train a signal transduction pathway that leads to apoptosis, but it does not always proceed that far, and signals emanating from ligated

Fas do not only lead to activation of the cell death mechanism, but may result in other cellular responses.

ANIMAL MODELS AND PATHOGENIC MECHANISMS

Loss of Fas expression in the *lpr* mice is due to integration of a transposable element into intron 2 of the Fas gene, leading to markedly reduced Fas expression (Adachi et al., 1993). Mice with a point mutation in the cytoplasmic domain of Fas that prevents signaling (*lpr*^{cg} mice) (Adachi et al., 1993), and Fas null mice generated by gene targeting (Adachi et al., 1996) exhibit similar phenotypes, characterized by accumulation of $CD4^-CD8^-CD45R^+$ T lymphocytes in the lymph nodes and spleen. The blood contains increased levels of IgG, anti-DNA antibodies, rheumatoid factor, and immune complexes, and the mice often develop glomerulonephritis and arthritis. A similar syndrome is seen in Fas ligand mutant (*gld*) mice and FasL gene-targeted mice (Karray et al., 2004), although the disease in the latter is slightly more severe.

The severity of lymphocyte accumulation and autoimmunity in *lpr* mice is strongly affected by the background genotype; for example, on the MRL background, it is much more severe than when the same mutation is on a C57BL6 background. This demonstrates that several other genes can influence the clinical manifestations of the disease (Vidal et al., 1998).

Although mutations in Fas and its ligand result in ALPS-like syndromes in mice, mutations to other components of the pathway, such as FADD and caspase 8, do not. Deletion of the FADD genes in mice results in embryonic lethality, and transgenic mice expressing a dominant negative FADD construct in their T cells do not develop an ALPS-like phenotype, but have defects in T-cell proliferation (Newton et al., 1998).

Mice do not have a gene homologous to human caspase 10, and deletion of caspase 8 genes results in embryonic lethality. Transgenic mice expressing the caspase 8 inhibitor CrmA in T-lineage cells (Smith et al., 1996) did not develop autoimmunity or accumulate $CD4^-CD8^-CD45R^+$ lymphocytes, even though the cells were profoundly resistant to Fas-induced apoptosis. Mice in which caspase 8 was deleted only in the T lineage, which rendered the cells completely resistant to induction of apoptosis following ligation of Fas (Salmena et al., 2003), had normal thymocyte development, but numbers of peripheral T cells were reduced, and they were immunodeficient. There was no accumulation of $CD4^-CD8^-CD45R^+$ T-lymphocytes or autoimmunity. These experiments suggest that the development of the disease manifestations associated with ALPS in mice, such as accumulation of lymphocytes and autoimmunity, requires interruption of Fas-signaling pathways upstream of caspases and FADD.

Autoimmune lymphoproliferative syndrome results from loss of signals from ligated Fas on both B and T lymphocytes. These signals include activation of NFκB and Jun transcription-factor pathways, processing of caspase 8, and apoptosis. MLR *lpr* mice expressing transgenic Fas in just the T-lineage did not develop splenomegaly or lymphadenopathy, but still generated autoantibodies and developed glomerulonephritis, whereas transgenic expression of Fas in both B and T lineages prevented both lymphocyte accumulation and autoantibody production (Fukuyama et al., 1998; 2002). This suggests that loss of Fas on T-lineage cells leads to persistence of the $CD4^-CD8^-CD45R^+$ T cells, but Fas signaling must be lost from B lymphocytes for autoimmunity to occur.

NONCLASSICAL FORMS OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME

Classical ALPS is due to either homozygosity for a null mutation to Fas (ALPS 0, which has been observed in three cases) or heterozygosity for a dominant negative mutation to Fas (ALPS Ia, found in approximately 100 cases). These mutations are usually found in the DD. There are a number of other classes of ALPS, namely ALPS Ib, ALPS II and ALPS III in which the cause of the syndrome is less well established.

The ALPS Ib form is due to mutations in the gene for FasL in humans, analogous to the gld mutation in mice. Only a single instance has been reported: a patient heterozygous for an 84 base-pair deletion in the gene for FasL (Wu et al., 1996). This patient, who presented with features typical of systemic lupus erythematosus (SLE) rather than classical ALPS, did not have severe lymphadenopathy or increased $CD4^-CD8^-CD45R^+$ T cells. Furthermore, because the defects in function of FasL expressed on the patient's lymphocytes were partial, it is not certain that the FasL deletion was the cause of his disease.

Patients with ALPS II have the classical clinical features of ALPS, and their lymphocytes are resistant to apoptosis caused by Fas ligation, but they have normal Fas and FasL genes. Mutations to caspase 10 have been found in two kindreds (Wang et al., 1999), but at this stage it has not been proven beyond doubt whether the nucleotide changes observed cause the disease, or whether these patients have some other abnormality. One of the genetic changes was subsequently found to occur in 0.5% of the Danish population, so might represent a polymorphism, rather than a loss of function mutation (Gronbaek et al., 2000).

If mutations in caspase 10 are indeed the cause of ALPS II it will be important to determine the mechanism by which they do so, but, because mice do not have genes for caspase 10, it is not possible to examine the analogous mutations in mice. The mechanism proposed is that the mutant caspase

10 molecules, which bear missense point mutations, act in a dominant negative way to interfere with caspase 8 function (Wang et al., 1999). Because complete loss of caspase 8 causes immunodeficiency rather than an ALPS phenotype (Chun et al., 2002; Salmena et al., 2003), if they are acting in a dominant negative way, the mutant caspase 10 proteins cannot inhibit all caspase 8 function.

Some patients with ALPS II have normal genes for caspase 10 (Rieux-Laucat et al., 2003), raising the possibility that other molecules involved in Fas-signaling pathways are involved in these, and possibly all, cases of ALPS II.

Approximately 20 patients with ALPS III have been described. They show mild clinical features of ALPS, including increased numbers of $CD4^-CD8^-CD45R^+$ T cells in the blood, but have no mutations in their genes for Fas or FasL. Some are reported to have lymphocytes with normal sensitivity to ligation of Fas *in vitro*, whereas in others it is reduced (Ramenghi et al., 2000). No errors have been found in genes for FADD, TNF receptors 1 and 2 (TNFR1, TNFR2), caspase 8, or caspase 10 (Chun and Lenardo, 2001; van der Werff ten Bosch et al., 2001; Rieux-Laucat et al., 2003). Suspicion now falls on other members of the TNFR family, such as receptors for the TNF-like cytokine TRAIL. However, to date, there have been no mutations reported, and mice engineered to have mutations in genes such as TRAIL do not exhibit an ALPS-like phenotype (Sedger et al., 2002).

Six children initially classed as having ALPS III, because they had autoimmunity, increased $CD4^-CD8^-$ T cells, and hypergammaglobulinemia, but normal sensitivity of their lymphocytes to Fas-induced apoptosis, were subsequently found to have Fas mutations in their $CD4^-CD8^-$ T cells, but not in DNA from their hair or skin cells (Holzelova et al., 2004). These investigators proposed that somatic mutations to the Fas gene (similar to those in ALPS Ia) arose in a hematopoietic precursor cell, resulting in mosaicism, and suggested that patients be denoted as belonging to ALPS type Im.

AUTOIMMUNITY

In ALPS, the autoimmunity is chiefly antibody mediated, with the usual autoantibodies found being antibodies to red blood cells, platelets, nuclear antigens, and rheumatoid factor. Although the major abnormal cell types are of the T lineage (namely the $CD4^-CD8^-CD45R^+$ T cells), and the defects in Fas-induced death are usually demonstrated in T lymphocytes, B cells are clearly important for development of the autoimmune manifestations. For autoimmunity to develop in *lpr* mice, the B cells must bear the mutation (Fukuyama et al., 1998; 2002). How then does loss of Fas signaling lead to production of autoantibodies?

It has been proposed that Fas is required for elimination of autoreactive B cells (Rathmell et al., 1995), creating a defect in peripheral tolerance. This possibility could be confirmed by expressing the gene for the caspase 8 inhibitor CrmA in the B cells of transgenic mice. It seems clear that, whatever the mechanism leading to autoimmunity, it will be complicated and influenced by many genes. In humans, the partial penetrance observed in families with ALPS demonstrates the effects of other genes, as do differences in the severity of autoimmune disease in mice with different genetic backgrounds. Mutations to many different genes that affect the number or survival of B cells in mice can lead to autoimmune syndromes. For example, transgenic expression of the antiapoptotic gene Bcl-2 (Strasser et al., 1990; 1991), or deletion of the genes for BIM (Bouillet et al., 2002) or IL-2 (Suzuki et al., 1997) develop SLE-like autoimmune syndromes when on certain genetic backgrounds.

TREATMENT AND OUTCOME

Autoimmune lymphoproliferative syndrome usually spontaneously decreases in severity (Fischer et al., 1999), and is compatible with normal life expectancy (Infante et al., 1998), so the treatments are conservative. Lymphadenopathy, splenomegaly, and autoimmune manifestations often regress without treatment, even though elevated IgG and increased numbers of $CD4^-CD8^-CD45R^+$ T cells often persist. Acute episodes of autoimmune disease such as hemolytic anemia and thrombocytopenia may occur, and lymphoma has developed in some patients. Management is, therefore, based on regular monitoring and treatment of acute exacerbations of disease.

To treat the more severe autoimmune disease manifestations a number of regimens have been tried, including steroids, mycophenolate mofetil (Alvarado et al., 2004), rituximab, and vincristine (Heelan et al., 2002), splenectomy, or bone marrow transplantation (Sneller et al., 1997; Sleight et al., 1998).

CONCLUDING REMARKS—FUTURE PROSPECTS

For researchers, the significance of ALPS has been from the insights it has provided into the mechanisms of apoptosis and autoimmunity, but because it is such a rare disease there is little incentive to develop specific therapies. The main burdens of disease faced by ALPS patients are due to the accumulation of lymphoid tissue and the flare ups of autoimmune disease. Recent discoveries of the role of the TNF-related cytokine BAFF as a B-cell growth and survival factor that is elevated in a number of SLE-like autoimmune syndromes (Vaux, 2002) provides hope that BAFF-blocking

agents might one day be also useful for the treatment of the autoimmune manifestations of ALPS.

References

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. U.S.A. 90*, 1756–1760.

Adachi, M., Suematsu, S., Suda, T., Watanabe, D., Fukuyama, H., Ogasawara, J., Tanaka, T., Yoshida, N., and Nagata, S. (1996). Enhanced and accelerated lymphoproliferation in Fas-null mice. *Proc. Natl. Acad. Sci. U.S.A. 93*, 2131–2136.

Alvarado, C.S., Straus, S.E., Li, S., Dale, J.K., Mann, K., Le, A., and Lauer, S.J. (2004). Autoimmune lymphoproliferative syndrome: a cause of chronic splenomegaly, lymphadenopathy, and cytopenias in children—report on diagnosis and management of five patients. *Pediatr. Blood Cancer 43*, 164–169.

Andrews, B.S., Eisenberg, R.A., Theofilopoulos, A.N., Izui, S., Wilson, C.B., McConahey, P.J., Murphy, E.D., Roths, J.B., and Dixon, F.J. (1978). Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J. Exp. Med. 148*, 1198–1215.

Boldin, M.P., Goncharov, T.M., Goltsev, Y.V., and Wallach, D. (1996). Involvement of mach, a novel mort1/fadd-interacting protease, in fas/apo-1- and tnf receptor-induced cell death. *Cell 85*, 803–815.

Bouillet, P., Purton, J.F., Godfrey, D.I., Zhang, L.C., Coultas, L., Puthalakath, H., Pellegrini, M., Cory, S., Adams, J.M., and Strasser, A. (2002). BH3—only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature 415*, 922–926.

Canale, V.C. and Smith, C.H. (1967). Chronic lymphadenopathy simulating malignant lymphoma. *J. Pediatr. 70*, 891–899.

Chinnaiyan, A.M., Tepper, C.G., Seldin, M.F., Orourke, K., Kischkel, F.C., Hellbardt, S., Krammer, P.H., Peter, M.E., and Dixit, V.M. (1996). Fadd/mort1 is a common mediator of cd95 (fas/apo-1) and tumor necrosis factor receptor-induced apoptosis. *J. Biol. Chem. 271*, 4961–4965.

Chun, H.J. and Lenardo, M.J. (2001). Autoimmune lymphoproliferative syndrome: types I, II and beyond. *Adv. Exp. Med. Biol. 490*, 49–57.

Chun, H.J., Zheng, L., Ahmad, M., Wang, J., Speirs, C.K., Siegel, R.M., Dale, J.K., Puck, J., Davis, J., Hall, C.G., Skoda-Smith, S., Atkinson, T.P., Straus, S.E., and Lenardo, M.J. (2002). Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature 419*, 395–399.

Deutsch, M., Tsopanou, E., and Dourakis, S.P. (2004). The autoimmune lymphoproliferative syndrome (Canale-Smith) in adulthood. *Clin. Rheumatol. 23*, 43–44.

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*, 1643–1649.

Duckett, C.S. (2002). Apoptosis and NF-kappa B: the FADD connection. *J. Clin. Invest, 109*, 579–580.

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.

Fischer, A., Rieux-Laucat, F., and Le Deist, F. (1999). A new peak in the ALPS. Nat. Med. 5, 876–877.

Fukuyama, H., Adachi, M., Suematsu, S., Miwa, K., Suda, T., Yoshida, N., and Nagata, S. (1998). Transgenic expression of Fas in T cells blocks lymphoproliferation but not autoimmune disease in MRL-lpr mice. *J. Immunol. 160*, 3805–3811.

Fukuyama, H., Adachi, M., Suematsu, S., Miwa, K., Suda, T., Yoshida, N., and Nagata, S. (2002). Requirement of Fas expression in B cells for tolerance induction. *Eur. J. Immunol. 32*, 223–230.

Gronbaek, K., Dalby, T, Zeuthen, J., Ralfkiaer, E., and Guldberg, P. (2000). The V4101 (G1228A) variant of the caspase-10 gene is a common polymorphism in the Danish population. *Blood 95*, 2184–2185.

Heelan, B.T., Tormey, V., Amlot, P., Payne, E., Mehta, A., and Webster, A.D. (2002). Effect of anti-CD20 (rituximab) on resistant thrombocytopenia in autoimmune lymphoproliferative syndrome. *Br. J. Haematol. 118*, 1078–1081.

Holler, N., Tardivel, A., Kovacsovics-Bankowski, M., Hertig, S., Gaide, O., Martinon, F., Tinel, A., Deperthes, D., Calderara, S., Schulthess, T., Engel, J., Schneider, P., and Tschopp, J. (2003). Two adjacent trimeric fas ligands are required for fas signaling and formation of a death-inducing signaling complex. *Mol. Cell. Biol. 23*, 1428–1440.

Holzelova, E., Vonarbourg, C., Stolzenberg, M.C., Arkwright, P.D., Selz, F., Prieur, A.M., Blanche, S., Bartunkova, J., Vilmer, E., Fischer, A., Le Deist, F., and Rieux-Laucat, F. (2004). Autoimmune lymphoproliferative syndrome with somatic Fas mutations. *N. Engl. J. Med. 351*, 1409–1418.

Infante, A.J., Britton, H.A., DeNapoli, T., Middelton, L.A., Lenardo, M.J., Jackson, C.E., Wang, J., Fleisher, T., Straus, S.E., and Puck, J.M. (1998). The clinical spectrum in a large kindred with autoimmune lymphoproliferative syndrome caused by a Fas mutation that impairs lymphocyte apoptosis. *J. Pediatr. 133*, 629–633.

Irmler, M., Thome, M., Hahne, M., Schneider, P., Hofmann, B., 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*, 190–195.

Karray, S., Kress, C., Cuvellier, S., Hue-Beauvais, C., Damotte, D., Babinet, C., and Levi-Strauss, M. (2004). Complete loss of Fas ligand gene causes massive lymphoproliferation and early death, indicating a residual activity of gld allele. *J. Immunol. 172*, 2118–2125.

Kischkel, F.C., Hellbardt, S., Behrmann, I., Germer, M., Pawlita, M., Krammer, P.H., and Peter, M.E. (1995). Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J. 14*, 5579–5588.

Lens, S.M.A., Kataoka, T., Fortner, K.A., Tinel, A., Ferrero, I., MacDonald, R.H., Hahne, M., Beermann, F., Attinger, A., Orbea, H.A., Budd, R.C., and Tschopp, J. (2002). The caspase 8 inhibitor c-FLIPL modulates T-cell receptor-induced proliferation but not activation-induced cell death of lymphocytes. *Mol. Cell. Biol. 22*, 5419–5433.

Muzio, M., Chinnaiyan, A.M., Kischkel, F.C., Orourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J.D., Zhang, M., Gentz, R., Mann, M., Krammer, P.H., Peter, M.E., and Dixit, V.M. (1996). Flice, a novel fadd-homologous ice/ced-3-like protease, is recruited to the cd95 (fas/apo-1) death-inducing signaling complex. *Cell 85*, 817–827.

Newton, K., Harris, A.W., Bath, M.L., Smith, K., and Strasser, A. (1998). A dominant interfering mutant of fadd/mort1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T Lymphocytes. *EMBO J. 17*, 706–718.

Ramenghi, U., Bonissoni, S., Migliaretti, G., DeFranco, S., Bottarel, F., Gambaruto, C., DiFranco, D., Priori, R., Conti, F., Dianzani, I., Valesini, G., Merletti, F., and Dianzani, U. (2000). Deficiency of the Fas apoptosis pathway without Fas gene mutations is a familial trait predisposing to development of autoimmune diseases and cancer. *Blood 95*, 3176–3182.

Rathmell, J.C., Cooke, M.P., Ho, W.Y., Grein, J., Townsend, S.E., Davis, M.M., and Goodnow, C,C. (1995). CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with CD4+ T cells. *Nature 376*, 181–184.

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*, 1347–1349.

Rieux-Laucat, F., Blachere, S., Danielan, S., De Villartay, J.P., Oleastro, M., Solary, E., Bader-Meunier, B., Arkwright, P., Pondare, C., Bernaudin, F., Chapel, H., Nielsen, S., Berrah, M., Fischer, A., and Le Deist, F. (1999). Lymphoproliferative syndrome with autoimmunity: A possible genetic basis for dominant expression of the clinical manifestations. *Blood 94*, 2575–2582.

Rieux-Laucat, F., Le Deist, F., and Fischer, A. (2003). Autoimmune lymphoproliferative syndromes: genetic defects of apoptosis pathways. *Cell Death Differ. 10*, 124–133.

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*, 1–20.

Salmena, L., Lemmers, B., Hakem, A., Matysiak-Zablocki, E., Murakami, K., Au, P.Y., Berry, D.M., Tamblyn, L., Shehabeldin, A., Migon, E., Wakeham, A., Bouchard, D., Yeh, W.C., McGlade, J.C., Ohashi, P.S., and Hakem, R. (2003). Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. *Genes Dev. 21*, 21.

Sedger, L.M., Glaccum, M.B., Schuh, J.C., Kanaly, S.T., Williamson, E., Kayagaki, N., Yun, T., Smolak, P., Le, T., Goodwin, R., and Gliniak, B. (2002). Characterization of the *in vivo* function of TNF-alpha-related apoptosis-inducing ligand, TRAIL/Apo2L, using TRAIL/Apo2L gene-deficient mice. *Eur. J. Immunol. 32*, 2246–2254.

Sleight, B.J., Prasad, V.S., DeLaat, C., Steele, P., Ballard, E., Arceci, R.J., and Sidman, C.L. (1998). Correction of autoimmune lymphoproliferative syndrome by bone marrow transplantation. *Bone Marrow Transplant 22*, 375–380.

Smith, K.G.C., Strasser, A., and Vaux, D.L. (1996). Crma expression in T lymphocytes of transgenic mice inhibits CD95 (Fas/APO-1)-transduced apoptosis, but does not cause lymphadenopathy or autoimmune disease. *EMBO J. 15*, 5167–5176.

Sneller, M.C., Straus, S.E., Jaffe, E.S., Jaffe, J.S., Fleisher, T.A., Stetler-Stevenson, M., and Strober, W. (1992). A novel lymphoproliferative/autoimmune syndrome resembling murine lpr/gld disease. *J. Clin. Invest. 90*, 334–341.

Sneller, M.C., Wang, J., Dale, J.K., Strober, W., Middelton, L.A., Choi, Y., Fleisher, T.A., Lim, M.S., Jaffe, E.S., Puck, J.M., Lenardo, M.J., and Straus, S.E. (1997). Clinical, immunologic, and genetic features of an autoimmune lymphoproliferative syndrome associated with abnormal lymphocyte apoptosis. *Blood 89*, 1341–1348.

Strasser, A., Harris, A.W., Vaux, D.L., Webb, E., Bath, M.L., Adams, J.M., and Cory, S. (1990). Abnormalities of the immune system induced by dysregulated bcl-2 expression in transgenic mice. *Curr. Top. Microbiol. Immunol. 166*, 175–181.

Strasser, A., Whittingham, S., Vaux, D.L., Bath, M.L., Adams, J.M., Cory, S., and Harris, A.W. (1991). Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. U.S.A. 88*, 8661–8665.

Suzuki, H., Hayakawa, A., Bouchard, D., Nakashima, I., and Mak, T.W. (1997). Normal thymic selection, superantigen-induced deletion and fas-mediated apoptosis of T cells in IL-2 receptor beta chain-deficient mice. *Intl. Immunol. 9*, 1367–1374.

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.

Ting, A.T., Pimentelmuinos, F.X., and Seed, B. (1996). Rip mediates tumor necrosis factor receptor 1 activation of nf-kappa-b but not fas/apo-1-initiated apoptosis. *EMBO J. 15*, 6189–6196.

van der Werff ten Bosch, J., Otten, J., and Thielemans, K. (2001). Autoimmune lymphoproliferative syndrome type III, an indefinite disorder. Leuk. *Lymphoma 41*, 501–11.

Vaux, D.L. (2002) The buzz about BAFF. *J. Clin. Invest. 109*, 17–18.

Vidal, S., Kono, D.H., and Theofilopoulos, A.N. (1998). Loci predisposing to autoimmunity in MRL-Fas lpr and C57BL/6-Faslpr mice. *J. Clin. Invest. 101*, 696–702.

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.

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.

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.

Yonehara, S., Ishii, A., and Yonehara, M. (1989). A cell-killing monoclonal antibody (anti-Fas) to a cell surface antigen co-downregulated with the receptor of tumor necrosis factor. *J. Exp. Med. 169*, 1747–1756.

CHAPTER

71

Cameos

IAN R. MACKAY

Department of Biochemistry and Molecular Biology, Monash University, Victoria, Australia

The previous edition (3rd) of 1998, Chapter 37, described various "contender" autoimmune diseases of which many now appear as full chapters in this edition. Here are presented (alphabetically) further diseases as cameos, either by reason of their rarity, controversial evidence on causation, or autoimmunity being just one of several elements in pathogenenesis.

ANOREXIA NERVOSA AND BULIMIA

Anorexia nervosa (AN) and bulimia are eating disorders that affect up to 3% of women in their lifetime (Walsh and Devlin, 1998), and are characterized by aversion to food, cachexia and amenorrhea (AN) or food aversion with bouts of overeating (bulimia). Since AN is usually interpreted as a behavioral disorder, patients often come into psychiatric care, although a neuropsychiatric basis has not been ascertained. The incidence in young women could prompt consideration of autoimmunity despite absence of other features. Fetissov et al. (2002) reported an analysis of serum autoantibodies from 57 women with eating disorders: AN, 28; bulimia, 22; and combined AN and bulimia, 7. The assay used was immunohistochemical labeling by serum of cells of rat pituitary gland and hypothalamus. Of the 57 sera, 42 (74%) had autoantibodies that bound to melanotropes and/or corticotropes in the pituitary gland, and certain of the sera were selectively reactive with hypothalamic neuronal cells and projections that expressed melanocyte-stimulating hormone. Further technical details are given by Fetissov et al. (2002). There has been no direct confirmation of these data.

ATHEROSCLEROSIS

Atherosclerosis is discussed in Chapter 64 in this volume with emphasis on autoimmune reactivity to heat shock protein 60 (hsp60), but the complexity of this multifactorial inflammatory disorder warrants a "second look." Schwartz and Mitchell (1962) illustrated mononuclear cell infiltrations, mainly lymphocytic, in the vascular adventitia in

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

atheroma, with surprise that "such prominent cellular accumulations should have received so little attention." They cited several antecedent reports on the frequency of these infiltrations, and tabulated data indicating that adventitial cellular infiltrations correlated with coronary arterial plaque severity and with recent arterial thrombosis. In an immunoepidemiologic study of 3407 adult residents of Busselton, Western Australia, in 1973, it was found that the prevalence of routinely tested autoantibodies not only increased with age, but also proved to be an independent risk factor for mortality and morbidity from coronary/cerebrovascular disease (Mathews et al., 1973); postulated mechanisms included reactivity of autoantibodies with antigens on the vascular surface. Subsequently, the presence of lymphocytes, predominantly T cells, in atherosclerotic plaques is repeatedly cited (Jonasson et al., 1986; Emeson and Robertson, 1988; Van der Wal et al., 1989; Xu et al., 1990; Libby and Hansson, 1991). Also the vascular lesion seen in long-standing human allografted organs—kidney, liver, or heart—as a thickening of the wall of medium-sized arteries with inflammatory features, and interpretable on the basis of a chronic host-versus-graft cellular immune attack, simulates atherosclerosis. Finally, there is the occurrence of accelerated atherosclerosis in multisystem autoimmune diseases, earlier interpreted as myocarditis (Mackay and Burnet, 1963), and recently well documented for systemic lupus erythematosus (SLE) (Asanuma et al., 2003; Roman et al., 2003) and rheumatoid arthritis (Kaplan and McCune, 2003).

Animal models have been developed to examine the immunopathogenesis of atherosclerosis. In rabbits with hypercholesterolemia, atherosclerotic lesions contain autoreactive T cells that respond to hsp60 (Wick et al., 1995; see Chapter 64). Mice with a "knockout" of the gene encoding apolipoprotein E, apoE$^{-/-}$ (Zhou et al., 1996), as for low density lipoprotein (LDL)-receptor deficient mice, are hypercholesterolemic and develop severe atherosclerosis with morphologic features very similar to the human counterpart (Lichtman et al., 1996). The lesions in these mice show numerous T lymphocytes, predominantly of the CD4^{+} phenotype with expression of the activation marker CD25, high expression of MHC class II molecules on adjacent tissue cells indicative of release of cytokines, e.g., interferon-γ (IFN-γ) from the infiltrating T cells, lipid-filled macrophages (foam cells), and hypertrophy of the smooth muscle of the arterial wall.

Studies in humans, have been directed to defining specific immune reactants that could promote atherogenesis, either directly or via immune complexes. These could include 1) pro-inflammatory oxidized phospholipids (Berliner and Watson, 2005; Piarulli et al., 2005; Tsimikas et al., 2005), and noting the reactivity of antibodies to phospholipids in SLE (Vaarala et al., 1993); 2) malondialdehyde which is an adduct formed on low density lipoprotein during oxidation (Salonen et al., 1992; Lopes-Virella and Virella 1996); or 3) hsp60, the mammalian equivalent of bacterial hsp65, when hyperexpressed on stressed arterial intimal cells (see Chapter 64). Laman et al. (1997) implicated an interrelationship between foamy macrophages and activated T cells via expression of coreceptor molecules, CD40 on the former and its ligand CD154 on the latter, so promoting inflammatory responses and cytokine release. Antigen dependence of CD154 expression by T cells in the lesion might be expected, but could occur in an inflammatory milieu independent of antigen presentation (Phipps, 2000). A further factor is infection, particularly with *Chlamydia pneumoniae*, by which induction of an anti-hsp60 response may occur (Benagiano et al., 2003). A contemporary assessment of the likely interrelated contributions to atherosclerosis of metabolism, infections, inflammation, and immunity is presented by Hansson (2005).

There is much work ahead on the immunopathology of atherosclerosis noting several studies implicating T cells of the Th1 subset (Benagiano et al., 2003), and findings in mice that a deficiency in T-bet signaling results in a switch to Th2 responsiveness that is atheroprotective (Buono et al., 2005). Other interesting leads that are being followed, or could be worth, following include effects in atherosclerosis-prone mice of hypo- or hyper-expression of pro-inflammatory cytokines or chemokines (MIF, GM-CSF, MCP-1, and others), and effects of depletion of regulatory T cells.

AUTOINFLAMMATORY DISEASES

"Autoinflammatory" is a term of convenience to describe intrinsic cytokine-mediated activation of inflammatory pathways independent of adaptive immune responses. The term is admittedly gray-edged and the perimeters for inclusion of diseases as autoinflammatory are indefinite. A prototypic disease would be familial Mediterranean fever (FMF) that mainly affects populations derived from the Mediterranean basin, and is characterized by periodic self-limited attacks of fever with features of serosal membrane inflammation. A culprit gene (*MERV*) that maps to the short arm of chromosome 16 encodes a regulator of leukocyte- and monocyte-specific inflammatory activity. Deficiency mutants of *MERV* result in unrestrained activity of predominantly Th1 polarized cytokines such as tumor necrosis factor-α (TNF-α) and IFN-γ (Centola et al., 2000). There is evidence for subclinical activation of inflammatory pathways even in symptom-free individuals (Musabak et al., 2004). While T cells are essential participants in FMF, there are no indicators for antigen-specificity in their activation.

As other examples, there are genetically less well-defined inflammatory pathways that can be activated in some way by lipids (or derivatives of these), examples being inflammatory responses associated with obesity (Wellen and

Hotarmisligil, 2005), or with triglyceride deposition in the liver. In the latter instance, non-alcoholic steatohepatitis (NASH) is emerging as a threatening variant of benign hepatic steatosis wherein the presence of lipid in liver cells provokes a progressive cytokine-dependent necroinflammatory response with fibrogenesis and cirrhosis. The factors that determine lipid accumulation in the liver in the first place, whether in NASH or the more familiar alcoholic liver disease, are reviewed by Browning and Horton (2004). These include metabolic shifts, oxygen species, mitochondrial dysfunction, and others and, superimposed on these, is the propensity in individuals at risk for NASH for T lymphocytes to be chemotactically attracted, and to release inflammatory cytokines, particularly TNF-α. Superimposed, there may be genetically dependent pathways that enhance fibrogenesis (Wynn, 2004). Extending yet further, tissue injury may be associated with abnormal cytoplasmic inclusions or misfolded proteins that are inadequately disposed of by chaperone molecules, ubiquination or autophagy, with cytotoxic consequences. Thus, in serpinopathy exemplified by deficiency of α1-antitrypsin, progressive inflammatory liver injury may supervene and, in certain CNS diseases, accumulations of beta amyloid or polyglutamine repeats may provoke cytotoxicity and a microglial-dependent inflammatory response (Taylor et al., 2002).

The extent to which autoinflammatory diseases require consideration in this present text is debatable, given the meager evidence for antigen-specific adaptive autoimmune responses in pathogenesis, yet a form of self-reactivity is seen as a contributory cause in at least some of these diseases.

BIRDSHOT RETINOPATHY

The slender reasons, additional to the piquancy of the title, for consideration of this rare ocular disease is the likely dependency on retinal S antigen in pathogenesis, and the remarkably high disease association with a class 1 MHC molecule, HLA A29. Considering inflammatory eye diseases in general (see Chapter 49), these take in responses to antigens in various of the ocular tissues, but mainly uvea and retina, although a serologic response to a well-characterized antigenic molecule is not so readily demonstrable as for autoimmune diseases of most other tissues. Birdshot retinopathy affects predominantly individuals of Northern European extraction and occurs usually in mid-adult life, with a slight female excess; it accounts for only about 1% of all cases presenting clinically as "uveitis." Birdshot retinopathy is seen ophthalmologically as multiple separate cream-colored spots on the post-equatorial fundus. Opportunity for pathologic examination is most infrequent but, from one study by Gasch et al. (1999), the retina was predominantly affected with a T-lymphocytic and granulomatous infiltrate. Serologic reactivity *in vitro* to retinal S antigen is not well documented. The risk conferred by the MHC allele HLA A29 (50- to 224-fold) is higher than that for any other disease, and is greater for the A29.1 than the A29.2 subtype. The only other human diseases with an HLA class 1 rather than a class II association are spondyloarthropathies with B27 (see Chapter 33) and psoriasis with Cw6 (see Chapter 58). Birdshot retinopathy presumably results from specific reactivity of $CD8^+$ cytotoxic T cells against retinal S antigen, but the provocation for this is unknown.

CHRONIC FATIGUE SYNDROME

This peculiar syndrome had its origin in 1955 from an outbreak of an illness affecting 292 hospital staff members with features of malaise, neck stiffness, lymphadenopathy, and fever (Medical Staff Royal Free Hospital, 1955). At that time, the term myalgic encephalomyelitis (ME) was proposed by someone described as "an unusually uncritical Lancet editorialist" (Byrne, 1988). Features include severe unexplainable fatigue, loss of energy, poor exercise tolerance with muscle discomfort, fibromyalgia, and various other nonspecific symptoms. A preceding viral illness is often implicated. "Myalgic encephalomyelitis" faded from use, but the syndrome continued to attract attention from various medical specialties and patient support groups, with protagonists for a functional basis and others for some unknown organic basis. By the 1980s, the syndrome was accepted as a real entity, and in 1987 was renamed chronic fatigue syndrome (CFS), with recommended diagnostic criteria by the Centers for Disease Control (CDC) in Atlanta, Georgia (Holmes et al. 1988). The two major criteria for diagnosis were 1) a new onset of fatigue lasting 6 months and reducing activity to less than 50%; and 2) any other condition usually producing fatigue ruled out, and the 11 minor criteria (of which eight should be fulfilled) included eight symptomatic criteria, and three physical criteria that comprised mild fever, nonexudative pharyngitis, and palpable cervical or axillary lymph nodes up to 2 cm in diameter. Claimed causes of CFS have included chronic viral infections, allergies, responses to unusual environmental exposures, or psychosomatic disorder. Despite evidence for immune activation and/or impaired indices of cell-mediated immunity in CFS (Lloyd et al., 1989, 1990; Buchwald and Komaroff, 1991), indications of inflammatory activity or tissue destruction are inconspicuous. Effects of immunomodulatory treatment with intravenous immunoglobulins, of clear efficacy in autoimmune disorders, are controversial, endorsed by some (Lloyd et al., 1990), and refuted by others (Peterson et al., 1990).

Analysis of an autoimmune component in CFS is beset by problems such as specifying a clear diagnosis, the overall

scarcity of affirmative reports indicative of a number of unpublished negative studies, and the occurrence of fatigue as a symptom of various well-defined autoimmune disorders. The question then is whether, among cases fulfilling CDC criteria for CFS, there can be identified a highly raised level of any autoantibody species, particularly antinuclear autoantibody (ANA). Among positive studies are those of Behan et al. (1985) (50 cases studied), and of Konstantinov et al. (1996) and von Mikecz et al. (1997) from the one laboratory (60 cases studied). The latter group cited frequencies of autoantibodies of up to 83% versus 17% in the control group, predominantly of ANA specificity, and directed to the nuclear envelope, together with various subspecificities, and the cytoplasmic staining patterns observed were of intermediate filament-vimentin type, indicative of viral infection (see Chapter 53). On the other hand, negative results for ANA in CFS were reported by Skowera et al. (2002), and only modestly positive results by Vernon and Reeves (2005).

The conclusions are that autoimmunity is unlikely to provide an overall explanation for CFS, and that autoantibodies, when present, may well be secondary to some other more basic cause. Perhaps autoimmunity is just one among a conglomerate of causes for CFS.

CONGENITAL HEART BLOCK

The observation was made in 1977 that newborn infants with congenital heart block (CHB) were delivered by mothers with an autoimmune disease, usually SLE or Sjögren syndrome, and with antibodies to the Ro/La (SS-A/SS-B) autoantigens, cited by Buyon et al. (2001) and Buyon and Clancy (2003). This remarkable experiment of nature provides striking examples of passive (transplacental) transfer of an autoimmune disease, and the pathogenic potential of humoral antibody even though the anti-Ro-bearing mother herself is spared. The fetal risk of CHB with an anti-Ro-positive mother is low (about 2%), but still immensely higher than that for all births. Other interesting features of anti-Ro-related CHB are the "window of susceptibility" between 18 and 24 weeks of gestation, an increased risk of recurrence in subsequent pregnancies, up to 18%, and a curiously low concordance in twin pregnancies (Buyon and Clancy, 2003). There is a gradation in degree of heart block from minor conduction defects to a state requiring replacement of the dysfunctional fibroinflammatory atrioventricular (AV) tissue by a permanent artificial pacemaker: in fact, the latter is the usual outcome.

While the complicity of transferred autoantibody in CHB is well established, the detailed pathogenesis is not so. The culprit autoantigen is the 52 kDa isoform of Ro (see Chapter 31), identified as a transcriptional regulator that is located intracellularly in all cells including ventricular cardiomyocytes, AV node, and impulse-conducting tissues. Possibly anti-Ro-positive sera contain another antibody species reactive with an accessible surface-expressed autoantigen, an L-type calcium channel (see Chapter 17), or development of the fetal heart involves remodeling by physiologic apoptosis that can generate autoantigenic fragments (see Chapter 15) that become translocated to the cell surface. However, neither of these ideas accounts for the subsequent over first pregnancy risk of anti-Ro for CHB. The likelihood that a specific antibody population is accountable arises from the finding of antibodies to an epitope sequence within the Ro 52 kDA protein (residues 200–239 called p200) that is contained within the leucine zipper region: these antibodies are highly associated with CHB (Salomonsson et al., 2005). Experimentally, female DA rats immunized with regular boostings with the p200 sequence of Ro52 developed a stable anti-p200 response without epitope spreading and, after mating, produced offspring among which the occurrences of AV block was 20% (Salomonsson et al., 2005). Highly focused monoclonal antibodies to the p200 region were used to test for binding to primary myocyte cultures from neonatal rat heart, so allowing for the identification of a particular subset of anti-p200 antibodies that bound directly to voltage-gated calcium channels on cardiomyocytes causing calcium overload and, eventually, apoptotic cell death (see Chapter 17). Otherwise stated, the pathogenic subset of autoantibodies does not primarily recognize apoptosis fragments, but rather induces apoptosis and thereby initiates a chain of events culminating in CHB. Consequently, there is now a means to identify pregnancies at high risk for CHB, and strategies can be visualized to prevent this.

ERYTHROID (PURE RED CELL) APLASIA

Erythroid or pure red cell aplasia (PRCA) describes severe anemia with a low blood reticulocyte count and scarce-to-absent erythroid precursors in the bone marrow. Pure red cell aplasia differs from aplastic anemia (see Chapter 44) wherein there is hypercellularity affecting all lineages in the bone marrow. Reports on PRCA go back to the 1950s, cited by Casadevall et al. (1996), some with varying solidity of evidence for an autoimmune basis, based on bioassays showing interference by disease serum of erythroid cell growth *in vitro*. Other cases seem clearly associated with infection by parvovirus of erythroid precursors (see Chapter 44). A further and intriguing association in some 15% of cases of spontaneously occurring PRCA is thymoma, sometimes with cure effected by thymectomy (Goldstein and Mackay, 1970; Dessypris, 1991). Currently, most cases can be attributed to autoantibodies to erythropoietin, the growth factor necessary for erythroid develop-

ment. Immunoassays show undetectable levels in blood of erythropoietin, and bioassays show absence of erythroid precursor cell growth in the presence of disease serum. In most cases, the patient usually has renal failure and is receiving hemodialysis and a preparation of recombinant erythropoietin (rEPO). Selected batches of rEPO have been incriminated in autoimmunogenicity, perhaps explained by differences in degree of glycosylation (Guest and Levitt, 2003).

The occurrence of an autoimmune reactant (anti-EPO) after repetitive adjuvant-free injection of a self antigen that is presumably only slightly modified, if at all, is interesting, and is reminiscent of autoantibody inhibitors of factor VIII after treatment of congenital hemophilia with preparations of recombinant factor VIII (see Chapter 45), or neutralizing antibody to type 1 interferons after therapy with such interferons. This could be regarded as induction of autoimmunity by a process somewhat akin to molecular mimicry and in the apparent absence of any elements of "damage-danger."

FOLATE DEFICIENCY SYNDROME

Maldevelopment of the embryonic neural tube resulting in spina bifida, anencephaly, or other defects occurs in infants at a prevalence of about 1 in 1000. It has been widely ascertained that periconceptual folic acid supplements to mothers strikingly alleviate this, even though the mothers usually do not have evident folate deficiency (Rothenberg et al., 2004). The observation was made that antiserum to folate receptors in pregnant rats resulted in embryonic maldevelopment, and this prompted the search for autoantibodies to folate receptors in women in whom a pregnancy had been followed by an infant affected with a neural tube defect (Rothenberg et al., 2004). The procedure used was the specific blocking of (^{3}H) folic acid to folate receptors on placental membranes and to indicator cell lines. The results were that 9 of 12 (versus 2 of 20 controls) of women with affected children had a receptor blocking antibody. In a further study (Ramaekers et al., 2005), the same authors investigated infantile-onset cerebral folate deficiency that develops 4–6 months after birth and is expressed as mental and psychomotor retardation, cerebellar ataxia, dyskinesis, seizures, visual disorder, and autism. There were low levels of 5-methyl tetrahydrofolate (5-MTHF) in the cerebrospinal fluid but normal levels in serum, and lack of evidence of extracerebral folate deficiency. Serum from 25 of 28 affected children, versus none of 28 controls, contained high-affinity blocking autoantibodies against membrane-bound folate receptors on the choroid plexus, indicating impediment to the passage of folic acid from serum to brain. This could be normalized by oral calcium folinate that led to clinical improvement. Notably, none of five tested mothers had autoantibodies. Perhaps the induction of the anti-folate receptor antibodies in these affected children was due to soluble folate-binding proteins in milk, or to other unknown antigens (Ramaekers et al., 2005). This story is indeed a true "cameo" in the universe of autoimmunity: anti-folate receptor autoantibody generated either in the pregnant mother or the newborn child with neural developmental disorders, prompting Schwartz (2005) to comment that "autoimmunization lurks behind every pillar"! And there is the added point that catastrophic consequence in this particular example can be alleviated by a very simple therapy, folic acid.

INTERSTITIAL CYSTITIS

Hunner (1914) reported eight cases of chronic recurrent edematous ulceration of the bladder in young women, but possible earlier descriptions of the disease are cited by Sant (1991). Subsequently, the term *interstitial cystitis* was introduced to accommodate the accompanying diffuse fibrosis of the bladder wall. Hand (1949) reported on 223 cases, 204 women and 19 men, who accounted for almost 5% of all urologically investigated patients, and Oravisto et al. (1970) on 54 cases from a urology unit over a 10-year period, of whom all were females aged 16–80 years, mean 59 years. Symptoms include urinary frequency and urgency, suprapubic pain, and hematuria. Cystoscopy reveals a greatly reduced bladder capacity and a bladder wall with stellate scars, clusters of granulations, and multiple punctuate hemorrhages, likened to an "angry scratch." Microscopy of the bladder wall shows bladder mucosal ulceration, edema, lymphoid-plasma cell infiltrations, and prominence of mast cells (Sant and Theoharides, 1994) and, in long-standing cases, pronounced fibrosis. The idea of autoimmunity as a cause of interstitial cystitis gained impetus from disease associations, particularly SLE (Fister, 1938; Shipton, 1965; Boye et al., 1979), Sjögren syndrome, and autoimmune thyroiditis (Oravisto, 1980). Other disease associations among 129 cases studied by Peeker et al. (2003) included hypersensitivity/allergic disorders, rheumatoid arthritis, and inflammatory bowel disease.

Present data on interstitial cystitis suggest a population frequency of approximately 1–2 in 1000, with a high predominance among women (90%). Sufficient interest in the condition exists for the creation in the USA of a support group (Interstitial Cystitis Association), and the development by the National Institute of Diabetes and Digestive and Kidney Diseases of the NIH (USA) of formal criteria for diagnosis. Subtypes of interstitial cystitis sometimes used in case classification are ulcerative (typical) or nonulcerative, and primary (sole disorder) or secondary (associated with some other immune-mediated disease, often SLE) (de la Serna and Alarcon-Segovia, 1981; Alarcon-Segovia, 1984).

Currently biomarkers are being sought to increase the reliability of diagnosis and assess efficacy of treatment.

Serologically, a finding of bladder-specific autoantibodies by Silk (1970) has not been confirmed. The frequency of ANA is controversial. Initially, raised frequencies were reported by Oravisto et al. (1970) and Jokinen et al. (1972). In the more modern era, the frequency of autoantibodies was assessed by Ochs et al. (1994) in 96 patients using immunofluorescence on tissue sections and the HEp-2 cell line, and a bladder epithelial cell line, and by western blotting. Specific autoantibodies to bladder cells were not demonstrable, but there was a clearly increased frequency of non-tissue-specific autoantibodies (cut-off titer 1:40), 36% versus 8% in female controls, mostly ANA, but antimitochondrial in three cases. The ANA patterns of the positive sera were speckled or nucleolar, unlike what pertains in SLE. Ochs et al. (1994) reasonably interpreted their findings as a consequence of chronic inflammation rather than a primary immune attack on the bladder wall, albeit with some potential to augment secondarily the inflammatory process.

Experimental models of autoimmune cystitis have been induced in mice and rats by immunization with bladder extracts. In a BALB/c-derived mouse strain, BALB/cAN, cystitis was induced, and histologic features included edema, fibrosis, and lymphocytic and mast cell infiltrations; specific reactivities of antibodies or T cells were not cited but disease was transferable adoptively by lymphoid cells (Bullock et al., 1992). In the Lewis rat, cystitis was similarly inducible, and could be adoptively transferred with splenocytes (Luber-Narod et al., 1996).

In conclusion, interstitial cystitis remains in the penumbra of autoimmunity. Neither pathogenic nor even marker autoantibodies are reliably demonstrable, earlier suggestions of damage by pathogenic immune complexes now seem speculative, and well-controlled studies showing sustained benefit from corticosteroid or immunosuppressive drugs are lacking. Hence, consideration is required of a pathogenesis other than autoimmunity, e.g., an adverse response to a constituent of urine to which the bladder wall is exposed.

IPEX SYNDROME

The immune dysregulation-polyendocrinopathy-X-linked syndrome (IPEX), the generic equivalent of scurfy disease in mice, is a usually lethal disease of male infants with florid autoimmune expressions. There is recessive inheritance of a mutant FOXP3 gene and disease expressions of IPEX/scurfy depend on a non-functional FOXP3 transcriptional factor (scurfin in mice) that is essential for proper development of CD4+CD25+ Treg cells. Results of molecular genetic analyses on IPEX/scurfy published in 2001 by Wildin et al. and Bennett et al. are cited in references to Chapter 9. The particular interest of IPEX/scurfy is that it could be a pointer to a dysfunctional FOXP3 as a genetic element in later-in-life autoimmune disease.

NEUROIMMUNOPATHIES

There are various "minor" autoimmune neurologic diseases that claim our attention yet cannot be accommodated under the major headings of multiple sclerosis, peripheral neuropathy, myasthenia gravis, or paraneoplastic disease. Some of these are described below.

Autonomic Neuropathy

Autonomic nerve fibers innervate cardiovascular, gastrointestinal, urogenital, thermoregulatory, sudomotor and pupillary structures. There exists a group of peripheral neuropathies of disparate cause that affect unmyelinated autonomic nerve fibers or ganglionic structures, with the clinical presentation of autonomic failure (dysautonomia). Paraneoplastic dysautonomia has been associated with a variety of other paraneoplastic expressions (Freeman, 2005), and with autoantibodies to the Hu antigen (see Chapter 68), nicotinic acetylcholine receptors in autonomic ganglia (Versino et al., 2000) and presynaptic voltage-gated calcium channels as in Lambert–Eaton syndrome (see Chapter 48) In some instances, autonomic neuropathy is "spontaneous," and apparently not associated with neoplasia (Vernino et al., 2000). Particular components of dysautonomia may be more prominent, for example, intestinal hypomotility seen as gastric stasis or constipation. In a case of severe gastric stasis associated with type 1 diabetes (T1D), histologic examination showed complete, and presumably autoimmune, depletion of the interstitial cells of Cajal of the myenteric plexus (He et al., 2001). In a model counterpart, rabbits immunized with the α3 subunit of the acetylcholine receptor developed an autonomic neuropathy (Lennon et al., 2003), with passive transfer to mice demonstrable with serum from affected rabbits and also, albeit with limited data, with sera from diseased humans (Vernino et al., 2004).

GADA-Associated Neuropathies

The discovery of the glutamic acid decarboxylase 65 kDa isoform (GAD65) as a diabetes autoantigen (see Chapter 36) arose from the detection of antibody to GAD65 (GADA) in a case of stiff-person syndrome (SPS, also known as stiff-man disease) with T1D. GAD65 is most abundant in neuroendocrine cells in pancreatic islets and the CNS, wherein it catalyzes the production of the inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Exactly how GAD65 as an intracellular enzyme is implicated in neuroendocrine autoimmunity is uncertain, although immunohistochemical staining shows GAD present at GABA-ergic nerve terminals

(Peltola et al., 2000). Several curious if not exotic diseases qualify as GADA-associated neuronopathy (GAN), of which the prototypic example is SPS (see Chapter 48). Interestingly, serum levels of GADA are substantially higher in GAN than in T1D, perhaps explaining selective positivity by immunoblot in the former, and there is often a different epitope selection including a short sequence in the N-terminal region of GAD65 rather than (or as well as) the highly conformational epitopes in the mid- and C-terminal regions typically engaged by T1D antisera (Myers et al., 2000). Further, as evidence for intrathecal synthesis of GADA, the CSF may contain monoclonal bands and have specific immunoreactivity (Peltola et al., 2000). Other examples of GAN include cerebellar ataxia that can be associated with high levels of GADA and polyendocrine autoimmunity (Saiz et al., 1997) and therapy-resistant epilepsy (Peltola et al., 2000); these expressions of GAN seem linked to the fact that the cells affected are GABA-ergic neurons that contain cellular reservoirs of GAD required to meet demands for GABA-dependent synaptic transmission.

Batten disease, a real curiosity, is a recessively inherited fatal neurodegenerative disease of children due to a mutation in both copies of a gene *CLN3*, thus leading to ceroid lipofuscin accumulation in neurons. A gene-disruption mouse model revealed altered expression of enzymes required for the synthesis of the neurotransmitter glutamate, and circulating antibodies to brain proteins including GAD65. This prompted a search for GADA in Batten disease itself, with positive results (Chattopadhyay et al., 2002). As yet, the role of GADA in the overall pathogenesis is uncertain.

Movement Disorders

There is a miscellaneous group of movement disorders marked by basal ganglion autoantibodies (BGA). These include Sydenham's chorea (jerky movements), Tourette syndrome (motor and vocal tics), and PANDAS, which is an acronym for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (Shulman, 1999). Speculative extensions have included obsessive–compulsive and attention-deficit disorders. The (weak) evidence for autoimmunity includes antecedent streptococcal infection with analogy drawn to rheumatic carditis (see Chapter 62), autoantibodies of various specificities but particularly to basal ganglia neurons, and capacity of sera for passive transfer of disease after direct injection into the striatum of rats. Not surprisingly, such claims are contested, and their validity remains an open question (Giovannoni, 2005).

Sandhoff Disease

Sandhoff disease (SD) is yet another genetic-metabolic autoimmune curiosity. It is a recessively inherited lysosomal storage disease of infancy in which neuronal cell death results from an enzyme deficiency that causes accumulation of GM2 gangliosides in lysosomes of brain cells. There is a murine equivalent created by knockout of the *hexb* gene that encodes the hexosamidase enzyme. It is claimed that anti-ganglioside antibodies in SD accelerate premature neuronal death, perhaps as complexes of antibody and ganglioside that cause inflammatory activation of microglial cells. Similar events are postulated for the human counterpart (Yamaguchi et al., 2004).

Epilepsy and Rasmussen Disease

Considerable interest was aroused in the early 1990s by suggestions that a severe form of childhood encephalitis with seizures (Rasmussen disease, RD) was attributable to antibodies to excitatory glutamate receptors in the CNS. Reminiscent of the serendipitous observations on experimental myasthenia gravis in rabbits immunized merely to raise an anti-acetylcholine receptor reagent (see Chapter 48), rabbits that were immunized to raise antibodies to the glutamate receptor subunit 3 (GluR3) developed seizures and histologic encephalitis, and this prompted a successful search for autoantibodies to glutamate receptors in RD (Barinaga, 1995). Thus some cases of idiopathic epilepsy could be due to autoimmunity affecting receptors for Glu3, so causing increased neuronal excitability; this idea remains current, but far from substantiated (see Chapter 48).

Narcolepsy

Narcolepsy is a sleep-wake disorder with onset during adolescence in which there is irresistible daytime sleepiness attributed to deficient neurotransmission dependent on orexin, a transmitter that promotes wakefulness. Prima facie this would seem hardly likely as an autoimmune disease, yet there is an extremely (and puzzlingly) high association with the class II MHC allele, HLA DQB1*0602. An autoantibody relevant to narcolepsy has never been demonstrated by conventional assays, but Smith et al. (2004) obtained such evidence by a bioassay based on passive transfer of IgG to mice, the readout being increased contractile responses of detrusor muscle strips to cholinergic stimulation; however, this research group considers that their data do not yet provide definitive evidence for an autoantibody-mediated loss of wakefulness, and are "very preliminary" (Gordon, T. personal communication, 2005).

Neuromyelitis Optica, Devic's Disease

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease that is more frequent among Asian than white populations. The selective effects of the disease on optic nerves, and longitudinally extensive spinal cord lesions, distinguish NMO from multiple sclerosis (MS),

which it otherwise resembles (see Chapter 46). The CSF contains inflammatory cells, but lacks oligoclonal bands as seen in MS. Since a routinely demonstrable marker autoantibody in MS has long been awaited, it is notable that serum in NMO is reactive by immunofluorescence on sections of mouse CNS tissue, specifically with the abluminal face of microvessels exclusive to the CNS (Lennon et al., 2004). The sensitivity of the assay was 58% and the specificity 100% for the clinical diagnosis of NMO, and sensitivity may well be enhanced by adaptations of the assay. The identity of the NMO autoantigen for which the presumed origin is astrocytic foot processes and the blood–brain barrier, has been defined as the widely distributed aquaporin-4 water channel (Lennon et al., 2005). Since the Ig reactant in NMO is likely generated extrathecally, and can be causally implicated, logical therapies would include plasma exchange or IVIG, followed by corticosteroids and azathioprine. The question remains open as to whether NMO represents a subcategory of MS or is an independent autoimmune CNS disorder.

OMENN SYNDROME

This eponym was applied to infantile familial reticuloendotheliosis with eosinophilia (Omenn, 1965), and is now expanded to cover intestinal and cutaneous expressions, absence of B lymphocytes in blood and lymphoid tissues and infiltration of oligoreactive T cells in skin, gut, liver, and spleen (Aleman et al., 2001). Omenn syndrome is the result of deficiency mutations of recombinase-activating genes (RAGs). A similar phenotype can ensue with a mutant allele of the gene encoding ARTEMIS, a DNA repair protein (Ege et al., 2005). There may be hypomorphic mutations that allow for some residual V(D)J recombination, such that T cells with restricted receptor specificity can expand in peripheral tissues, and it is these that cause the cutaneous and intestinal lesions. This scenario however implies a defect also in tolerance processes and T-cell regulation, and this was duly shown in two patients in whom there was additionally a greatly limited expression of the AIRE protein consequent on dysfunction of the *AIRE* gene (Cavadini et al., 2005). Omenn syndrome is thus an extraordinarily compound "experiment of nature" that vividly illustrates the complexities of establishment and maintenance of natural immune tolerance.

OSTEOARTHRITIS

Osteoarthritis (OA) is prototypically a degenerative articular disease with a late-in-life onset and a relationship to articular stresses, although an early inflammatory phase with an apparent acute onset is recognized. Cases of OA are routinely used as "disease controls" in tests for specificity of serologic reactants for the diagnosis of RA; however when citrullinated peptide or native collagen type II (CII) is used as the test antigen, some degree of reactivity in OA is usually observed. Xiang et al. (2004) noted that sera from cases of OA, unlike RA, react more with denatured than native CII, although, sera from RA, OA and relapsing polychondritis all do react with native CII. Accordingly, specific epitopes of CII were investigated in OA by Burkhardt et al. (2002), and the result was that, in contrast to RA sera, OA sera tended to engage a different epitope, CII-F4, that is localized to the C-terminal region of native CII, rather than the RA-related epitopes, C1 and M2.139. Notably, in mice, an anti-CII-F4 monoclonal antibody (MAb), in contrast to other epitope-specific MAbs, is not pathogenic on passive transfer; in fact, anti-CII-F4 inhibits the matrix-disruptive effects of antisera with other CII epitope specificities (Burkhardt et al., 2002). Xiang et al. (2004) further found that OA sera, as well as having anti-CII-F4 reactivity, also recognize another antigen identified as triose phosphate isomerase (TPI). Perhaps in OA, as for other conditions discussed herein, autoimmunity can develop in the setting of tissue injury of diverse cause and be associated with multiple reactants that perhaps contribute to clinical expressions.

PARATHYROID DYSFUNCTION

An autoimmune basis for hypoparathyroidism, proposed over 40 years ago by Solomon and Blizzard (1963), was consolidated by Seeman (1967) in a histologic study that revealed lymphocyte-plasma cell infiltrates, and hence it was called lymphocytic parathyroiditis. In the first edition of this text in 1985, Maclaren and Blizzard discussed the autoimmune polyendocrine syndrome (APS) types 1 and 2 and, for type 1, noted that three major components existed and appeared usually in a uniform order: candidiasis, hypoparathyroidism, and Addison's disease. Moreover, autoimmune hypoparathyroidism, albeit a characteristic feature of APS type 1, did not occur in any of their 224 cases of APS type 2. However serologic reactivity with parathyroid tissue was insufficiently convincing to generate clinical laboratory assays, although autoantibodies were reported to bind to the cell surface of human parathyroid cells and to inhibit parathyroid secretion. An autoimmune basis was clinched when Li et al. (1996) demonstrated reactivity with the calcium-sensing receptor (Ca-SR) on the cell surface in both APS1-associated and sporadic cases of hypothyroidism, and an immunoprecipitation assay showed that the extracellular domain of the Ca-SR contained the reactive epitopes. Notably this autoimmune reactivity, in 56% of 25 cases, was far more frequent among females than

males. In a further turn of events, cases were recognized wherein antibodies to the parathyroid Ca-SR had an inactivating effect, so rendering the glands insensitive to ambient calcium; the ensuing oversecretion of parathyroid hormone had effects akin to those seen with parathyroid adenomas, causing hypocalciuric hypercalcemia (Pallais et al., 2004). These antibodies were functionally active but nondestructive and, interestingly, were of the IgG4 subclass and associated in one case with autoimmune pancreatitis in which there are raised levels of IgG4 (see Chapter 56). Thus the clinical expressions of autoimmune parathyroiditis with anti-Ca-SR reactivity may be either hypoparathyroidism if the antibodies are non-blocking or if the glands are subject to lymphocytic destruction, or hyperparathyroidism if the antibodies are blocking and the glands retain functional activity.

PULMONARY ALVEOLAR PROTEINOSIS

Pulmonary alveolar proteinosis (PAP) moves us still closer to the fringes of autoimmunity. As reviewed by Trapnell et al. (2003), the three forms of PAP include *congenital* (gene mutations), *secondary* (macrophage deficiencies with pulmonary alveolar injury), and *acquired* (idiopathic), which is now suspected as autoimmune. Pulmonary alveolar proteinosis is rare (0.37 cases in 100,000) and is mostly of the acquired type. Symptoms include a history of smoking, breathlessness, cough, fever, chest pain, and hemoptysis; and diagnosis depends on suggestive radiologic findings, abnormal pulmonary function tests, a milky bronchoalveolar lavage fluid containing alveolar macrophages and lymphocytes, and lung biopsy findings of abundance of surfactant proteins. A clue to the nature of PAP was the unexpected occurrence of a similar disease in knockout mice deficient in granulocyte-macrophage colony-stimulating factor (GM-CSF) (Stanley et al., 1994). The pathogenesis was shown to be impaired clearance of surfactant proteins from the lung due to deficiency of GM-CSF, and GM-CSF replacement was efficacious. Further studies showed that the critically affected cell was the pulmonary alveolar macrophage, which failed to undergo maturation by reason of the presence in serum and pulmonary fluid of an inhibitor that proved to be an IgG class autoantibody with neutralizing capacity for the binding of GM-CSF to alveolar macrophages. A latex agglutination test for detection of this autoantibody had a sensitivity of 100% and specificity of 98% for the diagnosis of PAP (Trapnell et al., 2003). Also, current therapy with whole-lung lavage can now be rationally supplemented by various regimens of GM-CSF supplementation, with prospects for the eventual waning of autoimmunity to GM-CSF, i.e. "desensitization" (Schoch et al., 2002).

RELAPSING POLYCHONDRITIS

Relapsing polychondritis (RP) was so named by Pearson et al. (1960) to designate recurring inflammatory damage to cartilage throughout the body, but particularly nasal, auricular, tracheobronchial, and audiovestibular, rather than articular cartilage. There is clustering in some 30% of cases with other autoimmune diseases that include rheumatoid arthritis and SLE, mononuclear cell accumulation at affected sites, association with HLA DR4, and benefit from prednisolone (McAdam et al., 1976). Specific antibodies to cartilage were detected by immunofluorescence by Dolan et al. (1976) and Foidart et al. (1978), using indirect immunofluorescence and cartilage substrate from mouse leg, or human costochondral and tracheal cartilage with preincubation with hyaluronidase to remove masking proteoglycan. Autoantibodies were detected mostly in the acute stages of RP, were of IgG class and non-complement-binding, levels correlated with clinically assessed disease severity, and absorption exclusively with type II collagen removed reactivity from serum. In a study on a single case, Bergfeld (1978) detected deposits *in vivo* of IgG, IgA, and C3 in affected cartilage using immunofluorescence. In a further study on cartilage antibodies by immunofluorescence (Ebringer et al., 1981), positive results were obtained in six of nine patients, and there was clustering with organ (thyroid)-specific autoantibodies. Results of studies on T-cell-mediated immunity to cartilage in relapsing polychondritis, cited by Foidart et al. (1978) and Ebringer et al. (1981), were indecisive.

Sera of cases of RP are reactive with CII. Terato et al. (1990) studied 202 Japanese patients with RA, 26 with RP, and 92 with other rheumatic diseases for antibodies to human CII by ELISA, with positive results in 11 (42%) of the cases of RP, in contrast with 11% in RA and 0.3% in controls. Tests using peptides derived by cyanogen bromide (CB) digestion of CII indicated that RP sera reacted preferentially with the CB 9.7 peptide, unlike the anti-CII reactivity of sera from other diseases. However, subsequent studies have indicated that in RP the primary reactant might not be CII, since reactivity of T cells and serum antibodies was greater with collagens IX and XII (Yang et al., 1993). Also, NOD DQI transgenic mice that were immunized with CII developed an auricular chondritis simulating human RP, with reactivity to CIX as well as to CII (Taneja et al., 2003). Thus, anti-CII in RP may occur secondarily by epitope spreading. Also, the species of anti-CII detectable does not have the epitope specificity associated with erosive articular inflammation (Burkhardt et al., 2002). Moreover, there is a further candidate autoantigen in RP, the cartilage protein matrilin I (Hansson et al., 2004), which is abundant in cartilage at sites affected in RP (trachea, ears) but sparse in sites that are spared (joints). A model of RP could be induced in mice by active immunization with matrilin I, with intact B cells and complement factor V being

required for disease expression, and transfer of disease by a MAb to matrilin I indicated that RP is indeed an antibody-mediated autoimmune disease (Hansson et al., 2004).

REPERFUSION INJURY AUTOIMMUNITY

When interest recently became refocused on innate immunity as a first line of immune defense based on invariant recognition of pathogen-associated molecular patterns, this would have seemed an unlikely contributor to autoimmunity. However, innate immunity can not only promote adaptive immune responses (see Chapter 2) but of itself may directly participate in autoimmune reactions, exemplified by reperfusion injury (RI). This curious syndrome is due to acute inflammation provoked by tissue ischemia, usually a thrombotic infarction followed by restoration of blood flow. It has been studied experimentally after ischemia of intestine or skeletal muscle (Carroll and Holers, 2005), with findings that RI requires a source of natural IgM autoantibody produced by B-1 B-cells and integrity of complement pathways, since mice deficient in RAG-1 and complement are protected. The use of a panel of MAbs derived from hybridomas developed in RAG-1-deficient mice with RI enabled the identification of a single injury-reconstituting IgM with a specificity for an auto-(neo)-antigen generated by ischemic intestine. In other examples, such as renal ischemia-reperfusion, complement pathways alone appeared sufficient. It will be of interest to ascertain the molecular identity of the RI autoantigen, and ascertain its role in the human counterpart.

SARCOIDOSIS

An early suspicion that sarcoidosis could have an autoimmune connection (Mackay and Burnet, 1963) was not sustainable, although no other credible pathogenesis has emerged. The disease expressions depend on noncaseating granulomatous lesions in multiple sites—skin, lymph nodes, lung, liver or CNS—and pathogenicity depends either on inflammatory fibrosis as in the lungs, or pressure effects of lesions as in the CNS. The immune system is clearly implicated as judged by the granulomatous histopathology, but how? Ho et al. (2005) drew on the known Th1-biased $CD4^+$ T-cell response in sarcoidosis, together with possible involvement of the natural killer (NK) T-cell system. The NKT cells are activated by glycolipid antigens presented by CD1 molecules on antigen-presenting cells (APCs) (see Chapter 2). A particular class of CD1 (CD1d), after interaction with NKT cells with an invariant receptor (Vα24/JαQ, paired with Vβ11) exerts regulatory effects. In mice at least, the CD1-restricted repertoire includes autoreactive T cells (Park et al., 2001). Ho et al. (2005) ascertained in sarcoidosis a deficiency (for unknown reason) of Vα24 NKT cells, with ensuing loss of their normal regulatory effect on CD1d-dependent reactivity. However, for sarcoidosis to be ascribed to autoimmunity, the depletion of Vα24 NKT cells would need to be associated with persistent stimulation of the Th1 T-cell pathway by an endogenous autoantigen, for which there is meager evidence. In fact epidemiologic data implicate an environmental influence, with possible person-to-person transmission, or a shared response to a provocative agent.

BENEFICIAL (PROTECTIVE) AUTOIMMUNITY

It seems appropriate to conclude these cameos with brief comments on beneficial effects of autoimmunity. Earlier on, it was proposed by Grabar (1975) that "natural" autoantibodies existed, and served beneficially as *transporteurs* of products of tissue degradation. But there are now other intriguing examples of beneficial autoimmunity. One is the production of antibodies to cytokines. Such antibodies are well recognized as an unwanted complication of therapy of certain autoimmune diseases with recombinant cytokines such as type 1 interferons for multiple sclerosis, with ensuing loss of efficacy, comparable with effects of unwanted antibodies during therapy with the growth factor erythropoietin, or factor VIII, resulting in loss of efficacy. Now, surprisingly, there is reported the natural occurrence of beneficial autoantibodies to the pro-inflammatory mediator TNF-α, akin to what is attempted therapeutically with MAb to TNF-α in rheumatoid arthritis. These spontaneous autoantibodies to TNF-α were demonstrable during the course of adjuvant arthritis in rats, and also in patients with RA at much greater frequency than in cases of OA (Wildbaum et al., 2003). A further example may be neuroprotective autoimmunity. It was observed in rats that passive transfer of autoimmune T cells reactive with myelin basic protein reduced neuronal loss after injury to the CNS (Kipnis et al., 2002). Hence, it was presumed that the immune repertoire naturally contains CNS-reactive (myelin-reactive) T cells that promote the disposal of products of neural injury and thereby facilitate recovery from neural injury. Of interest, depletion of $CD4^+CD25^+$ regulatory T (Treg) cells enhanced this myelin-reactive T-cell protection, while augmentation of Treg effects had the opposite effect (Kipnis et al., 2002). Whether these findings can be extended more generally, beyond CNS injury is uncertain, as are the prospects for extension towards development of a vaccine to ameliorate effects in humans of traumatic or ischemic damage to the CNS (Hauben et al., 2001). So, like the contents of Pandora's box, the baneful diaspora of autoimmu-

nity may be accompanied by a glimmer of hope, namely benefit to the organism under particular circumstances, such as damping down inflammatory mediators, or facilitating recovery after tissue injury.

References

Alarcon-Segovia, D., Abud-Mendoza, C., Reyes-Gutierrez, E., Iglesias-Gammara, A., and Diaz-Jovanen, E. (1984). Involvement of the urinary bladder in systemic lupus erythematosus: A pathologic study *J. Rheum. 111,* 208–210.

Aleman, K., Noordzij, J.G., de Groot, R., van Dongen, J.J., and Hartwig, N.G. (2001) Reviewing Omenn syndrome. *Eur. J. Pediatr. 160,* 718–725.

Asanuma, Y., Oeser, A., Shintani, A.K., Turner, E., Olsen, N., Fazio, S., Linton, MacRae, F., Raggi, P., and Stein, C.M. (2003). Premature coronary-artery atherosclerosis in systemic lupus erythematosus. *N. Engl. J. Med. 349,* 2407–2415.

Barinaga, M. (1995) Antibodies linked to rare epilepsy. *Science 268,* 362–363.

Behan, P.O., Behan, W.M., and Bell E.J. (1985). The post-viral fatigue syndrome—an analysis of the findings in 50 cases. *J. Infect. 10,* 211–222.

Benagiano, M., Azzurri, A., Cierevo, A., Amedei, A., Tamburini, C., Ferrari, M., Telford, J.L., Baldari, T.C., Romagnani, S., Cassone, A., D'Elios, M., and Del Prete, G. (2003). T helper type 1 lymphocytes drive inflammation in human atherosclerotic lesions. *Proc. Natl. Acad. Sci. U.S.A. 100,* 6658–6663.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F., and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet. 27*, 20–21.

Bergfeld, W.F. (1978). Relapsing polychondritis with positive direct immunofluorescence (letter). *Arch. Dermatol, 114,* 127.

Berliner, J.A. and Watson, A.D. (2005). A role for oxidized phospholipids in atherosclerosis. *N. Engl. J. Med. 353,* 9–11.

Boye, E., Morse, M., Huttner, M., Erlanger, I., MacKinnon, K.J., and Klassen, J. (1979). Immune complex-mediated interstitial cystitis as a major manifestation of systemic lupus erythematosus. *Clin. Immunol. Immunopathol. 13,* 67–76.

Browning, J.D. and Horton, S.D. (2004). Molecular mediators of hepatic steatosis and liver injury. *J. Clin. Invest. 114,* 147–152.

Buchwald, D., Komaroff, A.L. (1991). Review of laboratory findings for patients with chronic fatigue syndrome. *Rev. Infect. Dis*, *13*(suppl 1), 512–518.

Bullock, A.D., Becich, M.J., Klutke, C.G., and Ratliff, T.L. (1992). Experimental autoimmune cystitis: A potential murine model for ulcerative interstitial cystitis. *J. Urol. 148,* 1951–1956.

Buono, C., Binder, C.J., Stavrakis, G., Witztum, J.L., Glimcher, L.H., and Lichtman, A.H. (2005). T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. *Proc. Natl. Acad. Sci. U.S.A. 102,* 1596–1601.

Burkhardt, H., Koller, T., Engstrom, A., Nandakumar, S.K., Turnay, J., Kraetsch, H.G., Kalden, J., Holmdahl, R. (2002). Epitope-specific recognition of type II collagen by rheumatoid arthritis antibodies is shared with recognition by antibodies that are arthritogenic in collagen-induced arthritis in the mouse. *Arthritis Rheum. 46,* 2339–2348.

Buyon, J.P. and Clancy, R.M. (2003) Maternal autoantibodies and congenital heart block: mediators, markers and therapeutic approach. *Semin. Arthritis Rheum. 33,* 140–154.

Buyon, J.P., Kim, M.Y., Copel, J.A., and Friedman, D.M. (2001). Anti-Ro/SSA antibodies and congenital heart block: necessary but not sufficient. *Arthritis Rheum. 44,* 1723–1727.

Byrne, E. (1988). Idiopathic chronic fatigue and myalgia syndrome myalgic encephalomyelitis: Some thoughts on nomenclature and aetiology. *Med. J. Aust. 148,* 80–82.

Carroll, M.C. and Holers, V.M. (2005). Innate autoimmunity. *Adv. Immunol. 86,* 137–157.

Casadevall, N., Molho-Sabatier, P., Dupoy, E., Tobelem, P., Varet, G.B., and Mayeux, P. (1996). Brief report: Autoantibodies against erythropoietin in a patient with pure red-cell aplasia. *N. Engl. J. Med. 334,* 630–633.

Cavadini, P., Vermi, W., Facchetti, F., Fontana, S., Nagafuchi, S., Mazzolari, E., Sediva, A., Marrella, V., Villa, A., Fischer, A., Notarangelo, L.D., and Badolato, R. (2005). AIRE deficiency in thymus of 2 patients with Omenn syndrome. *J. Clin. Invest. 15,* 728–732.

Centola, M., Wood, G., Frucht, D.M. Galon, J., Aringer, M., Farrell, C., Kingma, D.W., Horwitz, M.E., Mansfield, E., Holland, S.M., O'Shea, J.J., Rosenberg, H.F., Malech, H.L., and Kastner, D.L. (2000). The gene for familial Mediterranean fever, MEFV, is expressed in early leucocyte development and is regulated in response to inflammatory mediators. *Blood*, *95,* 3223–3233.

Chattopadhyay, S., Ito, M., Cooper, J.D., Brocks, A.L., Curran, T.M., Powers, J.M., and Pearce, D.A. (2002). An autoantibody inhibitory to glutamic acid decarboxylase in the neurodegenerative disorder Batten disease. *Hum. Mol. Genet. 11,* 1421–1431.

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,* 808–810.

Dessypris, E.N. (1991). The biology of pure red cell aplasia. *Semin. Haematol. 28,* 275–284.

Dolan, D.L., Lemmon, G.B., and Teitelbaum, S.L. (1976). Relapsing polychondritis. Analytical literature review and studies on pathogenesis. *Am. J. Med. 41,* 285–297.

Ebringer, R., Rook, G., Swana, G.T., Bottazzo, G.F., and Donaich, D. (1981). Autoantibodies to cartilage and type II collagen in relapsing polychondritis and other rheumatic diseases. *Ann. Rheum. Dis. 40,* 473–479.

Ege, M., Ma, Y., Burkhard, M., Kaiwak, K., Lu, H., Lieber, M.R., Schwarz, K., and Pannicke, U. (2005). Omenn syndrome due to ARTEMIS mutation. *Blood 105,* 4179–4186.

Emeson, E.E., Robertson, A.L. (1988). T lymphocytes in aortic and coronary intimas: Their potential role in atherogenesis. *Ann. J. Pathol. 130,* 369–376.

Fetissov, S.O., Hallman, J., Oreland, L., af Klintenberg, B., Grenbäck, E., Hulting, A-L., and Hökfelt, T (2002). Autoantibodies against—MSH, ACTH and LHRH in anorexia and bulimia nervosa patients. *Prac. Natl. Acad. Sci. U.S.A. 99,* 17155–17160.

Fister, G.M. (1938). Similarity of interstitial cystitis (Hunner's ulcus) to lupus erythematosus. *J. Urol. 40,* 37–51.

Foidart, J.-M., Abe, S., Martin, G.R., Zizic, T.M., Barnett, E.V., Lawley, T.J., and Katz, S.I. (1978). Antibodies to type II collagen in relapsing polychondritis. *N. Engl. J. Med. 299,* 1203–1207.

Freeman, R. (2005). Autonomic peripheral neuropathy. *Lancet 365,* 1259–1270.

Gasch, A.T., Smith, J.A., and Whitcup, S.M. (1999). Birdshot retinochoroidopathy. *Br. J. Opthalmol 83*, 241–249.

Giovannoni, G. (2005). Anti-neruronal antibodies and movement disorders. *J. Neuroimmunol. 163,* 5–7.

Goldstein, G. and Mackay, I.R. (1970). *The Human Thymus.* London: Heinemann.

Grabar, P. (1975). Hypothesis. Autoantibodies and immunological theories: An analytic review. *Clin. Immunol. Immunopathol. 4,* 453–466.

Guest, S.S. and Levitt L. (2003). Pure red-cell aplasia secondary to anti-erythropoietin antibodies. *N. Engl. J. Med. 349,* 2572–2573.

Hand, J.R. (1949). Interstitial cystitis: Report of 223 cases (204 women and 19 men). *J. Urol. 61,* 291–310.

Hansson, G. (2005). Inflammation, atherosclerosis and coronary artery disease. *N. Engl. J. Med. 352,* 1685–1695.

Hansson, A.-S., Johanneson, M., Svensson, L., Nandakumar, K.S., Heinegad, D., and Holmdahl, R. (2004). Relapsing polychondritis, induced in mice with Matrilin 1, is an antibody and complement-dependent disease. *Am. J. Path*ol. *164,* 959–66.

Hauben, E., Agranov, E., Gothilf, A., Nevo, U., Cohen, A., Smirnov, I., Steinman, L., and Schwartz, M., (2001). Posttraumatic therapeutic vaccination with modified myelin self-antigen prevents complete paralysis while avoiding autoimmune disease. *J. Clin. Invest. 108,* 591–599.

He, C., Soffer, E.E., Ferris, C.D., Walsh, R.M., Szurszewski, J.H., and Farrugia, G. (2001). Loss of interstitial cells of Cajal and inhibitory innervation in insulin-dependent diabetes. *Gastroenterology 121,* 427–434.

Ho, L.-P., Urban, B.C., Thickett, D.R., Davis, R.J.O., and McMichael, A.J. (2005). Deficiency of a subset of T cells with immunoregulatory properties in sarcoidosis. *Lancet 365,* 1062–1072.

Holmes, G.P., Kaplan, J.E., Gantz, N.M., Komaroff, A.L., Schonberger, L.B., Straus, S.E., Jones, J.F., Dubois, R.E., Cunningham-Rundles, C., Pahwa, S., et al. (1988). Chronic fatigue syndrome: A working case definition. *Ann. Intern. Med.*, 108, 387–389.

Hunner, G.L (1914). A rare type of bladder ulcer in women. *Trans. South Surg. Gynec. Assoc. 27*, 247–292.

Jokinen, E.J., Alfthan, O.S., and Oravisto, K.J. (1972). Anti-tissue antibodies in interstitial cystitis. *Clin. Exp. Immunol. 11,* 333–339.

Jonasson, L., Holm, J., Skalli, O., Bondjers, G., and Hansson, G.K. (1986). Regional accumulations of T cells, macrophages and smooth muscle cells in the human atherosclerotic plaque. *Atherosclerosis 6,* 131–138.

Kaplan, M.J. and McCune, W.J. (2003). New evidence for vascular disease in early rheumatoid arthritis. *Lancet*, *361,* 1068–1069.

Kipnis, J., Mizrahi, T., Hauben, E., Shaked, E., and Schwartz, M. (2002). Neuroprotective autoimmunity: Naturally occurring CD+ CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Prod. Natl. Acad. Sci. U.S.A. 99,* 15620–15625.

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,* 1888–1996.

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.

Lennon, V.A., Ermilov, L.G., Szurzewski, J.H., and Vernino, S. (2003). Immunization with neuronal nicotinic acetylcholine receptor induces neurological autoimmune disease. *J. Clin. Invest. 111,* 907–913.

Lennon, V.A., Wingerchuk, D.M., Kryzer, T.J., Pittock, S.J., Luchinetti, C.F., Fujihara, K., Nakashima, I., and Weinshenker, B.G. (2004) A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet 364,* 2106–2112.

Lennon, V.A., Kryzer, T.J., Pittock, S.J., Verkman, A.S., and Hinson, S.R. (2005). IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J. Exp. Med. 202,* 473–477.

Li, Y., Song, Y.-H., Rais, N., Connor, E., Schatz, D., Muir, A., and Maclaren, N. (1996). Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism *J. Clin. Invest*. *97,* 910–914.

Libby, P. and Hansson, G.K. (1991). Involvement of the immune system in human atherogenesis: Current knowledge and unanswered questions. *Lab. Invest*. *64,* 5–15.

Lichtman, A.H., Cybulsky, M., and Luscinskas, F.W. (1996). Immunology of atherosclerosis: The promise of mouse models. *Am. J. Pathol. 149,* 351–357.

Lloyd, A.R, Wakefield, D., Boughton, CR., and Dwyer, J.M. (1989). Immunological abnormalities in the chronic fatigue syndrome. *Med. J. Aust. 151,* 122–124.

Lloyd, A., Hickie, I., Wakefield, D., Boughton, C., and Dwyer, J. (1990). A double-blind placebo-controlled trial of intravenous immunoglobulin therapy in patients with chronic fatigue syndrome. *Am. J. Med. 89,* 561–568.

Lopes-Virella, M.F. and Virella, G. (1996). Cytokines, modified lipoproteins and arteriosclerosis in diabetes. *Diabetes 45*(Suppl. 3), S40–S44.

Luber-Narod, J., Austin-Ritchie, T., Banner, B., Hollins, C., Maramag, C., Price, H., and Menon, M. (1996). Experimental autoimmune cystitis in the Lewis rat: a potential animal model for interstitial cystitis. *Urol. Res. 24,* 367–373.

Mackay, I.R. and Burnet, F.M. (1963). *The Autoimmune Diseases: Pathogenesis, chemistry and therapy*, (pp. 78–79). Springfield IL: Charles C Thomas.

Maclaren, N.K. and Blizzard, R.N. (1985). Adrenal autoimmunity and autoimmune polyglandular syndromes. In N.R. Rose and I.R. Mackay (eds), *The Autoimmune Diseases,* 1st ed. (pp. 201–225). Orlando: Academic Press.

Mathews, J.D., Hooper, B.M., Whittingham, S., and Mackay, I.R. (1973). Association of autoantibodies with smoking, cardiovascular mortality and death in the Busselton population. *Lancet 2,* 754–758.

McAdam, L.P., O'Hanlan, M.A., Bluestone, R., and Pearson, C.M. (1976). Relapsing polychondritis. Prospective study of 23 patients and a review of the literature. *Medicine* (*Baltimore*) *55,* 193–215.

Medical Staff of the Royal Free Hospital (1957). An outbreak of encephalomyelitis in the Royal Free Hospital Group, London, in 1955. *Br. Med. J. 2,* 1436–1437.

Musabak, U., Sengul, A., Oktenli, C., Pay, S., Yesilova, Z., Kenar, L., Sanisoglu, S.Y., Inal, A., Tuzun, A., Erdil, A., and Bagci, S. (2004). Does immune activation continue during an attack-free period in familial Mediterranean fever? *Clin Exp Immunol 138,* 526–533.

Myers, M.A., Davies, J.M., Whisstock, J., Scealy, M., Mackay, I.R., and Rowley M.J. (2000) Conformational epitopes on the diabetes autoantigen GAD65 identified by peptide phage-display and molecular mimicry. *J. Immunol. 165,* 3830–3838.

Newman, L.S., Rose, C.S., and Maier, L.A. (1997). Sarcoidosis. *N. Engl. J. Med. 336,* 1224–1234.

Ochs, R.L., Stein, T.W., Jr., Peebles, C.L., Gittes, R.F., and Tan, E. (1994). Autoantibodies in interstitial cystitis. *J. Urol. 151,* 587–592.

Omenn G.S. (1965) Familial reticuloendotheliosis with eosimophilia. *N. Engl. J. Med. 273,* 427–432.

Oravisto, K.J. (1980). Interstitial cystitis as an autoimmune disease. A review. *Eur. Urol. 6,* 10–13.

Oravisto, K.J., Alftahn, O.S., Jokinen, E.J. (1970). Interstitial cystitis. Clinical and immunological findings. *Scand. J. Urol. Nephrol. 4,* 37–42.

Pallais, J.C., Kifor, O., Chen, Y.-B., Slovik, D., Brown, E.M. (2004). Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor. *N. Engl. J. Med. 351,* 362–364.

Park, S.-H, Weiss, A., Benlagha K, Kyin, T., Teyton, L., and Bendelac, A. (2001) The mouse CD1d-restricted repetoireis dominated by a few autoreactive T-cell families. *J. Exp. Med. 193,* 893–904.

Pearson, C.M., Kline, H.M., and Newcomer, V.D (1960). Relapsing polychondritis. *N. Engl. J. Med. 263,* 51–58.

Peeker, R., Atanasiu, L., and Logadottir, Y., (2003). Intercurrent autoimmune conditions in classic and non-ulcer interstitial cystitis. *Scand. J. Urol. Nephrol. 37,* 60–63.

Peltola, J., Kulmala, P., Isojärvi, J., Saiz, A., Latvala, K., Palmio, J., Savola, K., Knip, M., Keränen, T., and Graus, F. (2000) Antibodies to glutamic acid decarboxylase in patients with therapy resistant epilepsy. *Neurology 55,* 46–50.

Peterson, P.K., Shepard, J., Macres, M., Schenck, C., Crosson, J., Rechtman, D., and Laurie, N. (1990). A controlled trial of intravenous immunoglobulin G in chronic fatigue syndrome. *Am. J. Med. 89,* 554–560.

Phipps, R.P. (2000) Atherosclerosis: The emerging role of inflammation and the CD40–CD40 ligand system. *Proc. Natl. Acad. Sci. U.S.A. 97,* 6930–6932.

Piarulli, F., Lapolla, A., Sartore, G., Rossetti, C., Bax, G., Noale, M., Minicuci, N., Fiore, C., Marchioro, L., Manzato, R., and Fedele, D., (2005). Autoantibodies against oxidized LDLs in atherosclerosis in type 2 diabetes. *Diabetes Care 28,* 653–657.

Ramaekers, V.T., Rothenberg, S.P., Sequeira, J.M., Opladen, T., Blau, N., Quadros, E.V., and Selhub, J. (2005). Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *N. Engl. J. Med. 352,* 1985–1991.

Roman, M.J., Shanker, B.-A., Davis, A., et al. (2003). Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N. Engl. J. Med. 349,* 2399–2406.

Rothenberg, S.P., da Costa, M.P., Sequeira, J.M., Cracco, J., Roberts, J.L., Weedon, J., and Quadros E.V. (2004), Autoantibodies against folate receptors in women with a pregnancy complicated by neural-tube defect. *N. Engl. J. Med. 350,* 134–142.

Saiz, A., Arpa, J., Sagasta, A Casamitjana, R., Zarranz, J.J., Tolosa, E., and Graus, F. (1997). Autoantibodies to glutamic acid decarboxylase in three patients with cerebellar ataxia, late-onset insulin-dependent diabetes mellitus, and polyendocrine autoimmunity. *Neurology 49,* 1026–1030.

Salomonsson, S., Sonesson, S.-E., Ottosson, L., Muhallab, S., Olsson, T., Sunnerhagen, M., Kuchroo, VK., Thoren, P., Herlenius, E., and Wahren-Herlenius, M. (2005). Ro/SSA autoantibodies directly bind cardiomy cytes, disturb calcium homeostasis, and mediate congenital heart block. *J. Exp. Med. 201,* 11–17.

Salonen, JT., Ylä-Herttuala, S., Yamamoto, R., Butler, S., Korpela, H., Salonen, R., Nyyssonen, K., Palinski, W., and Witztum, J.L. (1992). Autoantibody against oxidized LDL and progression of carotid atherosclerosis. Lancet 339, 883–887.

Sant, G.R. (1991). Interstitial cystitis. *Mongor. Urol.* 12, 37–63.

Sant, G.R. and Theoharides, T.C. (1994). The role of the mast cell in interstitial cystitis. *Urol. Clin. N. Am. 21,* 41–53.

Schoch, O.D., Schanz, U., Koller, M., Nakata, K., Seymour, J.F., Russi, E.W., and Boehler, A. (2002). BAL findings in a patient with pulmonary alveolar proteinosis successfully treated with GM-CSF. *Thorax 57*, 277–280.

Schwartz, R.S. (2005). Autoimmune folate deficiency and the rise and fall of "horror autotoxicus." *N. Engl. J. Med. 352,* 1948–1950.

Schwartz, C.J. and Mitchell, J.R.A. (1962). Cellular infiltration of the human arterial associated with atheromatous plaques. *Circulation 28,* 73–78.

Seemann, N. (1967). Untersuchungen zur Häufigkeit der lymphozytären Parathyreoiditis. *Dtsch. med Wschr. 92,* 106–108.

Shipton, E.A. (1965). Hunner's ulcus (chronic interstitial cystitis). A manifestation of collagen disease. *Br. J. Urol. 37,* 443–449.

Shulman, S.T. (1999). Pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS) *Pediatr. Infect. Dis. J. 18,* 281–282.

Silk, M.R. (1970). Bladder antibodies in interstitial cystitis. *J. Urol. 103,* 307–309.

Skowera, A., Stewart, E., Davis, E.T., Cleare, A.J., Unwin, C., Hull, L., Ismail, K., Hossain, G., Wessely, S.C., and Peakman, M. (2002). Antinuclear autoantibodies in Gulf War-related illness and chronic fatigue syndrome patients. *Clin. Exp. Immunol. 129,* 354–358.

Smith, A.J., Jackson, M.W., Neufing, P., McEvoy, R.D., and Gordon, T.P. (2004). A functional autoantibody in narcolepsy. *Lancet 364,* 2122–21224.

Solomon, I.L. and Blizzard, R.M. (1963). Autoimmune disorders of the endocrine glands. *J. Pediatrics 63,* 1021–1033.

Stanley, E., Lieschke, G.J., Grail, D., Metcalf, D., Hodgson, G., Gall, J.A., Maher, D.W., Cebon, J., Sinickas, V., and Dunn, A.R. (1994). Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc. Natl. Acad. Sci. U.S.A 91,* 5592–5596.

Taneja, V., Griffiths, M., Behrens, M., Luthra, H.S., and David, C.S. (2003). Auricular chondritis in NOD.DQ8.Aßo (Ag7-/-) transgenic mice resembles human relapsing polychondritis. *J. Clin. Invest. 112,* 1843–1850.

Taylor, J.P., Hardy, J., and Fishbeck, K.H. (2002) Toxic proteins in neurodegenerative disease. *Science 296,* 1991–1995.

Terato, K., Shimozuru, Y., Katayama, K., Takemitsu, Y., Yamashita, I., Miyatsu, M., Fujii, K., Sagara, M., Kobayashi, S., Goto, M., Nishioka, K., Miyasaka, N., and Nagai, Y. (1990). Specificity of antibodies to type II collagen in rheumatoid arthritis. *Arthritis Rheum. 33*, 1493–1500.

Trapnell, B.C., Whitsett, J.A., Nakata, K. (2003). Pulmonary alveolar proteinosis. *N. Engl. J. Med. 345,* 2527–2539.

Tsimikas, S., Brilakis, E.S., Miller, E.R., McConnell, J.P., Lennon, R.J., Kornman, K.S., Witztum, JL, and Berger, P.B. (2005). Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. *N. Engl. J. Med. 353,* 46–57.

Vaarala, O., Alfthan, G., Jauhiainen, M., Leirsalo-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.

Van der Wal, A.C., Das, P.K., Bentz van de Berg, D., van der Loos, C.M., Becker, A.E. (1989). Atherosclerotic lesions in humans: *In situ* immunophenotypic analysis suggesting an immune mediated response. *Lab. Invest. 61,* 166–170.

Vernino, S., Low, P.A., Fealey, R.D., Stewart, J.D., Farrugia, G., and Lennon, V.A. (2000). Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N. Engl. J. Med. 343,* 847–855.

Vernino, S., Ermilov, L.G., Sha, L., Szurszewski, J.H., Low, P.A., and Lennon, V. (2004). Passive transfer of autoimmune autonomic neuropathy to mice. *J. Neurosci. 24,* 7037–7042.

Vernon, S.D. and Reeves, W.C. (2005). Evaluation of autoantibodies to common and neuronal cell antigens in chronic fatigue syndrome. *J. Autoimmune Dis. 2,* 5 *http://www/jautoindis.com/content/2/1/5*.

von Mikecz, H., Konstantinov, K., Buchwald, D.S., Gerace, I., and Tan E.M. (1997). High frequency of autoantibodies to insoluble cellular antigens in patients with chronic fatigue syndrome. *Arthritis Rheum. 40,* 295–305.

Walsh, B.T. and Devlin, M.J. (1998). Eating disorders: Progress and problems. *Science 280,* 1387–1390.

Wellen, K.E. and Hotarmisligil, G.S. (2005). Inflammation, stress and diabetes. *J. Clin. Invest. 115,* 1111–1119.

Wick, G., Schett, G., Amberger, A., Kleindienst, R., and Xu, Q. (1995). Is atherosclerosis an immunologically mediated disease? *Immunol. Today 16,* 27–33.

Wildbaum, G., Nahir, MA., and Karen, N. (2003). Beneficial autoimmunity to proinflammatory mediators restrains the consequences of self destructive immunity. *Immunity 19,* 679–688.

Wildin, R.S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J.L., Buist, N., Levy-Lahad, E., Mazzella, M., Goulet, O., and Perroni, L. (2001). X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet. 27*, 18–20.

Wynn, T.A. (2004). Fibrotic disease and the TH1/TH2 paradigm. *Nat. Rev. Immunol. 4,* 583–594.

Xiang, Y., Sekine, T., Nakamura, H., Imajoh-Ohmi, S., Fukuda, H., Nishioka, K., and Kato, T., (2004). Proteomic surveillance of autoimmunity in osteoarthritis: identification of triosephosphate isomerase as an autoantigen in patients with osteoarthritis. *Arthritis Rheum. 50,* 1511–1521.

Xu, Q.B., Oberhuber, G., Gruschwitz, M., and Wick, G. (1990). Immunology of atherosclerosis: Cellular composition and major histocompatibility complex class II antigen expression in aortic intima, fatty streaks, and atherosclerotic plaques in young and aged human specimens. *Clin. Immunol. Immunopathol. 56,* 344–359.

Yamaguchi, A., Katsuyama, K., Nagahama, K., Takai, T., Aoki, I., and Yamanaka, S. (2004). Possible role of autoantibodies in the pathophysiology of GM2 gangliosides. *J. Clin. Invest. 113,* 200–208.

Yang, C.L., Brinkmann, J., Rui, H.F., Vehring, K.H., Lehmann, H., Kakow, J., Wolff, H.H., Gross, W.L.J., and Muller, P.K. (1993). Autoantibodies to cartilage collagens in relapsing polychondritis. *Arch. Dermatol. Res. 285,* 245–249.

Zhou, X., Stemme, S., and Hansson, G.K. (1996). Evidence for a local immune response in atherosclerosis: CD4/T cells infiltrate lesions of APO-E deficient mice. *Am. J. Pathol. 149,* 359–366.

CHAPTER

72

Autoantibody Assays, Testing, and Standardization

MARVIN J. FRITZLER*
**Faculty of Medicine, University of Calgary, Calgary, Canada*

ALLAN WIIK†
†Statens Serum Institut, Copenhagen, Denmark

The first description of human autoantibodies and the development of the Wassermann test for syphilis that was initially based on agglutination and complement fixation dates to 1906 (Wassermann et al., 1906). In the early 1940s, the active component of the Wassermann test was found to be a phospholipid, which was subsequently named "cardiolipin" and the antibodies reactive to cardiolipin were named "reagin" (Pangborn, 1941; 1942). Serum reagins were detected by a flocculation or immunoprecipitation (IP) that employed a mixture of cardiolipin, cholesterol, and phosphatidylcholine in an assay that was referred to as the Venereal Disease Research Laboratory (VDRL) test. Early in the history of the application of the VDRL test, it became clear that some individuals, including certain systemic lupus erythematosus (SLE) patients, had a positive test without any other evidence for syphilis (Moore and Mohr, 1952; Moore and Lutz, 1955). Subsequent studies showed that this reactivity was due to the presence of phospholipid antibodies, and in particular anticardiolipin antibodies (Harvey and Shulman, 1966; Koike et al., 1984) (see Chapter 30). The importance of the false positive VDRL test in the diagnosis and classification of SLE was underscored by the inclusion of the false-positive VDRL test as one of the criteria in the original American Rheumatism Association's criteria (Cohen et al., 1971).

In 1948, just as the VDRL test was achieving some interest in its relationship to SLE, Hargraves and colleagues published the seminal paper on the lupus erythematosus (LE) cell (Hargraves et al., 1948). The identification of the LE cell, the development of the LE cell assay and the introduction of indirect immunofluorescence (IIF) and immunodiffusion (ID) assays stand as a historical pivotal point because it led to the development of other new diagnostic techniques and the rapid expansion of medical literature that documented numerous autoantibodies in a variety of conditions (Van Venrooij and Maini, 1993; Krapf et al., 1996; Peter and Shoenfeld, 1996). In the five decades that followed the development of the LE cell assay, the detection of autoantibodies in human sera has become an increasingly important approach to the diagnosis and management of patients with autoimmune conditions. At the outset, most laboratories prepared many of their own reagents for these assays, but increasing requests and a widening spectrum of autoantibodies heightened the need for supplies and a wide variety of reagents to perform these assays. This became an entry point for manufacturers and commercial vendors to develop and market a wide variety of diagnostic kits. Although some diagnostic laboratories still use assays that are developed in house, commercial kits have gained a significant foothold in many areas of autoimmune serology. The use of commercial kits has become so widespread because they are cost-effective, easy to use and can satisfy criteria for accreditation.

The discovery of autoantibodies also had an important impact on progress in the fields of basic and applied immunology. The mechanisms that led to the generation

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

of autoantibodies created an understanding of immune regulation. In addition, the ongoing identification of novel autoantibodies strengthens the foundation of disease-related autoantibody markers while adding new conditions to an expanding list of autoimmune diseases. Although autoantibodies are thought to be relevant in B-cell dominant conditions; autoantibodies are also found where T-cell-mediated processes are thought to predominate (Rose, 1996).

Many autoantibodies have become central to the practice of clinical immunology and immunopathology. For example, the diagnostic and classification criteria for many autoimmune diseases, such as rheumatoid arthritis (RA) (Arnett et al., 1988), SLE (Tan et al., 1982), mixed connective tissue disease (MCTD) (Kasukawa, 1987), and proposed criteria for type 1A diabetes mellitus (Seissler et al., 1998) have included autoantibodies as key components of their respective diagnostic algorithms. Taken together, the detection of autoantibodies has achieved a prominent role in clinical practice, particularly as an approach to the diagnosis of autoimmune conditions, and recently as markers of disease prognosis.

Box 72.1
Considerations regarding the clinical interpretation and application of autoantibody testing

- Normal sera contain autoantibodies
- Disease-specific autoantibodies can antedate disease
- Definition of a positive test is based on an empirically defined threshold
- Some assays such as indirect immunofluorescence are based on subjective interpretation
- Lack of standardized antibodies and reagents
- Variety of assays and diagnostic assay platforms
- Adoption of high throughput assays before validation of local performance
- Impact of short-term cost and budget restraints
- Long-term impact on total health care costs are mostly unknown

SPECTRUM OF AUTOANTIBODIES

The spectrum of autoantibodies in autoimmune diseases is continually expanding and the reader is referred to the relevant chapters in this book. Table 72.1 lists and summarizes some of the autoantibodies that are widely used as an approach to diagnosis and treatment of autoimmune and related conditions. To be sure, the list of autoantibodies found in these conditions is much wider. For example, in autoimmune conditions with multi-organ involvement such as SLE, more than 100 different autoantibodies have been described (Sherer et al., 2004) and over 20 have been found in systemic sclerosis (SSc) (Fritzler, 1993; Ho and Reveille, 2003). In most organ-localized diseases, the autoantibodies tend to be directed to only one or a few antigens harbored in the affected organ (see Table 72.1).

ASSAYS AND TECHNOLOGIES FOR AUTOANTIBODY TESTING

Many laboratories rely on commercial autoantibody assay kits that employ a variety of technologies, including IIF, ID, IP, immunoblotting (IB) and line immunoassays, enzyme-linked immunosorbent assays (ELISA), and, more recently, addressable laser bead assays (ALBA) and antigen arrays (Fritzler, 2002; Robinson et al., 2002). The use and application of these kits has been attended by certain limitations that are not always apparent to the clinician (Box 72.1). One of the more popular assay platforms is based on the ELISA or enzyme immunoassay (EIA) because it offers sensitivity, high throughput, and relatively low cost on the background of only modest equipment needed to perform the assay. Unfortunately, there has been little done to standardize these kits (Feltkamp, 1996) and post-marketing surveillance and quality assurance is largely left to the manufacturers (Fritzler et al., 2003a). Although the ELISA and EIA kits are constantly being improved, some high-titer antibodies to a variety of autoantigens (e.g., fibrillarin, PM/Scl, centromere, nuclear envelope) are often not detected. Hence, a report that indicates a "negative EIA or ELISA" screen should not be interpreted as "negative for all relevant autoantibodies" that might otherwise be detected by IIF and other diagnostic technologies.

A number of studies have evaluated the performance characteristics of kits for detecting ANA using IIF and EIA (Jansen et al., 1987; Avina-Zubieta et al., 1995; Jaskowski et al., 1995; Vancheeswaran et al., 1996; Bizzaro et al., 1998; Emlen and O'Neill, 1997; Cordiali et al., 1998; Fawcett et al., 1999; Bossuyt, 2000; Kern et al., 2000; Ulvestad et al., 2000; Fritzler et al., 2003b). Other studies focused on kits for antineutrophil cytoplasmic antibodies (ANCA) detection (Csernok et al., 2002). Studies that compared EIA kits from different manufacturers with conventional assays such as IIF and ID concluded that there was significant discordance between conventional assays and EIA (Jaskowski et al., 1996; Fawcett et al., 1999) and between kits from different manufacturers (Emlen and O'Neill, 1997). In one study, EIAs were found to be more sensitive than ID (Jaskowski et al., 1995) and another study that used a cross-section of serum referred to a rheumatology laboratory found moderate-to-good agreement between IIF and anti-DNA results with two commercial EIA kits (Ulvestad et al., 2000). Analysis of the design of some studies suggests that lack of agreement between EIA and conventional assays may

TABLE 72.1 Autoantibodies used in diagnosis of autoimmune diseases

Autoantibody	Disease	Sensitivity (%)/Specificity (%)	Assay
dsDNA	SLE DIL*	45–60/80–90	IIF, ELISA, IP
Sm (U2-U4-6 RNP)	SLE	10–20/95	ELISA, ID.IP
U1RNP	MCTD SLE	90/90–95 40/30	ELISA, ID, IP
SS-A/Ro 60	SjS SLE	50–70/80 45/25	ELISA, ID, IP
SS-B/La	SjS SLE	40/70–90 15/10	ELISA, ID, IP
Histone/chromatin	SLE DIL	70/20 80/20	ELISA, IB
Topoisomerase I/Scl-70	SSc	20/90	ELISA, IB, IP
U3 RNP/fibrillarin	SSc	15/95	IIF, IP
Centromere	SSc	50–60/90	IIF, ELISA, IB
Jo-1/histidyl tRNA synthetase	PM/DM	20–30/90–95	ELISA, IB
Citrullinated peptides and proteins	RA	65–85/85–95	ELISA
Proteinase 3	Wegener's granulomatosis	60–70/90–95	ELISA, IB, IIF (cANCA)
Myeloperoxidase	MPA	65/80	ELISA, IIF (pANCA)
Pyruvate dehydrogenase complex (M2)	PBC	75–80/85	IIF, IB, ELISA, IP
Smooth muscle F-actin	Chronic active hepatitis	70/25	IIF, ELISA
Intrinsic factor	Pernicious anemia	90/70	IIF, ELISA
Human tissue transglutaminase	Celiac disease	85/90	IIF, ELISA
Cardiolipin complex	APS	80/50	ELISA
β2-glycoprotein I	APS	80/90	ELISA
Basement membrane (α3 domain of type IV collagen)	Anti-GBM disease (Goodpasture syndrome)	80/80	IIF, ELISA
Acetylcholine receptor	Myasthenia gravis	80/90	ELISA, IB
Thyroid microsomes (thyroid peroxidase)	Hashimoto's thyroiditis	90/75	IIF, ELISA
Cadherins	Pemphigus vulgaris	90/90	IIF
Skin basement membrane zone	Bullous pemphigoid	80/80	IIF
Yo/Purkinje cell	PCD	70/95	IIF, IB
Hu	PEM	70/90	IIF, IB, IP

APS, antiphospholipid syndrome; cANCA and pANCA, cytoplasmic and perinuclear antineutrophil cytoplasmic antibodies; DIL, drug-induced lupus; ELISA, enzyme-linked immunoassay; IB, immunoblot; IIF, indirect immunofluorescence; IP, immunoprecipitation; MCTD, mixed connective tissue disease; MPA, microscopic polyangiitis; PBC, primary biliary cirrhosis; PCD, paraneoplastic cerebellar degeneration; PEM, paraneoplastic encephalomyelitis; PM, polymyositis; RNP, ribonucleoprotein; SjS, Sjögren syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TNF, tumor necrosis factor.

*Some RA patients treated with anti-TNF or sulfasalazine develop features of drug-induced lupus and anti-dsDNA antibodies.

depend on the diagnosis and/or the selection bias of the control patients under study (Jaskowski et al., 1995; Jaskowski et al., 1996; Emlen and O'Neill, 1997; Ulvestad et al., 2000).

A study by Tan et al. (1999) focused on nine different commercial EIA kits for the determination of antibodies to a number of nuclear and cytoplasmic autoantigens and highlighted deficiencies in intrinsic properties of these kits (sensitivities and specificities). A more recent study focused on the clinical laboratories themselves and, although one might expect academic laboratories to be rather proficient in the implementation of the EIA kits, that was not the case (Fritzler et al., 2003b). As in other studies (Feltkamp, 1996), it was suggested that quality-control procedures for daily performance of tests in the clinical laboratory setting should not be ignored, and that a minimal performance of EIA assays should be established (Fritzler et al., 2003b).

In the field of neutrophil-specific autoantibodies (NSA), which are found in many diseases where the chronic inflammation involves ongoing recruitment of neutrophils to the

inflammatory site (Wiik, 1980), the main emphasis has been on the study of ANCA (Hoffman and Specks, 1998; Wiik, 2001). Antineutrophil cytoplasmic antibodies are rather specifically directed against one of the two main azurophilic granule enzymes, proteinase 3 or myeloperoxidase, and thus fulfill criteria for being called ANCA, whereas the NSA found in RA, ulcerative colitis, primary sclerosing cholangitis, and autoimmune hepatitis are polyspecific, directed to a whole host of autoantigens that are not yet well known. It is important to mention that ANCA are found at high and intermediate levels in patients with small vessel necrotizing vasculitides, whereas borderline positivity can be found in many chronic inflammatory diseases and long-standing infections. Therefore, it is imperative to adjust positive cut-off of assays for proteinase 3 ANCA and myeloperoxidase ANCA at a sufficiently high level to assure sufficiently high diagnostic specificity for systemic vasculitis (Hagen et al., 1998). Results are best secured when both IIF of ANCA and ELISA quantitation of ANCA are studied and reported together (Hagen et al., 1998; Savige et al., 1999). Just a few studies have been undertaken to look at commercial assays for the determination of ANCA (Csernok et al., 2002).

Since there is considerable variation in the sensitivity of the various commercial diagnostic kits, the particular assay used to generate the test result should be made available to the clinician. In addition, because of the growing trend to use automated immunoassay systems with quantitation, these laboratories should follow standards of chemistry instrumentation and require analytical measured ranges and periodic calibrations. Clinical laboratories that adopt multiplexed assays (e.g., ALBA) must establish reference ranges and cut-off values for each analyte, and the sensitivity and specificity must be established with care as the laboratory findings may present a difficult interpretation problem. In the future, instrumentation may be developed for automated quantitation with measurement of avidity (affinity) and isotype profile of the specific autoantibody, which will help in determination of specificity and significance of the autoantibody.

CLINICAL APPLICATIONS OF AUTOANTIBODY TESTING

Before considering the application of autoantibody testing in the clinical setting, it is important to appreciate certain limitations to the interpretation and application of these tests (see Box 72.1) that may be understood by considering a number of features of autoimmunity and assays used to detect autoantibodies. First, any given human serum contains a wide range of autoantibodies of varying concentrations (Rose, 1996). Second, it is important to understand that the measurement and assignment of an abnormal or elevated autoantibody test requires an empirically defined threshold that is dependent on numerous factors (Fritzler et al., 2003a; Wiik et al., 2004). Third, some disease-specific autoantibodies can antedate overt disease by many years. For example, antibodies to centromere proteins (CENPs) may antedate the clinical diagnosis of SSc by many years (Wigley et al., 1992; Kallenberg et al., 1988) and antibodies to Scl-70 (topoisomerase I) were linked to the development of pulmonary fibrosis and higher mortality (Kuwana et al., 1994; Scussel-Lonzetti et al., 2002). In harmony with this, a large study of non-SLE patients harboring anti-dsDNA antibodies as detected by the Farr immunoassay were found to develop SLE within the course of 4 years (Swaak and Smeenk, 1985). Similarly, in 115 of 130 SLE patients, at least one autoantibody related to SLE could be traced back to a mean of 3.3 years before the clinical onset of SLE (Arbuckle et al., 2003). Recent evidence showed that antibodies to cyclic citrullinated peptide (CCP) are seen in patients with joint symptoms that antedate sufficient diagnostic criteria for RA (Kroot et al., 2000; Rantapää-Dahlqvist et al., 2003; Van Gaalen et al., 2004). It has been suggested that the appearance of type IA diabetes mellitus (DM) related autoantibodies also commonly antedates the clinical diagnosis (Atkinson and Eisenbarth, 2001). A sequential appearance and peak of antibodies to insulin (IAA), glutamic acid decarboxylase (GADA), and islet cell antigens ICA512/IA2A has been observed in children between the ages of 9 months and 3 years who then go on to develop DM. These autoantibodies tend to be persistent and most of the individuals that progress to overt diabetes express multiple anti-islet autoantibodies by the time of diabetes onset.

Although the detection of autoantibodies in human sera has become an important tool for experienced clinicians, the clinical application and validation of autoantibody testing has been hampered by a lack of standardized assays, divergent technologies, cost constraints, and subjective interpretation of results. New autoantibody assays are continually being developed and adopted with the anticipation that they will lead to better reproducibility and to higher sensitivity and specificity. If the ultimate goal is to improve patient care, it is imperative that the accuracy of autoantibody testing be validated by studies of sufficiently large patient populations before a new assay is accepted for clinical use. Since very few prospective, unbiased, and multicenter studies have been published, the clinical accuracy of many autoantibody tests is still uncertain. Studies based on literature review and meta-analysis have been published as "evidence-based guidelines" (Kavanaugh and Solomon, 2002; Solomon et al., 2002; Reveille and Solomon, 2003) but the translation of this information is hampered because of the wide variety of newer assays and assay parameters that may not apply to contemporary technologies used in many laboratories.

To achieve significant clinical utility, it is important that the ability of autoantibody testing to discriminate between disease and the absence of disease, or between disease and confounding clinical conditions is understood. The performance characteristics of each test must be known in order to avoid misinterpretation, incorrect diagnosis, and potentially harmful treatment. To aid in patient follow-up and the introduction of therapeutic strategies, the prognostic significance of a given autoantibody in a patient who may have a subclinical autoimmune disease should also be considered. For example, the finding of an autoantibody that indicates a poor prognosis in the context of early disease should lead to appropriate follow-up and monitoring for emerging organ involvement that would place the patient at risk for increased morbidity or mortality. Attention to these factors requires the close collaboration and cooperation between patients, dedicated clinicians, laboratory scientists, and the diagnostics industry (Fritzler et al., 2003a; Wiik et al., 2004). Once a diagnostic assay has been established and is widely utilized in a clinical setting, standardized postmarketing surveillance and quality assurance by manufacturers and laboratories alike should be mandatory (Tan et al., 2002).

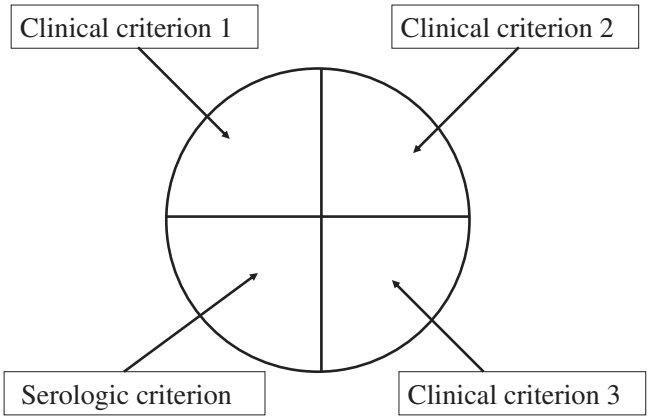


Figure 72.1 Relationship between clinical criteria and autoantibody results, illustrated as a situation where a positive antinuclear antibody is used as a disease criterion, but a specific antibody is indicative of a certain subtype of disease and, hence, prognosis. The fewer the clinical criteria, the more helpful is the addition of a serologic criterion or support for diagnosis.

AUTOANTIBODIES AND CLINICAL DIAGNOSTICS

Years of accumulated clinical experience and research have led to the development of clinical criteria to support a given diagnosis of a number of autoimmune diseases (Masi et al., 1980; Kasukawa, 1987; Alarcon-Segovia and Cardiel, 1989; Cassidy et al., 1989; Leavitt et al., 1990; Jennette et al., 1994; Savige et al., 1999; Wilson et al., 1999). Since systemic rheumatic diseases frequently involve multiple organ systems, multiple criteria are required to confirm a diagnosis. As a consequence, it is rare that a single pathognomonic criterion be translated into certainty that a given diagnosis is correct. To ensure that results from the immunology laboratory gain maximum utility for clinicians, it is important to study the performance of each assay as a diagnostics aid in early disease, since that is the time at which a serologic result will impact diagnostic and prognostic considerations the most (Fenger et al., 2004). This is best done by building a particular serum bank containing samples from patients at an early stage of disease, often drawn at a time when diagnosis has not yet been set with certainty due to lack of some clinical criteria. The clinical diagnosis then has to be established after months or years of clinical follow-up. The impact of adding a serologic criterion at a stage where clinical criteria are insufficient for diagnosis is illustrated in Figure 72.1. Although autoantibodies are included as a criterion in many diseases, the diagnosis of SSc stands out as one disease where the inclusion of disease-specific autoantibodies is lacking (Masi et al., 1980; LeRoy and Medsger, 2001). A recent study suggested that there is clinical value in including topoisomerase I and centromere autoantibodies in criteria for the classification of SSc (Nadashkevish et al., 2004). The more knowledgeable the clinician is with regard to the clinical and laboratory characteristics of diseases, the greater the chance that a diagnosis is correct. Many of the diseases listed in Table 72.1 have clinical subgroups with somewhat dissimilar manifestations and, hence, prognosis. Of relevance to this discussion, these subgroups are often associated with different autoantibody profiles and specificities (Permin et al., 1978; Cervera et al., 1993; Kuwana et al., 1994; Mustila et al., 2000; Wiik, 2001; Cervera et al., 2002; Scussel-Lonzetti et al., 2002; Targoff, 2002). Therefore, an autoantibody may be a valuable marker of a prognostic subgroup and used to tailor follow-up and therapeutic strategies (Franssen et al., 1998).

Autoantibodies with high disease specificity, regarded as disease-specific markers, tend to be rare (von Muhlen and Tan, 1995). Unfortunately, some data related to certain autoantibodies and their disease specificity relied on outdated technology and on studies published several decades ago. With the introduction and adoption of newer diagnostic technology platforms, autoantibodies that were thought to be specific for one disease may subsequently turn out to be associated with a variety of autoimmune diseases (van Eenennaam et al., 2002). The presence of multiple disease-related autoantibodies occurring in a single serum has strengthened the importance of autoantibody profiles, and changes of autoantibody expression in individual patients may be more indicative that a certain diagnosis is correct

than the presence of a single antibody (von Muhlen and Tan, 1995; Blass et al., 1999; Vasiliauskiene et al., 2001; Visser et al., 2002). Hence, multiplexed and autoantigen array technologies that are now emerging will provide even more extensive autoantibody and autoepitope profiles in a given patient, which in turn may alter approaches to diagnosis and therapeutics (Schachna et al., 2002; Robinson et al., 2002; Fritzler, 2002).

New technologies often focus on achieving high diagnostic sensitivity rather than on reaching high diagnostic specificity of specific autoantibodies. In addition, new assays are frequently released before the ability of the assay to accurately predict a specific diagnosis is fully known. Diagnostic specificity of an assay generally decreases when high sensitivity is achieved. Thus, when a new test is introduced, clinicians may overlook the loss of diagnostic specificity and focus on the improved sensitivity in predicting a disease. Evaluation of new assays is necessary to demonstrate that the diagnostic specificity of new assays does not suffer because of attempts to increase sensitivity. To achieve this, it is important that sera from local control patients with inflammatory autoimmune diseases are tested in order to measure the predictability of any given assay. This testing needs to be attended by consent from patients whose sera is being used in such studies and by close collaboration with experienced clinicians who are willing to monitor and record the clinical manifestations of each patient who donated serum for these studies. It is evident that a highly sensitive automated assay can be used for selection of sera that should be further tested using a very specific assay, since this may save time and money by focusing attention to few candidate positive sera (Wiik et al., 2004).

As laboratory equipment becomes more sophisticated and, as noted above, assays become more sensitive, adjustment of cut-off or screening titers to achieve higher specificity should be undertaken. New recommendations were recently suggested regarding IIF testing for ANA, using HEp-2 cell substrates (Tan et al., 1997). For years, the cut-off for normal results was considered to be at a serum dilution of 1:40 or 1:80. This multicenter international study revealed that 32% of normal individuals were positive at serum dilutions of 1:40. Thus, a titer of 1:160 was recommended as a more acceptable cut-off between normal and abnormal sera. Unfortunately, despite the best efforts of international committees to provide these recommendations, subsequent cursory surveys and review of subsequent literature suggest that these guidelines have not been widely applied. While it is appreciated that a value of screening at a dilution of 1:40 is to rule out a disease such as SLE, Sjögren syndrome (SjS) and SSc (Griner et al., 1981; Homburger, 1995), the disadvantage is the generation of a significant health care cost through referral and investigation of people that have a "positive" ANA but do not have a systemic rheumatic condition.

CLINICAL PRACTICE GUIDELINES

Because of the complexity of modern autoimmune serology, there is a pressing need for guidelines that outline the appropriate and economic use of serologic testing. If a limited number of clinicians are involved in ordering diagnostic testing and the laboratory serves a finite number of departments, it is easier to achieve an understood consensus on testing strategies. However, as exemplified by current trends, regional and national laboratories provide service to more extensive areas or populations and to family practitioners as well as specialists, and in these situations written guidelines may be required to develop screening criteria, and then a rational testing scheme that limits unnecessary testing (Wiik et al., 2004). Another strategy is to develop order forms in such a way that the doctor can choose between a tentative diagnosis, after which a few rational tests are done, or choose just one or two tests from this testing panel (Figure 72.2). A positive screening test may lead to the referral of a patient to a specialist, who will then plan the further investigation program including further serologic testing. It is important to realize that the pretest probability to detect a useful diagnostic laboratory result increases dramatically with each clinical feature that has been incorporated into the tentative diagnosis (Keren and Nakamura, 1997). Some clinics have agreed with laboratory specialists on the use of post-test algorithms to alleviate appropriate use of positive and negative laboratory results in relation to tentative diagnoses.

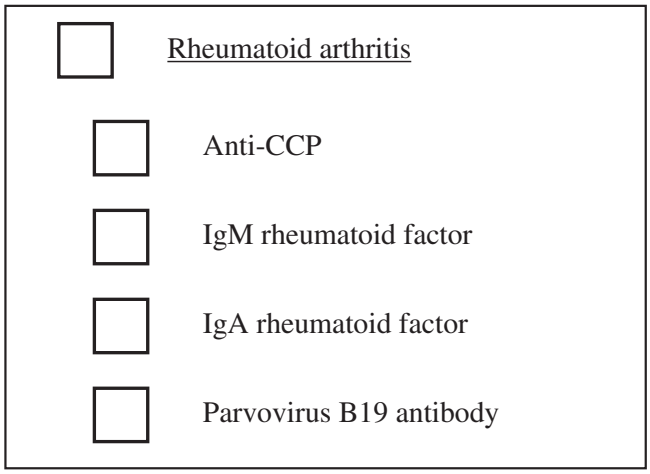


Figure 72.2 Example of a test order hierarchy from a laboratory order form based on tentative diagnosis or on single test(s).

LABORATORY REPORTS, INTERPRETATION, AND COST ANALYSIS

When an autoantibody is found, the positive result is usually communicated as a printed report that is mailed directly to the requesting physician. Unfortunately, in some jurisdictions, current regulatory constraints (Health Protection Acts and Freedom of Information Protection Acts) prohibit the transmission of a digital report directly to the doctor's personal computer or other digital device. Perhaps an international effort to develop consensus on the electronic medical record and the digital transmission of clinical data would help alleviate concerns about this technology (Vogt et al., 2004). A description of the most common diagnostic associations related to an autoantibody found in diagnostic testing is included with the result, for example: "Anticentromere antibodies are found in about 25% of patients with SSc. This antibody is most commonly seen in the limited form (CREST) subset of the disease."

To aid in interpretation of the results, information related to the sensitivity and specificity of a positive result should be tabulated and made accessible in a printed form and on the internet. Also the level of the antibody or the strength of expression should be mentioned together with cut-off towards disease control patients and the range of credible measurements. Some laboratories routinely accompany reports on positive findings with a short written comment on the most frequent clinical association(s) to help direct further diagnostic work-up.

Many laboratories use an algorithm that includes a rapid and inexpensive screening test followed by more specific tests as an approach to rationally screen for the presence of autoantibodies. For example, the IIF test or the whole cell ELISA is often used to detect autoantibodies in systemic rheumatic and other diseases. This serves three purposes: first, it serves as a triage for further testing; second, if the IIF or screening ELISA test is negative, unless there is a compelling clinical evidence to do so, no further testing is required and the result is reported accordingly; third, many autoantibodies can be accurately identified solely by this IIF screening approach. Examples include anticentromere antibodies that are seen primarily in patients with a limited subset of SSc or in a subpopulation of SjS patients (Vlachoyiannopoulos et al., 1993) and antibodies to parietal cells seen in pernicious anemia (see Table 72.1). If the IIF test is positive, the samples can be processed by more specific tests that include ELISAs, line assays that detect a variety of autoantibodies, or an addressable laser bead assay that has the ability to detect up to 100 different autoantibodies in a single serum sample (Fritzler, 2002; Robinson et al., 2002).

It is very important that all laboratory results can be validated and, in this context, borderline (low positive) results can be especially troublesome. It is recommended that borderline positive results must be confirmed or refuted by use of a second well-established independent technique to ensure that only certified positive results are reported to the physician or clinic. If controversy about the result persists, the laboratory should add a note of caution to the clinician that the result may have little significance in supporting a diagnosis and/or may recommend retesting a new serum sample in 1 to 3 months. Some regulatory agencies mandate that borderline positive tests should be reported as positive until proven otherwise through repeat or follow-up testing at appropriate intervals. High and intermediate positive results of a single credible technique can be reported without independent confirmation by a second technique. To aid in the interpretation of laboratory results, the chosen limit for positivity is stated on the report and ranges of low, intermediate, and strong positivity should be indicated.

A significant problem is that easy to perform and high throughput techniques are being adopted without proper clinical validation. Screening for autoantibodies by use of ELISA plates that have adsorbed complex mixtures of native and/or recombinant autoantigens or nuclear extracts is now used by many laboratories instead of ANA screening by IIF. This practice continues despite data showing that many patients with SjS, SSc, polymyositis/dermatomyositis, and juvenile RA score negative for ANA using such composite ELISA techniques (Keren and Nakamura, 1997; Fawcett et al., 1999). HEp-2-IIF screening of these "false-negative" sera reveals that most of these sera contain antibodies to nucleoli, nuclear matrix, nuclear envelope, nuclear pores, coiled bodies, PML domains, cell-cycle-specific antigens such as proliferating cell nuclear antigen or mitotic spindle apparatus components, or other cytoplasmic organelles and structures such as mitochondria, Golgi apparatus, signal recognition particles, or ribosomes (Bayer et al., 1999; Wiik, 2003a; 2003b; Stinton et al., 2004). Studies have shown that these autoantibodies are readily recognized by experienced technicians (Wiik and Lam, 2001) but are missed by ELISAs for ANA screening (Bayer et al., 1999). Some of these autoantibodies have defined clinical associations and should be identified as such (Table 72.2). On the other hand, autoantibodies that lack proven clinical value should not be reported until their diagnostic specificity and value have been clearly established. This approach mandates rigorous, multicenter studies of newly discovered autoantibodies to establish their clinical relevance or value. One of the most important aspects of autoantibody test results is the impact the result has on clinical decision making such as prognosis, follow-up strategy, and therapeutic intervention, since these factors are the ones that impact on the long-term outcome of disease.

There is a serious deficiency in our knowledge of the actual costs incurred through inappropriate laboratory testing, although the actual costs of laboratory diagnostic

TABLE 72.2 Antinuclear antibodies determined by indirect immunofluorescence patterns that may be useful in clinical diagnostics

Cellular structure	Molecular target	Disease associations
Coiled bodies	p80 coilin	Localized SSc, Raynaud syndrome
Golgi complex	Golgins, giantin	SLE, SjS, RA overlap syndromes, malignancy, viral infection
Mitotic spindle apparatus:		
Centrioles	Enolase, pericentrin, ninein	SSc, SjS, post-viral syndromes
NuMa pattern	NuMa 235	*Mycoplasma* infection
Spindle microtubules	HsEg5	SLE
Multiple nuclear dots	Sp-100, PML protein	Primary biliary cirrhosis
Nuclear envelope	Lamins A/C, B1, B2, LAP1/2	SLE, SjS, AIH, APS, SNP
Nuclear pore complex	p62, gp-210, Tpr	PBC, SjS
GW bodies	GW182	SjS, sensory/motor neuropathy

AIH, autoimmune hepatitis; APS, antiphospholipid antibody syndrome; NuMA, nuclear mitotic apparatus; PBC, primary biliary cirrhosis; RA, rheumatoid arthritis; SjS, Sjögren syndrome; SLE, systemic lupus erythematosus; SNP, seronegative polyarthritis; SSc, systemic sclerosis; Tpr, translocated promoter region.

Data from Peter and Shoenfeld, 1996; Bayer et al., 1999; Wiik, 2003a; Fritzler et al., 2003c; Stinton et al., 2004.

studies can easily be calculated. In Scandinavia, the estimated cost of all types of *in vitro* diagnostic testing in laboratories is between 2% and 3% of the total budget for healthcare. Diagnostic imaging techniques are now being used much earlier in spite of their much higher expense. Frequently, their clinical value with regard to the long-term prognosis has not been clearly elucidated. In the light of this significant expense, it is difficult to explain why low-cost and high-quality autoimmune serology is not given a higher priority by healthcare economists. The prospects for healthcare financing are very intimately linked to an accurate early diagnosis and treatment. Inappropriate laboratory testing (e.g., panel testing) is both costly and potentially misleading in the diagnostic workup. An estimation of long-term costs related to early accurate diagnosis and therapeutic intervention compared to a missed or a wrong diagnosis, with or without treatment, has to be performed to highlight the value of high-quality laboratory diagnostics. Factors that impact costs related to chronic diseases include the number of visits to clinics, length, and cost of stays in clinics, readmission rate, working days lost to patient and family, and productive years gained.

STANDARDIZATION AND QUALITY ASSURANCE

A set of standardized sera provided by the IUIS/AF/WHO/CDC Serology Committee (*www.ucalgary.ca/IUIS/index.htm*) is made available through the CDC in Atlanta (Smolen et al., 1997). The set of reference sera available through this program is continuously monitored and is currently being expanded to include antibodies to cardiolipin, β2-glycoprotein 1, fibrillarin, RNA polymerase I/III, ribosomal P proteins, proteinase 3 and myeloperoxidase (c- and p-ANCA, respectively). Sera used as standards for a particular methodology or technology platform need to be reviewed from time to time as exemplified by the re-evaluation of AF/CDC reference sera for immunoblotting purposes (Smolen et al., 1997). Although it would likely improve inter-laboratory variation in performance, standardized secondary antibodies are not widely available.

All clinical laboratories should participate in quality assurance and quality improvement programs such as the one administered by the College of American Pathologists (*www.cap.org*). The Clinical Laboratory Improvement Amendments of 1988 set standards for all laboratories engaged in clinical testing. These standards include requirements for trained and competent supervisory and testing personnel, record keeping and instrument maintenance, daily quality control practices, result reporting, and laboratory inspection and maintenance. It is not clear that these standards are being met in routine practice. In Europe the most widely used quality assurance and management program (DS/EN ISO/IEC 17025:2000) additionally sets laboratory standards aimed to prove that an assay actually gives results that are useful for clinical diagnostics.

SUMMARY

In contemporary medical practice, changes in laboratory diagnostics and new technologies are being introduced at a rapid pace. This is attended by concerns that certain key

matters (such as clinical utility) are not debated thoroughly between clinical and laboratory directors. It is important to avoid alienation of users and deliverers of potentially important information that should be used to set an early and precise diagnosis and estimate a likely prognosis. In many autoimmune diseases, autoantibodies are considered reporters of overt or subclinical organ or tissue damage. The detection of autoantibodies can serve as an indicator that focuses the clinician's attention to involved tissues or organs using classical diagnostic tools such as histopathology, imaging techniques, and organ function testing. Clinical guidelines to facilitate the communication between clinicians and laboratories can be formulated, mutually accepted algorithms for test ordering can be used, rules for reporting results must be agreed upon, and algorithms for optimal use of laboratory results should be adopted. A trade-off between sensitivity and diagnostic specificity always is ideally established before a test is made widely available, since cut-off values for a positive test, which are usually furnished by manufacturers, mainly separate a certain nosographic entity from the healthy population. This is not an ideal approach for differentiating between clinically related and overlapping autoimmune diseases.

The accuracy (sensitivity, specificity), reliability, and quality of many commercial kits have been a topic of study and concern. In many cases, the manufacturer has been assumed to be the root cause for these shortcomings; however, the use of commercial kits and their appropriate application in a clinical setting involves a rather complex chain of constituencies and events (Fritzler et al., 2003a). It has been suggested that a higher level of commitment and partnership between all of the participants is required to achieve the goal of improving the quality of patient care through the use of autoantibody testing and analysis (Wiik et al., 2004).

References

Alarcon-Segovia, D. and Cardiel, M.H. (1989). Comparison between 3 diagnostic criteria for mixed connective tissue disease: study of 539 patients. *J. Rheumatol. 16,* 328–334.

Arbuckle, M.R., McClain, M.T., Rubertone, M.V., Scofield, R.H., Dennis, G.J., James, J.A., and Harley, J.B. (2003). Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J Med. 349,* 1526–1533.

Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Laing, M.H., Luthra, H.S., Medsger, T.A., Mitchell, D.M., Neustadt, D.H., Pinals, R.S., Schaller, J.G., Sharp, J.T., Wilder, R.L., and Hunder, G.G. (1988). The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum. 31,* 315–324.

Atkinson, M.A. and Eisenbarth, G.S. (2001). Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet 358,* 221–229.

Avina-Zubieta, J.A., Galindo-Rodriguez, G., Kwan-Yeung, L., Davis, P., and Russell, A.S. (1995). Clinical evaluation of various selected ELISA kits for the detection of anti-DNA antibodies. *Lupus 6,* 370–374.

Bayer, P.M., Bauerfeind, S., Bienvenu, J., Fabien, N., Frei, P.C., Gilburd, B., Heide, K.G., Hoier-Madsen, M., Meroni, P.L., Monier, J.C., Monneret, G., Panzeri, P., Shoenfeld, Y., Spertini, F., and Wiik, A. (1999). Multicenter evaluation study on a new HEp2 ANA screening enzyme immune assay. *J. Autoimmun. 13,* 89–93.

Bizzaro, N., Tozzoli, R., Tonutti, E., Piazza, A., Manoni, F., Ghirardello, A., Bassetti, D., Villalta, D., Pradella, M., and Rizzotti, P. (1998). Variability between methods to determine ANA, anti-dsDNA and anti-ENA autoantibodies: a collaborative study with the biomedical industry. *J. Immunol. Methods 219,* 99–107.

Blass, S., Engel, J.-M., and Burmester, G.R. (1999). The immunologic homunculus in rheumatoid arthritis. *Arthritis Rheum. 42,* 2499–2506.

Bossuyt, X. (2000). Evaluation of two automated enzyme immunoassays for detection of antinuclear antibodies. *Clin. Chem. Lab. Med. 38,* 1033–1037.

Cassidy, J.T., Levinson, J.E., and Brewer, E.J. (1989). The development of classification criteria for children with juvenile rheumatoid arthritis. *Bull. Rheum. Dis. 38,* 5.

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., Galeazzi, M., Haga, H.J., Mathieu, A., Houssiau, F., Ingelmo, M., and Hughes, G.R.V. (1993). Systemic lupus erythematosus: Clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. *Medicine (Baltimore) 72,* 113–124.

Cervera, R., Piette, J.C., Font, J., Khamashta, M.A., 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.W.M., De Groot, P.G., Gromnica-Ihle, E., Baleva, M., Mosca, M., Bombardieri, S., Houssiau, F., and Gris, J.C. (2002). Antiphospholipid syndrome—Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum. 46,* 1019–1027.

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.

Cordiali, F.P., D'Agosto, G., Ameglio, F., Valesini, G., Alessandri, C., Farsi, A., Domenighetti, M.P., Passaleva, A., Scamardella, F., Neri, R., Bombardieri, S., Quinzanini, M., Franceschini, F., and Chiarotti, F. (1998). Determination of antibodies to extractable nuclear antigens by commercial kits: a multicenter study. *Int. J. Clin. Lab Res. 28,* 29–33.

Csernok, E., Ahlquist, D., Ullrich, S., and Gross, W.L. (2002). A critical evaluation of commercial immunoassays for antineutrophil cytoplasmic antibodies directed against proteinase 3 and myeloperoxidase in Wegener's granulomatosis and microscopic polyangiitis. *Rheumatology (Oxford) 41,* 1313–1317.

Emlen, W. and O'Neill, L. (1997). Clinical significance of antinuclear antibodies: comparison of detection with immunofluorescence and ELISA. *Arthritis Rheum. 40,* 1612–1618.

Fawcett, P.T., Rose, C.D., Gibney, K.M., Emerich, M.J., Athreya, B.H., and Doughty, R.A. (1999). Use of ELISA to measure antinuclear antibodies in children with juvenile rheumatoid arthritis. *J. Rheumatol. 26,* 1822–1826.

Feltkamp, T.E.W. (1996). Antinuclear antibody determination in a routine laboratory. *Ann. Rheum. Dis. 55,* 723–727.

Fenger, M., Wiik, A., Hoier-Madsen, M., Lykkegaard, J.J., Rozenfeld, T., Hansen, M.S., Samsoe, B.D., and Jacobsen, S. (2004). Detection of antinuclear antibodies by solid-phase immunoassays and immunofluorescence analysis. *Clin. Chem. 50,* 2141–2147.

Franssen, C., Gans, R., Kallenberg, C., Hageluken, C., and Hoorntje, S. (1998). Disease spectrum of patients with antineutrophil cytoplasmic autoantibodies of defined specificity: distinct differences between patients with anti-proteinase 3 and anti-myeloperoxidase autoantibodies. *J. Intern. Med. 244,* 209–216.

Fritzler, M.J. (1993). Autoantibodies in scleroderma. *J. Dermatol. 20,* 257–268.

Fritzler, M.J. (2002). New technologies in the detection of autoantibodies. In K. Conrad, M.J. Fritzler, M. Meurer, U. Sack, and Y. Shoenfeld (eds), *Autoantigens, Autoantibodies, Autoimmunity,* (pp. 50–63). Lengerich: Pabst Scientific Publishers.

Fritzler, M.J., Wiik, A., Fritzler, M.L., and Barr, S.G. (2003a). The use and abuse of commercial kits used to detect autoantibodies. *Arthritis Res. Ther. 5,* 192–201.

Fritzler, M.J., Wiik, A., Tan, E.M., Smolen, J.S., McDougal, J.S., Chan, E.K.L., Gordon, T.P., Hardin, J.A., Kalden, J.R., Lahita, R.G., Maini, R.N., Reeves, W.H., Rothfield, N.F., Takasaki, Y., Wilson, M., Byrd, M., Slivka, L., and Koziol, J.A. (2003b). A critical evaluation of enzyme immunoassay kits for detection of antinuclear antibodies of defined specificities. III. Comparative performance characteristics of academic and manufacturers' laboratories. *J. Rheumatol. 30,* 2374–2381.

Fritzler, M.J., Zhang, M., Stinton, L.M., and Rattner, J.B. (2003c). Spectrum of centrosome autoantibodies in childhood varicella and post-varicella acute cerebellar ataxia. *BMC Pediatrics 3,* 11.

Griner, P.F., Mayewski, R.J., Mushlin, A.I., and Greenland, P. (1981). Selection and interpretation of diagnostic tests and procedures. *Ann. Intern. Med. 94,* 553–600.

Hagen, E.C., Daha, M.R., Hermans, J., Andrassy, K., Csernok, E., Gaskin, G., Lesavre, P., Ludemann, J., Rasmussen, N., Sinico, R.A., Wiik, A., and van der Woude, F.J. (1998). Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int. 53,* 743–753.

Hargraves, M.M., Richmond, H., and Morton, R. (1948). Presentation of two bone marrow elements: the "tart" cells and the "L.E." cell. *Mayo Clin. Proc. 27,* 25–28.

Harvey, A.M. and Shulman, L.E. (1966). Connective tissue disease and the chronic biologic false positive test for syphyllis (BFP-STS reaction). *Med. Clin. North Am. 50,* 1271–1279.

Ho, K.T. and Reveille, J.D. (2003). The clinical relevance of autoantibodies in scleroderma. *Arthritis Res. Ther. 5,* 80–93.

Hoffman, G.S. and Specks, U. (1998). Antineutrophil cytoplasmic antibodies. Arthritis Rheum. 4, 1521–1537.

Homburger, H.A. (1995). Cascade testing for autoantibodies in connective tissue diseases. *Mayo Clin. Proc. 70,* 183–184.

Jansen, E.M., Deng, J., Beutner, E.H., Kumar, V., and Chorzelski, T.P. (1987). Comparison of commercial kits for the detection of anti-nDNA antibodies using *Crithidia lucilae*. *Am. J. Clin. Pathol. 87,* 461–469.

Jaskowski, T.D., Schroder, C., Martins, T.B., Mouritsen, C.L., and Hill, H.R. (1995). Comparison of three commercially available enzyme immunoassays for the screening of autoantibodies to extractable nuclear antigens. *J. Clin. Lab. Anal. 9,* 166–172.

Jaskowski, T.D., Schroder, C., Martins, T.B., Mouritsen, C.L., Litwin, C.M., and Hill, H.R. (1996). Screening for antinuclear antibodies by enzyme immunoassay. *Am. J. Clin. Pathol. 105,* 468–473.

Jennette, J.C., Falk, R.J., Andrassy, K., Bacon, P.A., Churg, J., Gross, W.L., Hagen, E.C., Hoffman, G.S., Hunder, G.G., Kallenberg, C.G.M., McCluskey, R.T., Sinico, R.A., Rees, A.J., Van Es, L.A., Waldherr, R., and Wiik, A. (1994). Nomenclature of systemic vasculitides. *Arthritis Rheum. 37,* 187–192.

Kallenberg, C.G., Wouda, A.A., Hoet, M.H., and Van Venrooij, W.J. (1988). Development of connective tissue disease in patients presenting with Raynaud's phenomenon: a six year follow up with emphasis on the predictive value of antinuclear antibodies as detected by immunoblotting. *Ann. Rheum. Dis. 47,* 634–641.

Kasukawa, R. (1987). Preliminary diagnostic criteria for classification of mixed connective tissue disease. In R. Kasukawa and G.C. Sharp (eds), *Mixed Connective Tissue Disease and Anti-Nuclear Antibodies,* (pp. 41–47). Amsterdam: Excerpta Medica.

Kavanaugh, A.F. and Solomon, D.H. (2002). Guidelines for immunologic laboratory testing in the rheumatic diseases: Anti-DNA antibody tests. *Arthritis Rheum. 47,* 546–555.

Keren, D.F. and Nakamura, R.M. (1997). Progress and controversies in autoimmune disease testing. *Clin. Lab. Med. 17,* 483–497.

Kern, P., Kron, M., and Hiesche, K. (2000). Measurement of antinuclear antibodies: assessment of different test systems. *Clin. Diagn. Lab. Immunol.* 7, 72–78.

Koike, T., Sueishi, M., Funaki, H., Tomioka, H., and Yoshida, S. (1984). Anti-phospholipid antibodies and biological false positive serological test for syphilis in patients with systemic lupus erythematosus. *Clin. Exp. Immunol. 56,* 193–199.

Krapf, A.R., Von Mühlen, C.A., Krapf, F.E., Nakamura, R.M., and Tan, E.M. (1996). *Atlas of immunofluorescent autoantibodies*. Munich: Urban & Schwarzenberg.

Kroot, E.J.J.A., De Jong, B.A.W., Van Leeuwen, M.A., Swinkels, H., van den Hoogen, F.H.J., Van't Hof, M., Van De Putte, L.B.A., Van Rijswijk, M.H., Van Venrooij, W.J., and Van Riel, P.L.C.M. (2000). The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum. 43,* 1831–1835.

Kuwana, M., Kaburaki, J., Okano, Y., Tojo, T., and Homma, M. (1994). Clinical and prognostic associations based on serum antinuclear antibodies in Japanese patients with systemic sclerosis. *Arthritis Rheum. 37,* 75–83.

Leavitt, R.Y., Fauci, A.S., Bloch, D.A., Michel, B.A., Hunder, G., Arend, W.P., Calabrese, L.H., Fries, J.F., Lie, J.T., Lightfoot, R.W.Jr., Masi, A.T., McShane, D.J., Mills, J.A., Stevens, M.B., Wallace, S.L., and Zvaifler, M.J. (1990). The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum. 33,* 1101–1107.

LeRoy, E.C. and Medsger, T.A., Jr. (2001). Criteria for the classification of early systemic sclerosis. *J. Rheumatol. 28,* 1573–1576.

Masi, A.T., Rodnan, G.P., Medsger, T., Jr., Altman, R.D., D'Angelo, W.A., Fries, J.F., LeRoy, E.C., Kirsner, A.B., Mackenzie, A.H., McShane, D.J., Myers, A.R., and Sharp, G.C. (1980). Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum. 23,* 581–590.

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, 297–316.

Moore, J.E. and Mohr, C.F. (1952). Biologically false positive serologic tests for syphilis. *J. Am. Med. Assoc. 150,* 467–473.

Mustila, A., Paimela, L., Leirisalo-Repo, M., Huhtala, H., and Miettinen, A. (2000). Antineutrophil cytoplasmic antibodies in patients with early rheumatoid arthritis—An early marker of progressive erosive disease. *Arthritis Rheum. 43,* 1371–1377.

Nadashkevish, O., Davis, P., and Fritzler, M.J. (2004). A proposal of criteria for the classification of systemic sclerosis. *Med. Sci. Monitor 10,* 615–621.

Pangborn, M.C. (1941). A new serologically active phospholipid from beef heart. *Proc. Soc. Exp. Biol. Med. 48,* 484–486.

Pangborn, M.C. (1942). Isolation and purification of a serologically active phospholipid from beef heart. *J. Biol. Chem. 143,* 247.

Permin, H., Hørbov, S., Wiik, A., and Knudsen, J.V. (1978). Antinuclear antibodies in juvenile chronic arthritis. *Acta Paediatr. Scand. 67,* 181–185.

Peter, J.B. and Shoenfeld, Y. (1996). *Autoantibodies.* Amsterdam: Elsevier.

Rantapää-Dahlqvist, S., De Jong, B.A.W., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., and Van Venrooij, W.J. (2003). Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum. 48,* 2741–2749.

Reveille, J.D. and Solomon, D.H. (2003). Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl–70, and nucleolar antibodies. *Arthritis Rheum. 49,* 399–412.

Robinson, W.H., Steinman, L., and Utz, P.J. (2002). Proteomics technologies for the study of autoimmune disease. *Arthritis Rheum. 46,* 885–893.

Rose, N.R. (1996). Foreword—The uses of autoantibodies. In J.B. Peter and Y. Shoenfeld (eds), *Autoantibodies,* (pp. xxvii-xxix). Amsterdam: Elsevier.

Savige, J., Gillis, D., Benson, E., Davies, D., Esnault, V., Falk, R.J., Hagen, E.C., Jayne, D., Jennette, J.C., Paspaliaris, B., Pollock, W., Pusey, C., Savage, C.O., Silvestrini, R., van der, W.F., Wieslander, J., and Wiik, A. (1999). International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am. J. Clin. Pathol. 111,* 507–513.

Schachna, L., Wigley, F.M., Morris, S., Gelber, A.C., Rosen, A., and Casciola-Rosen, L. (2002). Recognition of granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. *Arthritis Rheum. 46,* 1873–1884.

Scussel-Lonzetti, L., Joyal, F., Raynauld, J.P., Roussin, A., Rich, É., Goulet, J.R., Raymond, Y., and Senécal, J.L. (2002). Predicting mortality in systemic sclerosis—Analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. *Medicine (Baltimore) 81,* 154–167.

Seissler, J., de Sonnaville, J.J., Morgenthaler, N.G., Steinbrenner, H., Glawe, D., Khoo-Morgenthaler, U.Y., Lan, M.S., Notkins, A.L., Heine, R.J., and Scherbaum, W.A. (1998). Immunological heterogeneity in type I diabetes: presence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. *Diabetologia 41,* 891–897.

Sherer, Y., Gorstein, A., Fritzler, M.J., and Shoenfeld, Y. (2004). Autoantibody explosion in systemic lupus erythematosus. *Semin. Arthritis Rheum. 34,* 501–537.

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, Y., Van Venrooij, 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 immunoblotting. *Arthritis Rheum. 40,* 413–418.

Solomon, D.H., Kavanaugh, A.J., and Schur, P.H. (2002). Evidence-based guidelines for the use of immunologic tests: antinuclear antibody testing. *Arthritis Rheum. 47,* 434–444.

Stinton, L.M., Eystathioy, T., Selak, S., Chan, E.K.L., and Fritzler, M.J. (2004). Autoantibodies to protein transport and messenger RNA processing pathways: Endosomes, lysosomes, golgi complex, proteasomes, assemblyosomes, exosomes and GW Bodies. *Clin. Immunol. 110,* 30–44.

Swaak, T. and Smeenk, R. (1985). Detection of anti-dsDNA as a diagnostic tool: a prospective study in 441 non-systemic lupus erythematosus patients with anti-dsDNA antibody (anti-dsDNA). *Ann. Rheum. Dis. 44,* 245–251.

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.

Tan, E.M., Feltkamp, T.E.W., Smolen, J.S., Butcher, B., Dawkins, R., Fritzler, M.J., Gordon, T., Hardin, J.A., Kalden, J.R., Lahita, R.G., Maini, R.N., McDougal, J.S., Rothfield, N.F., Smeenk, R.J., Takasaki, Y., Wiik, A., and Koziol, J.A. (1997). Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum. 40,* 1601–1611.

Tan, E.M., Smolen, J., McDougal, J.S., Butcher, B.T., Conn, D., Dawkins, R., Fritzler, M.J., Gordon, T., Hardin, J.A., Kalden, J.R., Lahita, R.G., Maini, R.N., Rothfield, N.F., Smeenk, R.J., Takasaki, Y., Van Venrooij, W.J., Wiik, A., Wilson, M.R., and Koziol, J.A. (1999). A critical evaluation of enzyme immunoassays for the detection of antinuclear antibodies of defined specificities. I. Precision, sensitivity and specificity. *Arthritis Rheum. 42,* 455–464.

Tan, E.M., Smolen, J.S., McDougal, J.S., Klippel, J.H., Fritzler, M.J., Gordon, T.P., Hardin, J.A., Kalden, J.R., Lahita, R.G., Maini, R.N., Rothfield, N.F., Takasaki, Y., Wiik, A., Wilson, M.R., and Koziol, J.A. (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.

Targoff, I.N. (2002). Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. *Rheum. Dis. Clin. North Am. 28,* 859–90, viii.

Ulvestad, E., Kansetrom, A., Madland, T.M., Thomasse, E., Haga, H.J., and Vollset, S.E. (2000). Evaluation of diagnostic tests for antinuclear antibodies in rheumatological practice. *Scand. J. Immunol. 52,* 309–315.

van Eenennaam, H., Vogelzangs, J.H.P., Bisschops, L., Te Boome, L.C.J., Seelig, H.P., Renz, M., De Rooij, D.J., Brouwer, R., Pluk, H., Pruijn, G.J.M., Van Venrooij, W.J., and van den Hoogen, F.H.J. (2002). Autoantibodies against small nucleolar ribonucleoprotein complexes and their clinical associations. *Clin. Exp. Immunol. 130,* 532–540.

Van Gaalen, F.A., Linn-Rasker, S.P., Van Venrooij, W.J., De Jong, B.A., Breedveld, F.C., Verweij, C.L., Toes, R.E.M., and Huizinga, T.W.J. (2004). Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis—A prospective cohort study. *Arthritis Rheum. 50,* 709–715.

Van Venrooij, W.J. and Maini, R.N. (1993). *Manual of Biological Markers of Disease.* Dordrecht: Kluwer Academic Publishers.

Vancheeswaran, R., Black, C.M., David, J., Hasson, N., Harper, J., Atherton, D., Trivedi, P., and Woo, P. (1996). Childhood-onset scleroderma—Is it different from adult-onset disease? *Arthritis Rheum. 39,* 1041–1049.

Vasiliauskiene, L., Wiik, A., and Hoier-Madsen, M. (2001). Prevalence and clinical significance of antikeratin antibodies and other serological markers in Lithuanian patients with rheumatoid arthritis. *Ann. Rheum. Dis. 60,* 459–466.

Visser, H., Cessie, S., Vos, K., Breedveld, F.C., and Hazes, J.M.W. (2002). How to diagnose rheumatoid arthritis early. *Arthritis Rheum. 46,* 357–365.

Vlachoyiannopoulos, P.G., Drosos, A.A., Wiik, A., and Moutsoupoulos, H.M. (1993). Patients with anticentromere antibodies, clinical features, diagnoses and evolution. *Br. J. Rheumatol. 32,* 297–301.

Vogt, T.M., Aickin, M., Ahmed, F., and Schmidt, M. (2004). The prevention index: using technology to improve quality assessment. *Health Serv. Res. 39,* 511–530.

von Muhlen, C.A. and Tan, E.M. (1995). Autoantibodies in the diagnosis of systemic rheumatic disease. *Semin. Arthritis Rheum. 24,* 323–358.

Wassermann, V.A., Neisser, A., and Bruck, C. (1906). Eine serodiagnostische reaktion bei syphilis. *Deutsche Med. Wochenshrift 19,* 745–746.

Wigley, F.M., Wise, R.A., Miller, R., Needleman, B.W., and Spence, R.J. (1992). Anticentromere antibody as a predictor of digital ischemic loss in patients with systemic sclerosis. *Arthritis Rheum. 35,* 688–693.

Wiik, A. (1980). Granulocyte-specific antinuclear antibodies. Possible significance for the pathogenesis, clinical features and diagnosis of rheumatoid arthritis. *Allergy 35,* 263–289.

Wiik, A. (2001). Methods for the detection of anti-neutrophil cytoplasmic antibodies. Recommendations for clinical use of ANCA serology and laboratory efforts to optimize the informative value of ANCA test results. *Springer Semin. Immunopathol. 23,* 217–229.

Wiik, A. (2003a). Testing for ANA and ANCA—Diagnostic value and pitfalls. In M.C. Hochberg, A.J. Silman, J.S. Smolen, M.E. Weinblatt, and M.H. Weisman (eds), *Rheumatology,* (pp. 215–226). Edinburgh: Mosby.

Wiik, A.S. (2003b). Appropriateness of autoantibody testing in clinical medicine. *Clin. Chim. Acta 333,* 177–180.

Wiik, A. and Lam, K. (2001). Report to the European Commission: On the usability of extended DOORS software for education and training, quality assurance and consensus formation. Deliverable D 09, version 2.1. The EU CANTOR project HC4003(HC)2000.

Wiik, A.S., Gordon, T.P., Kavanaugh, A.F., Lahita, R.G., Reeves, W., Van Venrooij, W.J., Wilson, M.R., and Fritzler, M. (2004). Cutting edge diagnostics in rheumatology: on the role of patients, clinicians, and laboratory scientists in optimizing the use of autoimmune serology. *Arthritis Care Res. 51,* 291–298.

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.

CHAPTER

73

T Cell Assays

BART O. ROEP

Division of Autoimmunity, Leiden University Medical Center, Dept. of Immunohematology and Blood Transfusion, E3-Q, NL-2300 RC Leiden, The Netherlands

T cells play a critical role in the vast majority of autoimmune diseases. Although it is often not proven whether the actual tissue destruction is mediated by autoreactive T cells or by T-cell hyper-reactivity leading to loss of tolerance to self-determinants by other effector immune cells, efforts to identify and characterize the role of T cells in the pathogenesis of autoimmune diseases have focused on T-cell autoreactivity. Measuring T-cell reactivity in autoimmune disease is a different league compared with that in infectious diseases and transplantation. In marked contrast to T-cell reactivity against pathogens and allodeterminants, the targets of T-cell autoreactivity are often not known precisely, and the list of candidate autoantigens is endless. Many of the autoantigens are not target-tissue specific, and the reactivity against them is not disease specific. Moreover, T-cell autoreactivity is conceivably hampered by factors such as low precursor frequencies in circulation, no access to sites of inflammation, counteracting regulatory immune reactivities, ectopic expression of target autoantigens, and lack of appropriate co-stimulation. This chapter will focus on T-cell assays in human autoimmune disease, using type 1 (insulin-dependent) diabetes (T1D) mellitus as a model, which results from a T-cell-mediated autoimmune destruction of the pancreatic β cells in genetically predisposed individuals (see also Chapters 5, 36) (Roep, 1996; 2003). Therapies directed against T cells have been demonstrated to halt the disease process (Chapters 36, 76) and prevent recurrent β-cell destruction after islet transplantation. Less is known about the nature and function of these T cells, the cause of the loss of tolerance to islet autoantigens, why the immune system apparently fails to suppress autoreactivity, and whether (or which) autoantigen(s) are critically involved in the initiation or progression of disease.

ASSAYS TO DETECT T CELL AUTOREACTIVITY

Proliferation Assay

T-cell autoreactivity is not exclusive for any autoimmune disease. Consequently, efforts to determine T-cell autoreactivity linked with autoimmune diseases have been hampered by the false expectation that sensitive and specific technology exists that allows reproducible measures for disease activity (Box 73.1). Multiple and sometimes conflicting studies have identified a variety of aberrations in the cellular immune response to autoantigens in persons with the disease. Potential explanations for these discrepancies include incomparable techniques or culture conditions, diversity in the populations of patients or controls tested, and differences in autoantigen preparations.

Appreciative of the above notion, international workshops on T-cell autoreactivity are organized under the auspices of the international Immunology of Diabetes Society (IDS) to standardize immunoassays to allow comparison between different studies (Roep, 1999; Roep et al., 1999a; Peakman et al., 2001). First, the islet autoantigen quality

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Box 73.1
Issues affecting progress in T-cell research in type 1 diabetes

- Lack of sensitive and reproducible detection assay
- Quality of recombinant autoantigens; choice of target autoantigen or peptide epitope
- Choice of control subjects
- Discordance between experimental models and human disease
- Inaccessibility to inflammatory lesion
- Relevance of circulating autoreactive T cells
- Potential hyper-responsive immune status in recent-onset patients
- Low precursor frequencies of circulating autoreactive T cells
- Limited diagnostic value (in cross-sectional studies) due to prevalence of autoreactive T cells in patients and controls.
- Immunoregulation
- Discordance between cellular and humoral autoimmunity
- Lack of technologies to detect autoreactive T cells
- False expectations
- Required expertise

control analyses indicate that the quality of recombinant autoantigen preparations requires improvement. For example, several T-cell clones specific for glutamic acid decarboxylase (GAD65) were unable to cross-react with GAD65 expressed in baculovirus, yeast, or bacteria. Nonetheless, responses could be measured to all autoantigen preparations evaluated in the workshop. Second, all participating centers were able to reproducibly measure T-cell responses to two identical samples of tetanus toxoid, but there was significant interlaboratory variation in sensitivity and extent of the proliferative response measured. Third, the results using candidate autoantigens indicated that although a few laboratories could distinguish patients with T1D from non-diabetic controls in proliferative responses to individual islet autoantigens, in general, no differences in T-cell proliferation between the two groups could be identified. This first T-cell workshop on T-cell autoreactivity in T1D confirms that this is a difficult area for interlaboratory investigations, but it provided insight towards future efforts focused on standardizing autoreactive T cell measurements.

Assay Standardization

At present, it is clear that measuring T-cell autoreactivity requires different skills, and higher sensitivity and specificity than standard assays suitable for measuring alloreactivity or T-cell responses against pathogens. Assay standardization has become essential. Some previously reported conflicting results can in part be explained by the observed interlaboratory variability. The inability to discriminate normal controls from patients suggests that measuring proliferative responses in peripheral blood mononuclear cells (PBMC) represents an incomplete picture of the immune response, perhaps complicated by difficulties in identifying suitable antigens and assays for standardized use. There is an excellent track record on standardization of assays for the detection of diabetes-associated autoantibodies (see also Chapter 36) (Bingley et al., 2001). Unfortunately, the experience gained in the latter efforts could not easily be translated to T-cell assay standardization studies in humans. Disappointment that may have arisen from the slow progress achieved in the standardization efforts could be attributable to unrealistic expectations, and lack of recognition of multiple and sometimes unique limitations associated with human T-cell assays (Atkinson et al., 2000).

Limitations to Measure T-Cell Autoreactivity

The most obvious limitation is the inability (for ethical and practical reasons) to conduct experiments *in vivo*. Inaccessibility to the target organ severely hampers the *in vitro* studies and limits our efforts to define surrogate markers of insulitis in cases of T1D. Yet, in other autoimmune diseases such as rheumatoid arthritis (Chapter 32), autoimmune thyroiditis (Chapter 35) and celiac disease (Chapter 51), the site of inflammation is accessible, while in other cases (e.g., multiple sclerosis, Chapter 46), immune samples can be isolated from cerebrospinal fluid (CSF) yielding relevant surrogate markers of disease activity. Levels of autoreactive T cells in circulation are much lower than those in inflammatory lesions. This contrasts the situation with autoantibodies. Unfortunately, the *in vitro* manipulation may introduce misleading artifacts that are associated with factors like the isolation procedure, antigen concentration, and source of serum in culture medium, etc. In addition, unlike autoantibody molecules, T cells cannot be frozen and thawed without affecting their functional capacities. In addition to these factors, simple enumeration of T cells before and after antigen stimulation is in many cases not mathematically feasible because the responding cells are present in very small frequencies in peripheral blood (i.e., less than 1 in 100,000 cells in the total cell population). Finally, the purity of the antigen preparations needed for detection of autoreactive T cells has been proven to be a critical variable to allow or prevent accurate measurements of circulating autoreactive T cells (Roep, 1999; Roep et al., 1999a; Peakman et al., 2001).

Perspectives for Assay Improvement

Due to the lack of technologies to determine T-cell autoreactivity, the insufficient quality of antigen preparations, and the difficulty of defining relevant immunogenic synthetic peptide epitopes of islet autoantigens, progress has been slow. In addition, the need for appropriately selected control subjects for comparison with diabetic individuals seems underestimated. Appreciating the contribution of HLA polymorphism in T-cell repertoire selection, tolerance induction, and antigen presentation, and the experience that autoimmunity becomes less pronounced with age, it is critical to choose HLA- and age-matched control subjects as references to determine the disease association of a given T-cell response. In fact, non-diabetic siblings of T1D patients have frequently been shown to respond more similar to their T1D relatives than unrelated controls. Considering the possibility that clinical onset of autoimmune disease may be accompanied by a generalized hyperimmune response, it should also be considered to include control subjects suffering from chronic inflammation unrelated to the autoimmune disease of study (Roep et al., 1995).

The T-cell committee of the Immunology of Diabetes Society has recommended a sensitive and reproducible assay for the detection of autoreactive T cells (Table 73.1). This assay provided useful surrogate markers for insulitis. Autoreactive T-cell reactivity against islet granules was associated with presence of insulitis in recent-onset diabetes, while established T1D and healthy controls showed intermediate and low reactivity, respectively. Reactivity against the non-diabetic recall antigen Tetanus Toxoid was comparable for the three groups (Figure 73.1). This T-cell assay was able to distinguish between control and T1D patient groups (Roep et al., 1995). However, on the individual basis, T-cell proliferation assays did not qualify as diagnostic, due to the lack of reactivity in a considerable proportion of new-onset patients (i.e., poor sensitivity), and increased responses to islet autoantigen in non-diabetic subjects (i.e., insufficient specificity) that are inherent to this type of technology. Nonetheless, due to the limited interassay variation of the assay in a given individual, this methodology was shown to provide useful and interpretable information when applied longitudinally in the context of islet reconstitution therapy (Roep et al., 1999b).

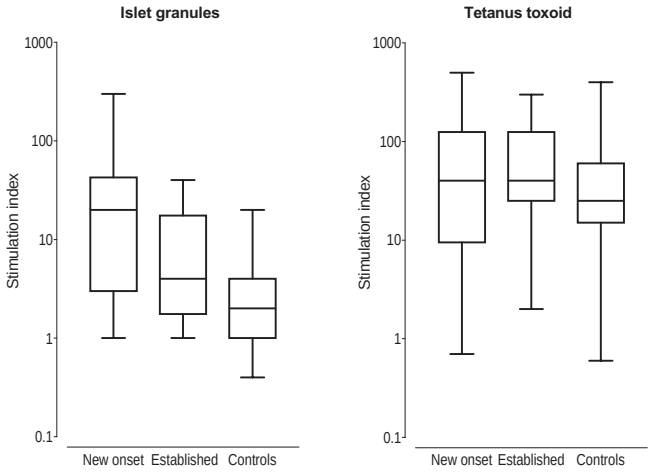


Figure 73.1 Proliferation Assay. T-cell proliferation provides a surrogate marker for the presence of autoimmune insulitis. *Left,* Children with newly diagnosed type 1 diabetes (T1D) have increased proliferative responses in peripheral blood mononuclear cells (PBMC) against insulin-secretory granule membrane preparations, compared with non-diabetic children with unrelated chronic inflammations. T1D patients with the disease for 6 months or more (with presumably no insulitis) still express increased autoimmunity, albeit less than patients at diagnosis. *Right,* T-cell responses to the recall antigen tetanus toxoid are not different between these three groups of subjects. T-cell responsiveness to antigen is indicated in stimulation indices (proliferactive (counts per minute of ^{3}H-thymidine in DNA) of PBMC to antigen divided by proliferation of PBMC in medium alone)

Modified from Roep et al., 1995.

Limiting Dilution Assay

One of the factors determining the magnitude of an immune response is the number of antigen-specific lymphocytes available to respond at the time of an antigenic challenge. Unlike regular proliferation assays that provide the total reactivity as a readout for mixed cell populations, culturing the T cells by limiting dilution can be used to estimate the actual number (frequency) of reactive T cells to various autoantigens. Limiting dilution analysis (LDA) is an established technique to assess immune response in humans in a quantitative manner at the level of the cell. It is a powerful *in vitro* tool for investigating the expressed human T-cell repertoire. Historically, LDA was developed to permit enumeration of cells induced to proliferate by *in vitro* exposure to antigen (Lefkovits and Waldmann, 1984). Past a certain cell dilution, an all-or-none response is seen, depending on the presence or absence of one type of cell, critical for reactivity. The application of Poisson statistics on the number of negative wells per cell concentration can be used to estimate the frequency of precursor T cells (cells undergoing clonal expansion and proliferation in response to a specific antigenic stimulus). Limiting dilution analysis also bears the advantage of relatively short culture periods *in vitro* and, consequently, results likely reflect the situation *in vivo* rather than T-cell clones that have undergone extensive stimulation *in vitro*.

Equal numbers of $CD4^{+}$ T cells recognizing myelin basic protein (MBP) and proteolipid protein (PLP) have been found in the circulation of normal individuals and multiple sclerosis (MS) patients (Zhang et al., 1994) (Chapter 46). While there were no differences in the frequencies of MBP- and PLP-reactive T cells after primary antigen stimulation,

TABLE 73.1 Protocol for proliferation assay for autoreactive T cells in peripheral blood mononuclear cells (PBMC)

Step	Details	Comments
Draw blood	B&D vacutainer system; Na or Li Heparin; 170 IU.	Shake tubes gently to avoid clotting
Ship sample	Room temperature	Heparin does not work at 4°C; make sure that the assay is performed within 24 hours after blood draw. Ship blood in heparin; do not ship Ficoll interphase
Ficoll gradient	Dilute up to 20 mL of blood to 35 mL in Hanks' balanced salt solution (HBSS) in 50 mL tube. Put 10 mL-pipet filled to top (~14 mL) with Ficoll (density 1.077 g/mL) under blood solution and release Ficoll passively under blood. Spin for 20 min at RT, 350 g, no brake.	Make sure that all solutions (Ficoll, media) are at RT. Keep blood >1 hour in heparin tube at RT before putting on Ficoll.
Remove interphase and wash interphase twice with HBSS and resuspend in IMDM supplemented with HS (20%)	280 g, RT, brake 2 mL IMDM/20% HS per 10 mL of heparinized blood	IMDM is not appropriate for cryopreservation (use RPMI 1640 w/o Hepes). Check each individual serum before pooling in mixed lymphocyte culture to ensure that sera qualify for proliferation assays. Also check pooled serum in MLR. HS is heterogeneous; reserve single pool for longitudinal and comparative studies.
Count mononuclear cells; dilute cells to 1.5×10^6/mL IMDM/20% HS	Dilute cells 1:10 in Türk solution, which lyses erythrocytes and stains nuclei	Counting of thawed cells is recommended in eosin, to exclude dead/dying (i.e., red) cells. ***N.B. cryopreservation affects monocytes, T-cell blasts and autoreactivity, and removes IL-10 from system.***
Add antigen preps to round-bottom 96 well plate.	Concentrate Ag prep 2 × in 100 µL per well; Ag medium: IMDM (no serum). Test dose response (final concentration of most recombinant antigens: 5–10 µg/mL; peptides: 2–10 µg/mL) Use tissue-coated plates.	Round-bottom plates are required for primary assays to ensure optimal cell–cell contact, while flat-bottom plates are recommended after initial *in vitro* stimulation. Adding Ag to wells first prevents spillover of cells (round-bottom!), and ensures that cells are kept under unfavorable conditions (pH, humidity, temperature) for minimum time. Include IL-2 triplicate as control for viability of T cells, PHA for control for APC function and proliferative capacity, and recall antigen (e.g., tetanus toxoid) for Ag-specific T-cell response.
Add cells to plate	100 µL ~150,000 cells/well	Add cells quickly with repetition pipette and move plate immediately to incubator.
Incubate	5 days, 37°C, 5% CO_2, 90% humidity.	Do not pile up more than 5 plates, to ensure gas exchange for medium buffering.
Add 0.5–1.0 µCi ^{3}H-thymidine; incubate overnight.	In 50 µL RPMI 1640.	
Harvest DNA	Semi-dry glass fiber filters are recommended for optimal signal/noise ratio (e.g., Wallac 1295–004) Betaplate™ 96-well Harvester (Pharmacia).	Ensure water is completely removed to prevent quenching of scintillation signal.
Measure cpm	E.g., Wallac 1205 liquid scintillation counter	

Ag, antigen; APC, antigen-presenting cell; cpm, counts per minute; HS, human pool serum; IL-2, interleukin 2; IMDM, Iscove's modified Dulbecco's medium; MLR, mixed lymphocyte reaction; RT, room temperature.

the frequency of MBP or PLP but not tetanus toxoid-reactive T cells generated after primary recombinant interleukin (rIL-2) stimulation was significantly higher in MS patients compared with control individuals. In the CSF of MS patients, MBP-reactive T cells generated with primary rIL-2 stimulation were 10-fold more frequent than in paired blood samples. In contrast, MBP-reactive T cells were not detected in CSF obtained from patients with other neurologic diseases (Zhang et al., 1994). These results provide definitive *in vitro* evidence of an absolute difference in the

activation state of myelin-reactive T cells in the central nervous system of patients with MS and provide evidence of a pathogenic role of autoreactive T cells in the disease.

In T1D, a progressive decrease in the number of negative cultures at increasing cell concentrations that was represented by a low goodness of fit (GoF, low chi square), was seen with the tetanus toxoid-response in new-onset patients, their siblings and parents; precursor frequencies and GoF were similar in all three groups. Reactivity to insulin, however, showed low precursor frequencies in patients and siblings, and the LDA to insulin demonstrated dramatic decreases in the number of positive cultures at higher cell concentrations leading to a high GoF in patients and siblings compared with parents (Naik et al., 2004). This saw-toothed pattern of reactivity to insulin is indicative of multiple hit kinetics and implies that the response is regulated. Consequently, the precursor frequency of insulin autoreactive cells in patients and their siblings are probably much higher than calculated.

Other Proliferation Assays

Recently, a novel assay was developed for antigen-specific human T-cell proliferation (Mannering et al., 2003). Peripheral blood mononuclear cells were labeled with the fluorescent dye 5,6-carboxylfluorescein diacetate succinimidyl ester (CFSE) and cells that proliferated in response to antigen, with resultant reduction in CFSE intensity, were measured directly by flow cytometry. This assay appeared more sensitive than ^{3}H-thymidine incorporation, and detected the proliferation of rare antigen-specific $CD4^+$ T cells at lower antigen concentrations. Using the CFSE assay, the investigators could measure directly the proliferation of human $CD4^+$ T cells from healthy donors in response to the T1D autoantigens glutamic acid decarboxylase (GAD) and proinsulin (PI), implying that this approach may not (yet) distinguish between affected and unaffected subjects.

HLA Tetramers

Fluorochrome-labeled major histocompatibility complex tetramers may enable investigators to enumerate antigen-reactive T cells. Soluble HLA-DR tetramers containing a peptide epitope from human GAD65 were used to analyze peripheral blood T cells of newly diagnosed T1D patients and at-risk subjects (Reijonen et al., 2002). Peripheral blood mononuclear cells were expanded on APCs presenting GAD65 peptide and subsequently activated with specific plate-bound class II-peptide monomers. T-cell activation defined in flow cytometry by CD4(high) and/or CD25 markers were observed in all T1D patients and some at-risk subjects, but not in normal control subjects. The activated T cells stained positive with tetramers containing the selected GAD65 epitope. Tetramer-positive cells were CD4(high) T cells with high avidity for an immunodominant GAD65 T-cell epitope. These results demonstrated that phenotyping of T cells utilizing HLA class II tetramers provides a new tool to characterize the autoimmune response in T1D. Shortcomings of this approach for identifying class II restricted cells are that the studies can only be done in individuals with certain HLA types, with reactivity to specific antigen peptides, and that it is necessary to expand the cells *in vitro* because of the low precursor frequency in the peripheral blood. Alternative assays using soluble MHC-multimers have also proven to be valuable tools for the stimulation of as well as the analysis of antigen-specific T cells *in vitro*. Dimeric major histocompatibility complexes coupled to immunoglobulin (HLA-Ig) and loaded with peptide epitopes have been used to visualize antigen-specific T cells and potentially stimulate immune responses as part of an artificial APC (Fahmy et al., 2002).

Cytokine Assays

Cytokines and chemokines are essential components in the communication between different components of the immune system, as well as in directing leukocytes to inflammatory lesions (see Chapter 18). Much interest has focused on the phenotype of the T-cell response that differentiates after activation (Chapter 7). Little is known of which cytokines and chemokines are associated with the pathogenesis of autoimmune diseases in humans, and to what extent they could reflect attempts of the immune system to counteract immune abnormalities including autoimmunity. There is a common belief that T1D, rheumatoid arthritis, autoimmune thyroiditis, and myasthenia gravis are Th1-associated diseases, i.e., associated with or accompanied by pro-inflammatory cytokines and chemokines, while Th2 cells could be beneficial or at least benign in fighting β-cell destruction. According to the quality of response they mediate, autoreactive T cells recognizing autoantigenic peptides could represent both disease effectors in the development of autoimmune disease and directors of tolerance in healthy individuals or those undergoing preventative immunotherapy. The rarity of these cells, inadequate technology, and poorly defined epitopes, however, has hampered examination of this paradigm.

Enzyme-Linked Immunospot Assays

The identification of sensitive assay formats capable of distinguishing islet autoreactive T cells directly *ex vivo* in blood is a major goal in autoimmunity research. Recently, much interest has been shown in the cytokine-enzyme-linked immunospot (ELISPOT) assay. The ELISPOT provides a highly sensitive assay to detect single cells secreting cytokines (Figure 73.2). The higher sensitivity of the ELISPOT compared with an ELISA is due to the plate-

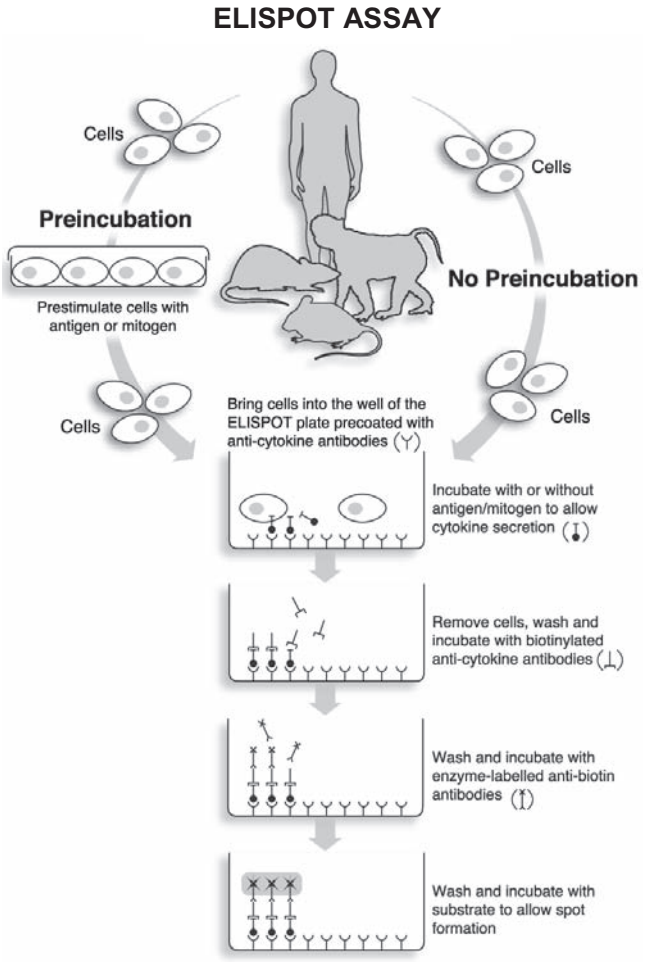


Figure 73.2 Flow diagram of the enzyme-linked immunospot (ELISPOT) assay. Cells are incubated for a defined length of time in the wells of the ELISPOT plate precoated with a high-affinity monoclonal antibody to which the cytokine, produced during incubation, will bind. Subsequently, cells are lysed and debris is washed away. Areas in which the cytokine has been captured by the coating antibody are detected with a combination of biotinylated anticytokine detector antibodies and enzyme-labeled streptavidin or antibiotin antibodies. The last step in the assay is the addition of a substrate yielding a colored zone ('spot'), which reveals the site of cytokine secretion. Numbers and sizes of spots can be read by light microscopy or through designated spot-readers followed by computer microimaging and analysis (Arif et al., 2004).

This diagram was kindly provided by Dr. Peter H. van der Meide, U-CyTech, Utrecht University, The Netherlands.

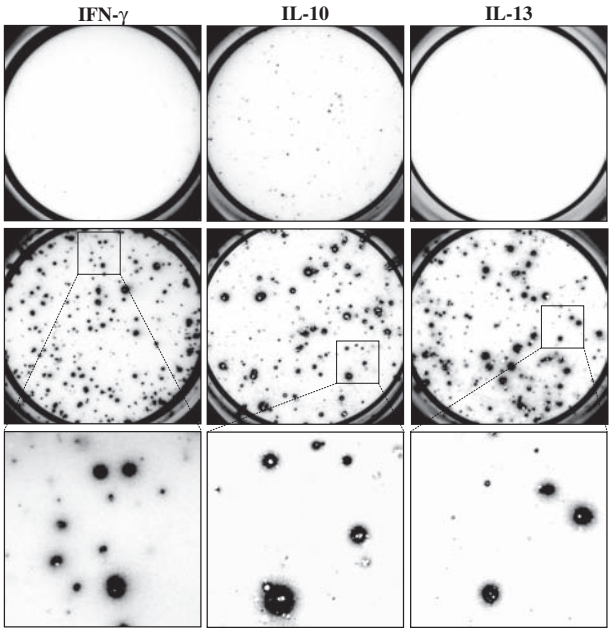


Figure 73.3 The ELISPOT Assay. Examples of interferon-γ *(left)*, IL-10 *(middle)* and IL-13 *(right)* specific spots produced by 2.5×10^4 human PBMC of a healthy subject (preincubation: 42 hours; incubation ELISPOT: 5 hours). *Top,* no stimulus (magnification 25×). *Middle,* concanavalin A stimulation (magnification 25×). *Bottom,* concanavalin A stimulation (magnification 140×).

Data provided by Dr. Peter H. van der Meide, U-CyTech, Utrecht University, The Netherlands.

bound antibodies directly capturing the cytokine secreted in the immediate vicinity of the cell before it is diluted in the supernatant, trapped by high-affinity receptors, or degraded by proteases (Figure 73.3).

A recent breakthrough that allowed distinction between T1D patients and non-diabetic controls was achieved by applying the ELISPOT assay using a panel of naturally processed islet epitopes by direct elution from APCs bearing HLA-DR4 (Arif et al., 2004; Herold, 2004). The quality of autoreactive T cells in patients with T1D showed extreme polarization toward a proinflammatory Th1 phenotype. Furthermore, rather than being unresponsive, the majority of non-diabetic, HLA-matched control subjects also manifested a response against islet peptides, but one that showed extreme bias to T regulatory cell (Treg, IL-10-secreting) bias. Development of T1D depends on the balance of autoreactive Th1 and Treg cells, which may be open to favorable manipulation by immune intervention (Chapters 9 and 10). The interferon-γ (IFN-γ) responses to combined pro-insulin and IA-2 peptides allowed for the first time to discriminate between T1D patients and control subjects. Moreover, the cells from the control subjects did not produce an IFN-γ response to the proinsulin peptides. IL-10 is a likely regulatory cytokine and would thus affect the cells recruited to the inflammatory site. The specificity of the immune regulation is imparted by the specificity of the responding T-cell receptor.

These recent findings also imply that a difference between healthy subjects and T1D patients is not in the repertoire and quantity of T-cells but is in the quality of the response, i.e., pathways of differentiation activated in response to antigen. Since the ELISPOT assay enumerates the number of cells producing a given cytokine, rather than

the total amount of this cytokine being produced, this approach did not allow for assessment of quantitative differences in terms of amounts of cytokine produced in responses to the antigens, so the full extent of differences in cytokine production between individuals with different rates of disease progression cannot be entirely determined. Yet, the qualitative differences suggest the mechanism described above.

Other Cytokine Assays

Given the low precursor frequencies of autoreactive T cells as evidenced by proliferation assays, limiting dilution assays, HLA tetramer studies, and ELISPOT analyses, it may prove very difficult to detect autoantigen-specific T cells after intracellular staining of cytokines after *in vitro* stimulation of PBMC by FACS analysis. Nonetheless, this approach has been reported to be applicable in infectious disease, tumor therapy, and transplantation to identify adaptive immune responsiveness. Preliminary studies suggested that new techniques such as the cytokine bead assay may turn out to be more useful to provide relevant surrogate markers of autoimmune disease. The advantage of such assays is that they require relatively small numbers of PBMCs, are less laborious, fast, and sensitive, while they can provide information on multiple cytokines and chemokines in small aliquots of supernatants of stimulated PBMCs.

T Regulatory Cells

The mechanisms involved in maintenance of peripheral tolerance and suppression of autoimmunity include a specialized subset of Treg within the $CD4^+CD25^+$ T-cell population. Recent interest has focused on these Tregs that represent a naturally occurring $CD4^+$ T-cell population expressing CD25 that arises from the thymus and seeds into the periphery, creating a cohort of cells with profound T-cell immunosuppressive qualities. No single phenotypic marker exists that is specific for Treg. $CD4^+CD25^+$ cells can be detected in peripheral blood in humans (Baecher-Allan et al., 2001) and are able to suppress proliferation and cytokine production from both $CD4^+$ and $CD8^+$ T-cells *in vitro* in a cell contact-dependent manner. Measuring the frequency and function of Treg might provide helpful information regarding the immunopathogenesis of the disease process. Hopefully, it will be a matter of time that testing of the frequency and function of circulating Treg becomes an important test parameter in clinical research involving patients. Recent studies indicate that the function and phenotype of these cells in human autoimmune diseases may differ from their counterparts in infection or alloreactivity, since their immune suppressive function *in vitro* appears to be impaired (Viglietta et al., 2004; Lindley et al., 2005).

Box 73.2
Perspectives for T-cell studies in autoimmune disease

- Unraveling of immunopathogenesis
- Definition for targets for immunotherapy
- Monitoring of immunologic and clinical efficacy of immunointervention trials
- Identification of triggers initiating autoimmune disease process
- Development of appropriate immunotherapy
- Guidance of immune regulatory therapy
- New technologies (ELISPOT assays, HLA tetramers, CFSE staining, LDA)

APPLICATION

Autoreactive T cells have proven to be valuable targets to study pathogenic or autoimmune disease related processes (Box 73.2). Measuring T-cell autoreactivity also provided critical information to determine the fate of islet allografts transplanted to T1D patients. Furthermore, these studies have provided proof of operational immunologic tolerance to islet allografts as well as valuable information to improve and customize immunosuppressive therapy. Presently, technologies to detect T-cell auto- and alloreactivity in T1D recipients of islet allografts are applied to monitor islet allograft survival in relation to various immunosuppressive therapies, and to guide tapering of these therapies after successful restoration of insulin production.

With the development of new technologies, such as ELISPOT assays and HLA tetramers that allow quick detection of peptide-specific T cells in the context of their restriction element, the stage has been set to improve the qualitative and quantitative detection of autoreactive T cells in autoimmunity. This will help to understand the cause of autoimmune diseases, and design and monitor immunotherapeutic intervention strategies. Although it is generally appreciated that studies on cellular auto- and alloimmunity are hampered by the complex nature of such immune responses and the required technical and physical skills, it has been a worthwhile quest to unravel the role of T cells in the pathogenesis of autoimmune disease.

References

Arif, S., Tree, T.I., Astill, T.P., Tremble, J.M., Bishop, A.J., Dayan, C.M., Roep, B.O., and Peakman, M. (2004). Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J. Clin. Invest. 113*(3), 451–463.

Atkinson, M.A., Honeyman, M.C., Peakman, M., and Roep, B.O. (2000). T-cell markers in type 1 diabetes: progress, prospects and realistic expectations. *Diabetologia 43,* 819–820.

Baecher-Allan, C., Brown, J.A., Freeman, G.J., and Hafler, D.A. (2001). CD4+CD25high regulatory cells in human peripheral blood. *J. Immunol. 167,* 1245–1253.

Bingley, P.J., Bonifacio, E., Ziegler, A.G., Schatz, D.A., Atkinson, M.A., and Eisenbarth, G.S. (2001). Proposed guidelines on screening for risk of type 1 diabetes. *Diabetes Care 24*(2), 398.

Fahmy, T.M., Bieler, J.G., and Schneck, J.P. (2002). Probing T cell membrane organization using dimeric MHC-Ig complexes. *J. Immunol. Methods 268,* 93–106.

Herold, K.C. (2004). Achieving antigen-specific immune regulation. *J. Clin. Invest. 113*(3), 346–349.

Lefkovits, I. and Waldmann, H. (1984). Limiting dilution analysis of the cells of immune-system 1. The clonal basis of the immune-response. *Immunol. Today 5*(9), 265–269.

Lindley, S., Dayan, C.M., Bishop, A., Roep, B.O., Peakman, M., and Tree, T.I. (2005). Defective suppressor function in CD4+CD25+ T-Cells from patients with type 1 diabetes. *Diabetes 54,* 92–99.

Mannering, S.I., Morris, J.S., Jensen, K.P., Purcell, A.W., Honeyman, M.C., van Endert, P.M., and Harrison, L.C. (2003) A sensitive method for detecting proliferation of rare autoantigen-specific human T cells. *J. Immunol. Methods 283*(1–2), 173–183.

Naik, R.G., Beckers, C., Wentwoord, R., Frenken, A., Duinkerken, G., Brooks-Worrell, B., Schloot, N.C., Palmer, J.P., and Roep, B.O. (2004). Precursor frequencies of T-cells reactive to insulin in recent onset type 1 diabetes mellitus. *J. Autoimm 23*, 55–61.

Peakman, M., Tree, T.I., Endl, J., van Endert, P., Atkinson, M.A., and Roep, B.O. (2001). Characterization of preparations of GAD65, proinsulin, and the islet tyrosine phosphatase IA-2 for use in detection of autoreactive T-cells in type 1 diabetes: report of phase II of the Second International Immunology of Diabetes Society Workshop for Standardization of T-cell assays in type 1 diabetes. *Diabetes 50*(8), 1749–1754.

Reijonen, H., Novak, E.J., Kochik, S., Heninger, A., Liu, A.W., Kwok, W.W., and Nepom, G.T. (2002) Detection of GAD65-specific T-cells by major histocompatibility complex class II tetramers in type 1 diabetic patients and at-risk subjects. *Diabetes 51*(5), 1375–1382.

Roep, B.O. (1996). T-cell responses to autoantigens in IDDM—The search for the Holy Grail. *Diabetes 45*(9), 1147–1156.

Roep, B.O. (1999). Standardization of T-cell assays in type I diabetes. Immunology of Diabetes Society T-cell Committee. *Diabetologia 42*(5), 636–637.

Roep, B.O. (2003). The role of T-cells in the pathogenesis of type 1 diabetes: From cause to cure. *Diabetologia 46*(3), 305–321.

Roep, B.O., Kallan, A.A., Duinkerken, G., Arden, S.D., Hutton, J.C., Bruining, G.J., and de Vries, R.R. (1995). T-cell reactivity to beta-cell membrane antigens associated with beta-cell destruction in IDDM. *Diabetes 44*(3), 278–283.

Roep, B.O., Atkinson, M.A., van Endert, P.M., Gottlieb, P.A., Wilson, S.B., and Sachs, J.A. (1999a). Autoreactive T cell responses in insulin-dependent (Type 1) diabetes mellitus. Report of the first international workshop for standardization of T cell assays. *J. Autoimmununol. 13*(2), 267–282.

Roep, B.O., Stobbe, I., Duinkerken, G., Van Rood, J.J., Lernmark, A., Keymeulen, B., Pipeleers, D.G., Claas, F.H.J., and De Vries, R.R.P. (1999b). Auto- and alloimmune reactivity to human islet allografts transplanted to insulin-dependent diabetes mellitus patients. *Diabetes 48,* 484–490.

Viglietta, V., Baecher-Allan, C., Weiner, H.L., and Hafler, D.A. (2004). Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med. 199,* 971–979.

Zhang, J., Markovic-Plese, S., Lacet, B., Raus, J., Weiner, H.L., and Hafler, D.A. (1994). Increased frequency of interleukin 2-responsive T cells specific for Myelin Basic Protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *J. Exp. Med. 179,* 973–984.

CHAPTER

74

Prediction of Autoimmune Disease

JENNIFER M. BARKER AND GEORGE S. EISENBARTH

Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, 4200 E. 9th Avenue B-140, Denver CO 80262

Many autoimmune diseases are chronic in nature with a long asymptomatic preclinical period marked by laboratory abnormalities (e.g., the presence of autoantibodies; Table 74.1). Therefore, individuals with asymptomatic disease can be identified prior to developing tissue destruction with immunologic and immunogenetic testing. Given the prevalence of autoimmune disorders, immunologic and immunogenetic testing is likely to become an important component of what has been termed "personalized medicine." Accurate prognostication is dependent upon sensitive and specific assays in combination with an understanding of pathophysiology and the natural history of disease. In this review, we will emphasize studies of endocrine autoimmunity, especially type 1 diabetes (T1D) where techniques for disease prediction have developed since the mid-1980s.

OVERVIEW OF PREDICTION

Understanding of key concepts such as positive predictive value (PPV) and negative predictive value (NPV), sensitivity and specificity, and Bayes' theorem is essential to prediction of disease.

Sensitivity is the probability of a positive test, given that the individual has disease. Specificity is the probability of a negative test given that the individual does not have the disease. Sensitivity and specificity are generally thought of as independent of disease prevalence. The PPV is the probability of disease given that the test is positive. Conversely, the NPV is the probability of no disease given a negative test result (Table 74.2). Both PPV and NPV are influenced by disease prevalence.

Table 74.3 demonstrates the influence of disease prevalence on PPV and NPV. In this table, assume a test with sensitivity of 90% and specificity of 99% with a low prevalence state of 0.3% and a high prevalence state of 5% in a hypothetical population of 100,000 individuals. Prevalence was chosen to approximate T1D in the general population (0.3%) and for first-degree relatives of patients with T1D (5%). Prevalence highly influences the PPV of a study. As the prevalence of a disease increases the proportion of positives that are "true positives" increases. Therefore, when attempting to predict disease, it is essential to understand the prevalence of the disease in the population of interest and the ability of a test to identify those with disease correctly (the sensitivity and specificity of the test).

Bayes' theorem mathematically relates the probability of a disease in an individual after a study is performed (post-test probability) to the probability of a disease before the study is performed (pre-test probability), the sensitivity and

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

specificity of a study and disease prevalence by the following equation:

$$\text{Post-test probability} = \frac{\text{Pre-test probability} \times \text{test sensitivity}}{(\text{Pre-test probability} \times \text{test sensitivity}) + (1 - \text{disease prevalence}) \times (1 - \text{specificity})}$$

When the pre-test probability of disease is the prevalence of disease, this equals the PPV of a test.

It should be noted that Bayes' theorem is a simplification of complex probabilities. It assumes a binary study outcome and independence of study results and pre-test probability. Predicting post-test probability with the use of a test is most helpful in cases of intermediate pre-test probability where a positive or negative study will influence diagnosis. In a diagnostic algorithm that incorporates multiple studies, the probability of disease increases with each positive test.

It is sometimes thought that to increase sensitivity one must decrease specificity. That is correct for any given specific test or algorithm of tests but, in the real world of clinical testing, we can change the test (e.g., a new method that increases sensitivity and specificity) and apply a different testing algorithm. An example is the analysis of three anti-islet autoantibodies, rather than a single autoantibody for prediction of T1D. The presence of two or more anti-islet autoantibodies has a very high sensitivity and specificity compared with single positive anti-islet autoantibody.

TABLE 74.1 Representative disease-associated autoantibodies

Disease	Autoantibodies
Type 1 diabetes	Islet cell autoantibodies
	Insulin, GAD65, IA-2
Thyroid disease	Anti-thyroid peroxidase
	Anti-thyroglobulin
Addison's disease	Anti-21-hydroxylase
Celiac disease	Anti-tissue transglutaminase
Multiple sclerosis	Anti-myelin oligodendrocyte glycoprotein
	Anti-myelin basic protein (MBP)
Rheumatoid arthritis	Rheumatoid factor (IgM anti-IgG)
	Anti-cyclic citrullinated peptide

TYPE 1 DIABETES MELLITUS AS A MODEL FOR PREDICTION OF AUTOIMMUNE DISEASE

The chronic disease model for T1D (Figure 74.1) is a useful model for the prediction of T1D. In this model, the mass of insulin-producing pancreatic β-cells is depicted on the y-axis and time on the x-axis (Eisenbarth, 1986). As the disease progresses, the β-cell mass decreases and markers of autoimmunity such as autoantibodies against islet-specific proteins and islet-specific T cells emerge. As the β-cell mass diminishes, metabolic abnormalities develop and ultimately clinical diabetes manifests. Studies can be performed at each step along the way to identify individuals at risk for clinical diabetes.

Genetics

Although more than 85% of individuals with T1D do not have a family history of T1D, the risk for diabetes in a first-degree relative of an individual with T1D is approximately 15 times greater than that of individuals from the general population (6% vs. 0.4%) (Tillil and Kobberling, 1987). The major histocompatibility complex (MHC) located on chromosome 6 (6p21.3) is thought to contribute 50% of the genetic risk for T1D (Nerup et al., 1974). Noble et al. (1996)

TABLE 74.2 Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)

Study	Disease present	Disease absent
Positive	A (true positive)	B (False positive)
Negative	C (false negative)	D (True negative)
Sensitivity = A/(A+C)	PPV = A/(A+B)	
Specificity = D/(B+D)	NPV = D/(C+D)	

TABLE 74.3 Effect of disease prevalence on positive predictive value (PPV) and negative predictive value (NPV) with identical test specificity (99%) and sensitivity (90%)

	Low prevalence (0.3%)			High prevalence (5%)		
Test	**Disease present**	**Disease absent**	**N**	**Disease present**	**Disease absent**	**N**
Positive	270	997	1267	4500	950	5450
Negative	30	98703	98733	500	94050	94550
Total	300	99700	100000	5000	95000	100000
PPV	270/1267 = 21.3%			4500/5450 = 82.6%		
NPV	98703/98733 = 99.97%			94050/94550 = 99.5%		

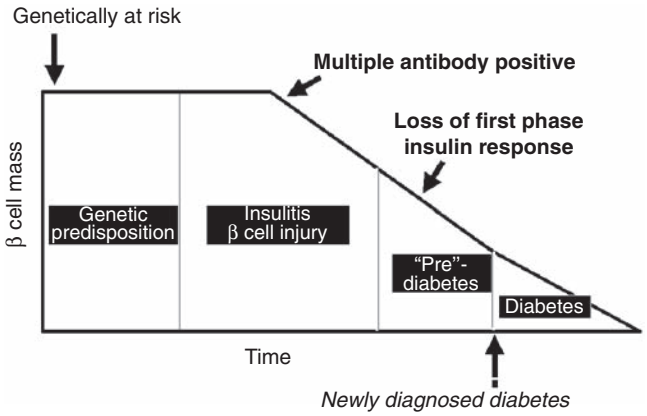


Figure 74.1 Stages in the development of type 1 diabetes. A model for the natural history of the autoimmunity leading to type 1A diabetes. Individuals who are genetically susceptible to disease are triggered to initiate insulitis or β cell injury. This is marked by the production of autoantibodies against the pancreas. As loss of β cell mass continues, metabolic abnormalities such as a loss of first-phase insulin response begin, and ultimately when enough β cells are lost, diabetes develops.

and Erlich et al. (1993) have shown that both DQ and DR alleles have been associated with T1D. The HLA haplotypes associated with the highest risk for T1D are DR3-DQ2 (DQA1*0501, DQB1 *0201) and DR4-DQ8 (DQA1*0302 DQB1*0302) (Ziegler et al., 1991).

It is estimated that approximately 6% of the population with DR3-DQ2/DR4-DQ8 will develop T1D. However, the risk of diabetes in first-degree relatives with these high-risk HLA haplotypes is much higher. Approximately 40% of first-degree relatives with DR3-DQ2/DR4-DQ8 will develop T1D. This suggests that other genetic loci within the MHC may also contribute to risk of T1D in that relatives with these fixed class II alleles usually share complete MHC haplotypes. These high-risk genotypes also impact the age at which T1D develops. Approximately 50% of those who develop T1D prior to 5 years of age are heterozygotes for DR3-DQ2/DR4-D8 compared with 2.4% of Colorado newborns (Redondo et al., 2001).

Specific HLA haplotypes protect individuals from disease. Similar to many studies, Pugliese et al. (1995) have reported that DR2 (DRB1*1501, DQA1*0102, DQB1*0501) and DR7 with DQA1, DQB1*0301, and DRB1*1401 are protective for the development of T1D.

Additional genetic loci such as the insulin gene (Bell et al., 1984; Bennet et al., 1997), CTLA-4 (Nistico et al., 1996; Lowe et al., 2000), lymphocyte specific tyrosine phosphatase (gene-*PTPN22*) (LYP) (Bottini et al., 2004) and other loci with as yet undetermined gene products have also been associated with risk for the TID.

HLA haplotypes and, to a lesser extent, insulin gene polymorphisms are the only genetic markers that have been used to identify individuals from the general population at high-risk for the development of T1D. Studies such as the Diabetes Autoimmunity Study in the Young (DAISY) in Denver, Colorado, USA (Rewers et al., 1996) and the Finnish Type 1 Diabetes Prediction and Prevention project (DIPP) (Kupila et al., 2001) follow children from the general population who are at high-risk for the development of T1D based upon the presence of moderate- or high-risk HLA genotypes.

The presence of high-risk HLA genotypes increases the likelihood of T1D by an order of magnitude. Individuals with the highest-risk HLA haplotypes and no family history of diabetes have a risk for T1D of approximately 6%, compared with a risk of 0.4% in the overall population (Tillil and Kobberling, 1987). In first-degree relatives this risk increases from approximately 6% to approximately 40% in those with the highest-risk HLA genotypes. Therefore, knowledge of HLA genotype improves predictive ability. However, risk is not absolute, with a significant subset of individuals with high-risk HLA genotypes never developing islet autoimmunity. Further evaluation, including the use of anti-islet autoantibodies will refine prediction.

Laboratory Markers of Autoimmunity (Including Autoantibodies and T-cell Assays)

Since the discovery of islet-cell autoantibodies (ICA), detected by incubation of serum with sections of frozen human pancreas (Lendrum, 1975; Bottazzo et al., 1980), autoantibodies have been used to identify individuals at risk for T1D. The ICA level is measured in Juvenile Diabetes Foundation (JDF) units with positive often defined as ≥10 JDF units. Bonifacio et al. (1990) and Chase et al. (1991) have shown that the risk for diabetes increases as the ICA level increases. In first-degree relatives, the risk for T1D increases from 40% with a low cut-off (≥4 JDF units) to 100% (in one study) with a high cut-off (≥80 JDF units) (Bonifacio et al., 1990). The relationship between ICA level and PPV for diabetes has also been observed in cohorts without an affected first-degree relative (Chase et al., 1991).

The specific antigens of ICA include the 65 kDa form of glutamic acid decarboxylase (GAD65) and the protein tyrosine phosphatase ICA512 (IA2). Insulin autoantibodies are not detected with the staining of frozen pancreas. These three autoantibodies are the current so-called "biochemical autoantibodies." Sensitive and specific radioimmunoassays (RIAs) have been developed for autoantibodies to insulin, GAD65, and IA2. When ICA are present in the company of biochemical autoantibodies, the risk for T1D increases. Individuals who expressed ICA alone were at a much lower risk for T1D (5%) compared with those positive for ICA and biochemical autoantibodies (66.2%) (Maclaren et al., 1999). Yu et al. (2001) demonstrated that higher levels of ICA are associated with expression of one or more biochemical

autoantibodies. Therefore, the risk conferred by ICA can also be identified through testing for biochemical autoantibodies.

Life-table analysis has shown that expression of multiple biochemical autoantibodies is associated with a greater risk for T1D (Figure 74.2). Individuals who express three anti-islet autoantibodies are at an approximately 70% risk for T1D after 5 years, compared with 12% for those who express one anti-islet autoantibody (Verge et al., 1996).

Studies from the USA and Finland have shown that autoantibody production can begin in the first year of life (Rewers et al., 1996; Kimpimaki et al., 2001). Individuals can persist with positive autoantibodies many years prior to the development of T1D (Bingley et al., 1994; 1997). The risk for T1D does not appear to dissipate over time for individuals with multiple autoantibodies. Gardner et al. (1999) followed first-degree relatives with multiple autoantibodies and found a progressive increase in incidence of T1D over time, such that at 15 years 66% had developed diabetes. The development of diabetes did not appear to be decreasing at the end of the study. This has led to the hypothesis that all individuals who express multiple autoantibodies will develop diabetes given enough time. In contrast, the expression of a single biochemical autoantibody is associated with a long-term risk of approximately 20% in relatives of patients with T1D. Transient expression of autoantibodies in the DAISY study was not associated with high-risk genotypes or family history of diabetes (Yu et al., 2000) and the development of T1D (Barker et al., 2004a). Conversely, Pietropaolo et al. (2002) have observed first-degree relatives with high-risk HLA genotypes developing classic insulin deficiency and diabetes without the presence of autoantibodies, suggesting that there are immunologic abnormalities that are not captured with standard autoantibody assays.

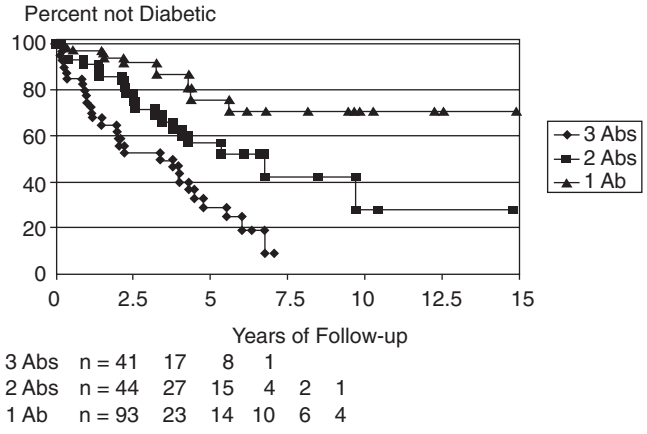


Figure 74.2 Progression to diabetes versus number of autoantibodies (GAD, ICA512, insulin). First degree relatives of individuals with T1D were followed for development of diabetes. Those relatives who expressed more than 2 or 3 autoantibodies were at a greater risk for the development of diabetes compared with those who expressed one autoantibody. The numbers along the bottom of the figure indicate the numbers of subjects in each group still followed at that time period.

Adapted from Verge, et al., 1996.

LaGasse et al. (2002) have used biochemical autoantibodies to screen school children in Washington State, USA. Approximately 4500 school children were screened, 12 children were found to have two or three positive autoantibodies. Six children developed diabetes during the 8-year follow-up. All expressed at least two autoantibodies. None of the children with zero or one autoantibody developed diabetes. This indicates that autoantibodies can be used in a large population to screen for disease.

The DAISY study has identified children with biochemical autoantibodies and followed them for the onset of diabetes. Children diagnosed with this intensive screening program tend to be found at an earlier stage of disease and have less diabetic ketoacidosis (Barker et al., 2004b). These children also have lower blood glucoses at the time of diagnosis (Figure 74.3).

The use of autoantibody assays has identified individuals previously classified as type 2 diabetics as having an autoimmune process associated with a faster progression to insulin dependency compared with autoantibody-negative individuals. Approximately 45% of such adults positive for ICA required insulin at 6 years post-onset of diabetes compared with 5% of those with no autoantibodies. This so-called "latent autoimmune diabetes of adults (LADA)" was analyzed in the UKPDS study (Turner et al., 1997). Similarly, Fuchtenbusch et al. (1997) have demonstrated that, in women with gestational diabetes, the presence of one, two, or three autoantibodies conferred a risk for insulin dependency of 17%, 61%, and 84%, respectively, during follow-up of approximately 2 years. Autoantibodies can be used in adults diagnosed with diabetes as a prognostic factor for insulin dependency.

Multiple international workshops evaluating different assay formats for islet autoantibodies in mouse models of T1D and in humans indicate that standard ELISA formats

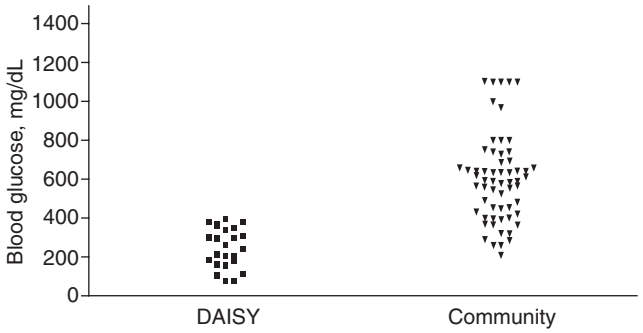


Figure 74.3 Blood glucose levels at diagnosis in children diagnosed with diabetes through an intensive screening program (Diabetes Autoimmunity Study in the Young [DAISY]) compared with those diagnosed from the community.

are inadequate in contrast to fluid phase radioassays. For example, ELISA anti-insulin autoantibody assays could detect insulin antibodies following subcutaneous insulin therapy but could not detect pre-diabetic insulin autoantibodies (Greenbaum et al., 1992) (Figure 74.4). A recent GAD65 atypical autoantibody enzyme-linked immunosorbent assay (ELISA) was configured with a very low density of GAD65 on the plate so that only a single binding site of the autoantibody reacted. The presence of autoantibody was detected by "fluid-phase" reaction of tagged GAD65 and performed as well as fluid-phase radioassays in the 2004 Diabetes Autoantibody Standardization Program (oral communication workshop results). A similar format for detection of IA2 autoantibodies was not as sensitive. In general, fluid-phase radioassays utilizing specific precipitants (e.g., protein A) provide the best sensitivity and specificity and can be performed in 96-well formats. It is relatively easy to develop such assays for protein antigens, with *in vitro* transcription and translation of cDNA to produce labeled autoantigen.

Type 1 diabetes is known to be a T-cell-mediated disease. Autoantibodies are likely only a marker of the autoimmune process and are not themselves directly pathogenic. Therefore, efforts are underway to develop reliable T-cell assays for T1D (see Chapter 73). Tetramer analysis has allowed for the detection of T cells that react to β cell antigens. Tetramer analysis employs the use of multimeric peptide-MHC complexes to detect T cells. Using this technology, soluble HLA-DR401 or DR404 tetramers with a GAD65 epitope were found to react with T cells of newly diagnosed T1D and individuals at risk for T1D (Reijonen et al., 2002; 2003). Further refinement of the tetramer assay is necessary in order to use these assays for disease prediction. Enzyme-linked immunospot (ELISPOT) assays are also being evaluated. In particular, a $CD8^+$ T-cell response to a peptide of islet amyloid polypeptide (IAPP) has been described (Panagiotopoulos et al., 2003).

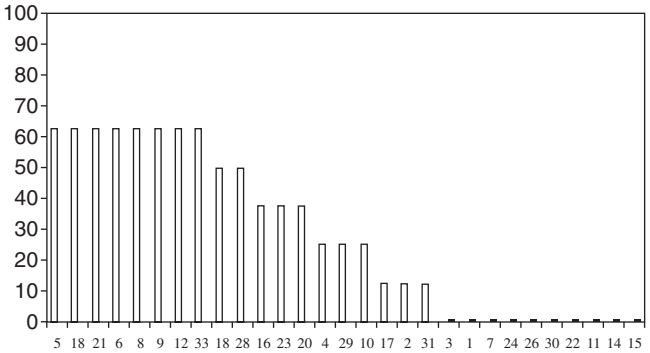


Figure 74.4 Percentage of positive insulin autoantibodies (IAA) (defined as ≥3 SD above the mean) by laboratory in healthy individuals who became diabetic (n = 8). Numbers along the y-axis indicate different laboratories. Open bars are laboratories using radioimmunoassay (RIA) and closed bars are those using enzyme-linked immunosorbent assay (ELISA).

Adapted from Greenbaum et al. (1992).

Metabolic Studies

Metabolic abnormalities begin to appear as the β cell mass diminishes to the point where it cannot appropriately respond to a glucose load. The first metabolic abnormality identified is the loss of the first-phase insulin response (FPIR) on the intravenous glucose tolerance test (IVGTT). The FPIR is the sum of the insulin levels at 1 and 3 minutes after an intravenous glucose load. In the presence of islet autoantibodies, levels that are less than the first percentile of age-matched normal controls identify individuals that are at a 50% risk for the development of overt diabetes at 5 years and 90% at 10 years (Bleich et al., 1990; Bingley et al., 1992). This relationship remains true in individuals who are positive for anti-islet autoantibodies (Bleich et al., 1990). In the DIPP study, Keskinen et al. (2002) have followed children with multiple anti-islet autoantibodies and shown that they have lower FPIR than children with ICA only (Figure 74.5), and that half of the children with abnormal FPIR developed overt diabetes during the time period of the study. The metabolic state of an individual at risk for T1D is dynamic and β cell loss may be ongoing, so repeat evaluation is necessary in order to be maximally sensitive. In addition to being able to correctly identify individuals at risk for overt diabetes, a FPIR greater than the 10th percentile has a

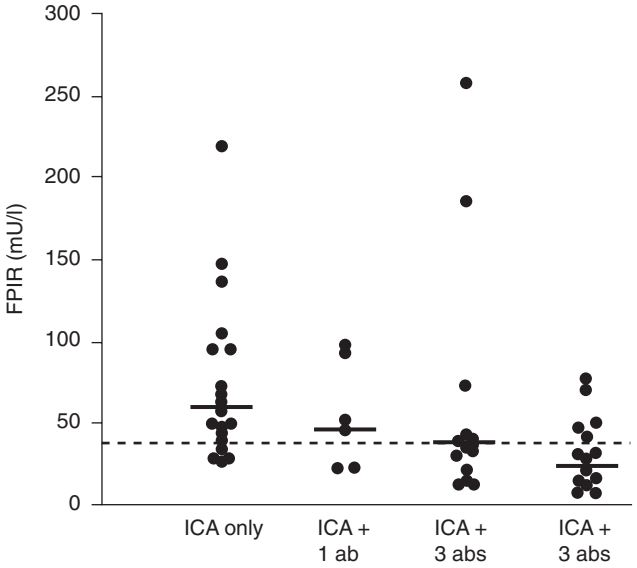


Figure 74.5 The level of first-phase insulin secretion (FPIR) is associated with the number of positive autoantibodies (abs), children with three autoantibodies expressed the lowest FPIR. The dotted line represents the first percentile in age-matched normal controls. (ICA, islet-cell autoantibodies.)

Adapted from Keskinen et al., 2002.

good NPV. Relatives with FPIR greater than the 10th percentile rarely progress to T1D over a 3-year period. Using the FPIR in addition to autoantibody data led to the development of a model of risk for T1D. In this model, the time remaining before the development of T1D was dependent upon the FPIR and insulin autoantibodies (IAA) level (Jackson et al., 1988).

The FPIR is not without problems. It is a measure of β cell function, and, therefore, only an indirect measure of β cell mass. As such, situations that increase insulin resistance such as puberty and obesity may affect the results. In addition, there is significant intra-subject variability with coefficients of variation ranging between 4 and 36% (Smith et al., 1988; Allen et al., 1993; Arslaninan and Austin, 1993). The timing of loss of FPIR differs in the progression to T1D in different groups. For example, in young children who express autoantibodies, the FPIR may already be lost at the time of first autoantibody expression (Keskinen et al., 2002) and some individuals maintain FPIR into their first year of diagnosis of T1D.

As the β cell mass continues to diminish, the metabolic abnormalities become more pronounced and may be detected in routine oral glucose tolerance testing, initially with impaired glucose tolerance (glucose 2 hours after oral glucose ≥140 mg/dL and <200 mg/dL). It is of note that early in the time course of T1D, the metabolic abnormality may not be observed in the fasting state. At this point, the individual is not symptomatic but may rapidly progress to symptomatic diabetes.

ORGAN-SPECIFIC AUTOIMMUNE DISEASES

Thyroid

Autoimmune thyroid disease (AIT) manifested as either hypothyroidism or hyperthyroidism is very common in the general population. Approximately 5% of the population is hypothyroid and 1.3% hyperthyroid (Hollowell et al., 2002). Autoimmune thyroid disease occurs at an increased frequency in individuals with T1D (28% [Umpierrez et al., 2003]), Addison's disease (AD) (14–21% [Betterle et al., 2002; Zelissen et al., 1995; Kasperlik-Zaluska et al., 1998]), celiac disease (CD) (up to 12% [Ansaldi et al., 2003]), and other autoimmune diseases. Therefore, the first step in prediction requires knowledge of the individual's medical history. Routine screening for thyroid disease is generally recommended in these individuals as there is a high pre-test probability of disease and the studies are relatively non-invasive.

Risk for AIT is weakly related to HLA genotypes that vary dependent upon the population. Sanatamaria et al. (1994) reported that *DRB1*0201* increased the risk for AIT in T1D. Kim et al. (2003) reported that *DQB1*0401* increased the risk in the Korean diabetic population. *DQB1*0302* has been associated with AIT in the Czech diabetic population (Sumnik et al., 2003). DR3 and DR5 have been associated with thyroid autoimmunity in German blood donors (Boehm et al., 1993).

Autoimmune thyroid disease is accompanied by autoantibodies to thyroid antigens thyroid peroxidase (TPO) and thyroglobulin (TG) (Vakeva et al., 1992). These autoantibodies are positive in approximately 10% of the general population (Hollowell et al., 2002) and in around 90% of the hypothyroid population (Vakeva et al., 1992). Prospective follow-up by Vanderpump et al. (1995) in the Whickham study has shown that women with positive TPO autoantibodies and normal thyroid stimulating hormone (TSH) have a 2.1% per year risk of developing hypothyroidism with an overall risk of 27% of hypothyroidism at 20 years.

In high-risk populations such as T1D, progression to AIT is related to the presence of TPO and TG antibodies, with approximately 80% of individuals with T1D who are positive for TPO antibodies progressing to AIT compared with 15% of those who are TPO-negative (Umpierrez et al., 2003) (Figure 74.6). Of note, individuals negative for TPO autoantibodies still progressed to AIT but at a much lower rate.

Similar to T1D in which abnormalities of glucose metabolism identify individuals at a greater risk for clinical diabetes, individuals who have abnormalities of thyroid metabolism are at an increased risk for the development of overt hypothyroidism. Of those with an elevated TSH and normal thyroid hormone, 55% were hypothyroid at 20 years, with a 4.3% yearly incidence.

Screening for thyroid autoimmunity in high-risk populations such as T1D with TPO and/or TG autoantibodies may

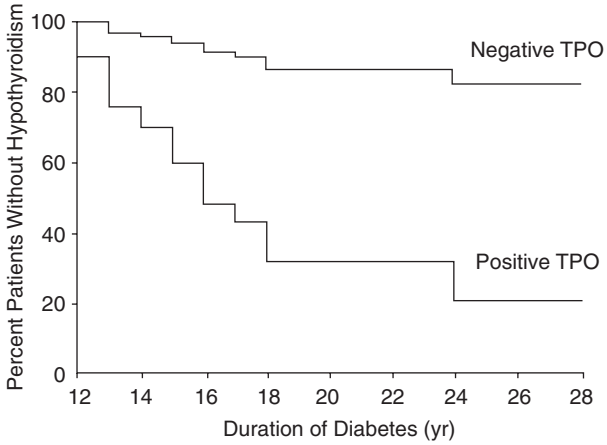


Figure 74.6 The development of hypothyroidism in the diabetic population is dependent upon the production of thyroid autoantibodies. Individuals who express autoantibodies to thyroid peroxidase (TPO) are at a much higher risk for hypothyroidism compared with those who do not.

Adapted from Umpierrez et al., 2003.

identify individuals at an increased risk for autoimmune thyroid disease for closer monitoring. The efficacy of this screening has not been fully evaluated.

Addison's Disease

Addison's disease is an autoimmune endocrine disease resulting in primary adrenal insufficiency. It is a rare disease in the general population, occurring in approximately 1 in 10,000 individuals. However, it does occur with other autoimmune endocrine diseases including T1D and AIT. The combination of two of the three autoimmune diseases (T1D, AD, and/or AIT) is known as autoimmune polyendocrine syndrome II (APS-II).

Genetic risk for AD is conferred by the MHC on chromosome 6. This includes the HLA class II region. AD is associated with DRB1*0404 DQ8 (Yu et al., 1999). Gambelunghe et al. (1999) and Park et al. (2002) have independently shown that polymorphisms of other loci within the MHC including MIC-A are associated with AD both in the diabetic and nondiabetic populations. Follow-up of individuals with T1D and 21-hydroxylase has shown that homozygosity for the MIC-A5.1 polymorphism is associated with an increased progression to clinical AD (Barker et al., in press). MIC-A encodes a ligand for a receptor found on T cells. This interaction may be important in thymic maturation of T cells (Hue et al., 2003) providing an attractive hypothesis for the importance of MIC-A in the development of autoimmune disease.

Addison's disease is accompanied by the production of autoantibodies against the adrenal cortex (Anderson et al., 1957). Since this report, Winqvist et al. (1992) and Baumann-Antczak et al. (1992) have identified the cytochrome p450 enzyme 21-hydroxylase as the major antigen for these antibodies. Falorni et al. (1997) have shown that 21-hydroxylase autoantibodies are highly sensitive and specific for AD. Reflecting the increased risk for AD in people with T1D, individuals with T1D express adrenal autoantibodies at a rate of 1–2% (Brewer et al., 1997; Peterson et al., 1997; Yu et al., 1999).

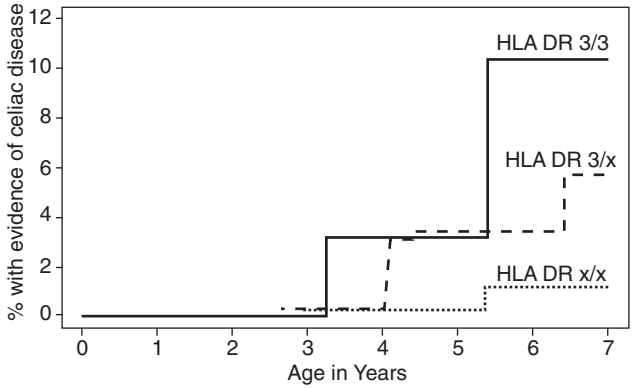
Adrenal function is tested using tetracosactide (cortrosyn; ACTH) stimulation. Baseline blood work consisting of ACTH, cortisol and plasma renin activity (PRA) are obtained and then a stimulatory dose of tetracosactide is given and cortisol levels are measured 30–60 minutes later. Boscaro et al. (1994) have proposed a sequence of events starting with the expression of adrenal autoantibodies and culminating in full-blown adrenal insufficiency. In their cohort, the first metabolic abnormality detected was an elevated baseline PRA. In our group of patients with T1D and 21-hydroxylase autoantibodies, elevated ACTH was a sensitive and specific predictor of abnormalities of stimulation testing (Barker et al., in press).

Celiac Disease

Celiac disease is an autoimmune disease characterized by gluten insensitivity. Symptoms range from malabsorption, diarrhea, and stunted growth to less specific symptoms of fatigue and malaise. Celiac disease can be found in the company of T1D and AIT, occurring in as many as 10% of individuals with T1D (De Vitis et al., 1996) and AIT (Ansaldi et al., 2003).

Celiac disease occurs in association with specific HLA genotypes. In individuals with T1D who are homozygous for HLA DQ2, approximately one-third are positive for autoantibodies to tissue transglutaminase (tTG) (Bao et al., 1999). Ninety-five percent of individuals with CD have HLA DQA1*0501, DQB1*0201, compared with 36% of the general population (Hall et al., 1991). DQA1*0501, DQB1*0201 generally occurs with DR3-DQ2 (DQA1*0501 DQB1*0201) and DR5, DR7 heterozygotes (DRB1*0701 DQA1*0501). Hoffenberg et al. (2003) have prospectively followed neonates from the general population with the high-risk DR3-DQ2 HLA haplotypes in Denver, Colorado. Children with high-risk haplotypes were enrolled and followed for the development of autoantibodies to tTG. At age 5 years, 3.2% of children DR3/3 homozygous developed positive tTG compared with 3.4% of heterozygotes and 0.3% of children negative for DR3 (Figure 74.7).

Celiac disease is associated with the expression of autoantibodies. Antibodies against gliadin (IgG and IgA) are sensitive but not specific and, therefore, have a low PPV. In contrast, anti-endomysial autoantibodies (EMA) are highly sensitive and specific; they are detected with indirect immunofluorescense against human umbilical cord.



3/3	84	80	75	63	47	29	13	3
3/x	506	495	448	325	268	149	75	13
x/x	397	392	366	307	268	208	149	38
All	987	967	889	695	583	386	237	54

Figure 74.7 Percentage of the general population with tissue transglutaminase by HLA DR type. The production of tissue transglutaminase autoantibodies is highly dependent on the presence of HLA DR3. The numbers along the bottom of the figure indicate the numbers of subjects in each group still followed at that time period.

Adapted from Hoffenberg et al., 2003.

Recently tissue transglutaminase (TG2) has been identified as the autoantigen of EMA and highly sensitive and specific assays have been developed to detect TG2 autoantibodies (Dieterich et al., 1997; Fasano and Catassi, 2001; Gillett et al., 2001) (see Chapter 51).

Diagnosis is made by biopsy of the small intestinal mucosa showing the characteristic pathologic changes and confirmed with resolution of these findings on biopsy after institution of a gluten-free diet. Because of the invasiveness of the procedure, biopsy should be performed only in individuals with a high likelihood of being positive. Factors that increase the pre-test probability of disease include individuals who are symptomatic of disease and individuals with higher levels of autoantibody as measured by the radioimmunoassay. Liu et al. (2003) have shown that higher levels of TG2 in a radioimmunoassay had a higher PPV for biopsy positivity. The standard cut-off of 0.05 (99% specificity) had a PPV of 76% compared with 96% with a cut-off of 0.5. In addition, the level of TG2 autoantibody has been shown to correlate with severity of findings on biopsy (Tursi et al., 2003). Therefore, level of autoantibody can be incorporated in an algorithm to decide whether or not to perform the biopsy. The autoantibody levels also decrease on a gluten-free diet and are used to monitor compliance with therapy.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disease resulting in neurologic deficits. It occurs in the setting of specific HLA alleles. DRB1*1501 has consistently been shown to predispose to MS (Allen et al., 1994; Laaksonen, et al., 2002). In a large group of patients, this has been shown to have a dose effect with two copies of the susceptible haplotype associated with a higher risk of MS compared with one or none. Those individuals who express DRB1*1501 on both HLA DRB1 alleles tend to have a more severe disease course (Barcellos et al., 2003).

Autoantibody determinations in these groups are less well defined compared with the other autoimmune disease discussed. Berger et al. (2003) have used western blot analysis looking for antibodies against the autoantigens myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) to predict "clinically definite multiple sclerosis" after a first demyelinating event. Individuals who were positive for anti-MOG and anti-MBP had a quicker relapse (7.5 ± 4.4 months) compared with those who were positive for only one autoantibody (14.6 ± 9.6 months) and those positive for neither (45.1 ± 13.7 months). In addition, a much higher proportion of individuals positive for one (83%) or two (95%) autoantibodies progressed to clinically evident MS compared with those who were negative for both antibodies (23%). This initial report requires confirmation, as determination of disease-specific autoantibodies with western blot assays in MS has proven difficult.

NON-ORGAN-SPECIFIC DISEASE

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic autoimmune disease. It is characterized by joint inflammation, ultimately leading to joint destruction and disability. At diagnosis, individuals often show signs of advanced joint destruction. Landewe et al. (2002) have shown that early treatment decreases disease severity. Therefore, the ability to predict disease and diagnose individuals early would have high clinical utility.

Rheumatoid arthritis is associated with HLA-DR4 (HLA-*DRB1* 0401* and *DRB1*0404*) with the strongest association to *DRB1*0404* (Silman and Pearson, 2002).

Rheumatoid arthritis is accompanied by the production of autoantibodies. Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (CCP) autoantibodies are commonly identified. Rheumatoid factor is an IgM (rarely IgG) autoantibody directed against the Fe piece of IgG. Citrulline is an amino acid that is generated by post-translational modification of arginine by the enzyme peptidylarginine deiminase (PAD) (Vossenaar et al., 2003); polymorphisms of the gene PAD4 are associated with RA (Vossenaar et al., 2004). Cyclic citrullinated peptide autoantibodies are detected in 80% of patient with RA and are produced within the synovium and may have some impact on disease processes (Suzuki et al., 2003). Molecular modeling has shown that *DRB1*0401* binds citrullinated peptides (Hill et al., 2003) and the presence of *DRB1*0401* is correlated with autoantibodies to CCP (Goldback-Mansky et al., 2000). Hill et al. (2003) have associated the genetic risk for RA to the presence of anti-CCP.

Rheumatoid arthritis is thought to have a long preclinical phase that is marked by the presence of autoantibodies without the symptoms of arthritis. Aho et al. (1991) followed over 7000 Finnish individuals over the age of 30 years for the development of RA. Twenty-one individuals developed RA, 15 of whom had positive RF in serum samples prior to the development of disease (Aho et al., 1991). Halldorsdottir et al. (2000) have prospectively determined that persistently positive RF is associated with development of disease. Nielen et al. (2004) retrospectively studied frozen serum samples obtained prior to the diagnosis of RA. Approximately 50% of individuals were positive for either RF or anti-CCP for a median of 4.5 years prior to symptom onset compared with approximately 1% of the general population control. The sensitivity of positivity for RF or CCP was 36.5%, with a specificity of 98.1%. The PPV for a high-risk population positive for either autoantibody positive was 43.8%.

Rantapaa-Dahlqvist et al. (2003) have shown similar findings. However, the PPV of CCP autoantibodies with an estimated population prevalence of 1% is 16%. Therefore, the majority of individuals positive for these autoantibodies

in the general population will not progress to disease and may not be candidates for early therapy. Majka and Holers (2003) have postulated that these autoantibodies may have a better utility in individuals with a high pre-test probability of disease such as those with high risk HLA haplotypes or those with a family history of disease.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by multiorgan involvement. The disease can have a relapsing remitting course or become persistent with fulminant organ involvement.

Genetic risk is conferred by HLA class II alleles in particular DR2 or DR3. In addition C4 null alleles occur in increased frequency in individuals in many ethnic groups (Christiansen et al., 1991).

Autoantibodies are produced in SLE and are characteristic of the disease. However, many of the autoantibodies are also produced in individuals who never develop disease. The antinuclear antibody is a good screening test for disease, in that 95% of individuals with SLE will be positive for this antibody. However, as many as 5% of the general population will also be positive for this autoantibody, albeit at a low titer. Therefore, it is a sensitive but not specific marker of disease. Anti-double stranded DNA antibodies are more disease specific. Specific autoantibodies and quantification using radioimmunoassays have been associated with disease activity. For example, Yamamoto et al. (2000) have described a radioimmunoassay for ribonuclear proteins (RNPs) and found that in individuals with SLE 44% were positive and there was a correlation with SLE nephritis. The use of combinations of autoantibodies can improve sensitivity and specificity for SLE, and levels of autoantibodies can be associated with disease activity scores (Ignat et al., 2003).

CONCLUSION

Effective prediction of autoimmune disease requires an understanding of the epidemiologic concepts of sensitivity, specificity, PPV, NPV, and Bayes' theorem and usually requires assays with specificities ≥99%. Though assays and algorithms for T1D are routinely set at this level, for most rheumatologic disorders immunohistochemical or ELISA assays with lower specificities are standard. It is likely that newer fluid-phase methodologies would also improve prediction for these disorders. Using laboratory studies in populations that are at a high risk for disease, defined by family history or the presence of a high-risk HLA haplotype, may also help improve prognostication for these individuals.

Prediction of disease is especially important when early diagnosis and intervention positively impact the patients. For RA, early treatment modifies the disease course and portends a better outcome in those patients. For T1D, the early identification of individuals at risk for disease by positive autoantibodies decreased the hospitalization for ketoacidosis compared with controls (Barker et al., 2004). As effective prevention measures are developed for autoimmune diseases, the ability to correctly identify individuals at a high risk for disease will become very important.

Autoimmune diseases are often accompanied by the production of autoantibodies. Certain characteristics of autoantibody production such as level, affinity, duration of positivity, and epitope recognition may be associated with disease development. Autoantibody assays can be used in a cohort of susceptible individuals to identify people at an increased risk for the development of disease. Autoantibody production can precede the development of disease for many years and some individuals who are autoantibody positive may never develop disease. Therefore, precise prognostication for a single individual is difficult. For T1D, the presence of two or more anti-islet autoantibodies is associated with very high risk of progression.

The presence of one autoimmune disease identifies an individual at a high risk for a second autoimmune disease (Pearce and Leech, 2004). The heightened risk for disease in this group increases the pre-test probability of disease. Therefore, using autoantibody assays and/or T-cell assays in this group will be more efficacious. A positive result will have a high post-test probability of disease in a single individual.

Many autoimmune diseases are T-cell dependent. The development of reliable T-cell assays that are sensitive and specific for autoimmune disease will be a major advance in the ability to predict disease. When used in combination with autoantibody assays, the T-cell assays may refine our diagnostic ability and improve PPV and NPV.

The use of predictive algorithms that incorporate genetic risk expressed by HLA haplotypes or family history of disease, autoantibody production, and early physiological changes such as abnormal FPIR (T1D), mildly elevated TSH (AIT), or elevated PRA, ACTH (AD) will identify individuals at different points along the natural history of disease with increasing risk for disease development. These algorithms can be used to identify individuals who might benefit and be willing to participate in intervention studies that could change the course of disease. We believe it is likely that most autoimmune disorders are preceded by a long prodrome and that waiting for clinical presentation can be associated with irreversible morbidity (bone erosions at diagnosis of patients with RA, osteoporosis with CD or presentation with T-cell lymphoma) or even mortality (e.g., death at diabetes onset from cerebral edema, hypotensive crisis of AD). As the field of "personalized" medicine progresses, utilizing knowledge of personal risk factors (genetic, laboratory, clinical) to guide individual care, we believe that early detection of autoimmune disease risk will

be increasingly important and will depend on improved tests as well as development of preventive therapies.

Acknowledgments

Research supported by NIH grant DK 32083, DK 32493, A139213 and S-M01-RR00051 (Clinical Research Centers Program) and Diabetes Endocrine Research Center (P30 DK57516 from NIH; JDF grant 1-1999-679), Children's Diabetes Foundation, Autoimmunity Center of Excellence (U19 A146374), and Autoimmunity Prevention Center (U19 A150864). Dr Barker is supported by the Juvenile Diabetes Research Foundation/Lawson Wilkins Pediatric Endocrine Fellowship 13-2002-444.

References

Aho, K., Heliovaara, M., Maatela, J., Tuomi, T., and Palosuo, T. (1991). Rheumatoid factors antedating clinical rheumatoid arthritis. *J. Rheumatol. 18,* 1282–1284.

Allen, H.F., Jeffers, B.W., Klingensmith, G.J., and Chase, H.P. (1993). First-phase insulin release in normal children. *J. Pediatr. 123,* 733–738.

Allen, M., Sandberg-Wollheim, M., Sjogren, K., Erlich, H.A., Petterson, U., and Gyllensten, U. (1994). Association of susceptibility to multiple sclerosis in Sweden with HLA class II DRB1 and DQB1 alleles. *Hum. Immunol. 39,* 41–48.

Anderson, J.R., Goudie, R.B., Gray, K.G., and Timbury, G.C. (1957). Autoantibodies in Addison's disease. *Lancet 272,* 1123–1124.

Ansaldi, N., Palmas, T., Corrias, A., Barbato, M., DAltiglia, M.R., Campanozzi, A., Baldassarre, M., Rea, F., Pluvio, R., Bonamico, M., Lazzari, R., and Corrao, G. (2003). Autoimmune thyroid disease and celiac disease in children. *J. Pediatr. Gastroenterol. Nutr. 37,* 63–66.

Arslanian, S. and Austin, A. (1993). Determinants of first and second phase insulin secretion in healthy adolescents. *Pediatr. Res. 3*(Suppl,), S74.

Bao, F., Yu, L., Babu, S., Wang , T., Hoffenberg, E.J., Rewers, M., and Eisenbarth, G.S. (1999). One third of HLA DQ2 homozygous patients with type 1 diabetes express celiac disease associated transglutaminase autoantibodies. *J. Autoimmun. 13,* 143–148.

Barcellos, L.F., Oksenberg, J.R., Begovich, A.B., Martin, E.R., Schmidt, S., Vittinghoff, E., Goodin, D.S., Pelletier, D., Lincoln, R.R., Bucher, P., Swerdlin, A., Pericak-Vance, M.A., Haines, J.L., and Hauser, S.L. (2003). HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. *Am. J. Hum. Genet. 72,* 710–716.

Barker, J.M., Goehrig, S.H., Barriga, K., Hoffman, M., Slover, R., Eisenbarth, G.S., Norris, J.M., Klingensmith, G.J., and Rewers, M. (2004). Clinical characteristics of children diagnosed with type 1 diabetes through intensive screening and follow-up. *Diabetes Care 27,* 1399–1404.

Barker, J.M., Barriga, K.J., Yu, L., Miao, D., Erlich, H.A., Norris, J.M., Eisenbarth, G.S., and Rewers, M. (2004). Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). *J. Clin. Endocrinol. Metab. 89*(9), 3896–3902.

Barker, J.M., Ide, A., Hostetler, C., Yu, L., Miao, D., Fain, P.R., Eisenbarth, G.S., and Gottlieb, P.A. (in press) Endocrine and immunogenetic testing in individuals with type 1 diabetes and 21-hydroxylase autoantibodies: Addison's disease in high risk population. *J. Clin. Endocrinol. Metab.*

Baumann-Antczak, A., Wedlock, N., Bednarek, J., Kiso, Y., Krishnan, H., Fowler, S., Smith, B.R., and Furmaniak, J. (1992) Autoimmune Addison's disease and 21-hydroxylase. *Lancet 340,* 429–430.

Bell, G.I., Horita, S., and Karam, J.H. (1984) A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. *Diabetes 33,* 176–183.

Bennet, S.T., Wilson, A.J., Esposito, L., Bouzekri, J., Undlien, D., Cucca, F., Nisticò, L., Buzzetti, R., the IMDIAB Group, Bosi, E., Pociot, F., Nerup, J., Cambon-Thomsen, A., Pugliese, A., Shield, J.P.H., McKinney, P.A., Bain, S.C., Polychronakos, C., and Todd, J.A. (1997). Insulin VNTR allele-specific effect in type 1 diabetes depends on identity of untransmitted paternal allele. *Nat. Genet. 17,* 350–352.

Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F., and Reindl, M. (2003). Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N. Engl. J. Med. 349,* 139–145.

Betterle, C., Dal Pra, C., Mantero, F., and Zanchetta, R. (2002). Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr. Rev. 23,* 327–364.

Bingley, P.J., Colman, P., Eisenbarth, G.S., Jackson, R.A., McCulloch, D.K., Riley, W.J., and Gale, E.A.M. (1992). Standardization of IVGTT to predict IDDM. *Diabetes Care 15,* 1313–1316.

Bingley, P.J., Christie, M.R., Bonifacio, E., Bonfanti, R., Shattock, M., Fonte, M.-T., Bottazzo, G.-F., and Gale, E.A.M. (1994). Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. *Diabetes 43,* 1304–1310.

Bingley, P.J., Bonifacio, E., Williams, A.J.K., Genovese, S., Bottazzo, G.F., and Gale, E.A.M. (1997). Prediction of IDDM in the general population: strategies based on combinations of autoantibody markers. *Diabetes 46,* 1701–1710.

Bleich, D., Jackson, R.A., Soeldner, J.S., and Eisenbarth, G.S. (1990). Analysis of metabolic progression to type I diabetes in ICA+ relatives of patients with type I diabetes. *Diabetes Care 13,* 111–118.

Boehm, B.O., Kuhnl, P., Loliger, C., Ketzler-Sasse, U., Holzberger, G., Seidl, S., Bauerle, R., Schifferdecker, E., and Usadel, K.H. (1993). HLA-DR3 and HLA-DR5 confer risk for autoantibody positivity against the thyroperoxidase (mic-TPO) antigen in healthy blood donors. *Clin. Investig. 71,* 221–225.

Bonifacio, E., Bingley, P.J., Shattock, M., Dean, B.M., Dunger, D., Gale, E.A., and Bottazzo, G.F. (1990). Quantification of islet-cell antibodies and prediction of insulin-dependent diabetes *Lancet 335,* 147–149.

Boscaro, M., Betterle, C., Sonino, N., Volpato, M., Paoletta, A., and Fallo, F. (1994). Early adrenal hypofunction in patients with organ-specific autoantibodies and no clinical adrenal insufficiency. *J. Clin. Endocrinol. Metab. 79,* 452–455.

Bottazzo, G.F., Dean, B.M., Gorsuch, A.N., Cudworth, A.G., and Doniach, D. (1980) Complement-fixing islet-cell antibodies in type I diabetes: possible monitors of active β-cell damage. *Lancet 3170,* 668–672.

Bottini, N., Musumeci, L., Alonso, A., Rahmouni, S., Nika, K., Rostamkhani, M., MacMurray, J., Meloni, G.F., Lucarelli, P., Pellecchia, M., Eisenbarth, G.S., Comings, D., and Mustelin, T. (2004). A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat. Genet. 36,* 337–338.

Brewer, K.W., Parziale, V.S., and Eisenbarth, G.S. (1997). Screening patients with insulin-dependent diabetes mellitus for adrenal insufficiency. *N. Engl. J. Med. 337,* 202.

Chase, H.P., Garg, S.K., Butler-Simon, N., Klingensmith, G., Norris, L., Ruskey, C.T., and OBrien, D. (1991). Prediction of the course of pretype I diabetes. *J. Pediatr. 118,* 838–841.

Christiansen, F.T., Zhang, W.J., Griffiths, M., Mallal, S.A., and Dawkins, R.L. (1991). Major histocompatibility complex (MHC) complement deficienty, 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.

DeVitis, I., Ghirlanda, G., and Gasbarrini, G. (1996). Prevalence of coeliac disease in type I diabetes: a multicentre study. *Acta Paediatr. Suppl. 412,* 56–57.

Dieterich, W., Ehnis, T., Bauer, M., Donner, P., Volta, U., Riecken, E.O., and Schuppan, D. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat. Med. 3,* 797–801.

Eisenbarth, G.S. (1986). Type I diabetes mellitus. A chronic autoimmune disease. *N. Engl. J. Med. 314,* 1360–1368.

Erlich, H.A., Zeidler, A., Chang, J., Shaw, S., Raffel, L.J., Klitz, W., Beshkov, Y., Costin, G., Pressman, S., Bugawan, T., and Rotter, J.I. (1993) HLA class II alleles and susceptibility and resistance to insulin dependent diabetes mellitus in Mexican-American families. *Nat. Genet. 3,* 358–364.

Falorni, A., Laureti, S., Nikoshkov, A., Picchio, M.L., Hallengren, B., Vandewalles, C.L., Gorus, F.K., Tortoioli, C., Luthman, H., Brunetti, P., and Santeusanio, F. (1997). 21-hydroxylase autoantibodies in adult patients with endocrine autoimmune diseases are highly specific for Addison's disease. *Clin. Exp. Immunol. 107,* 341–346.

Fasano, A. and Catassi, C. (2001). Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology 120,* 636–651.

Fuchtenbusch, M., Ferber, K., Standl, E., and Ziegler, A.G. (1997). Prediction of type 1 diabetes postpartum in patients with gestational diabetes mellitus by combined islet cell autoantibody screening: a prospective multicenter study. *Diabetes 46,* 1459–1467.

Gambelunghe, G., Falorni, A., Ghaderi, M., Laureti. S., Tortoioli, C., Santeusanio, F., Brunetti, P., and Sanjeevi, C.B. (1999). Microsatellite Polymorphism of the MHC Class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. *J. Clin. Endocrinol. Metab. 84,* 3701–3707.

Gardner, S.G., Gale, E.A., Williams, A.J., Gillespie, K.M., Lawrence, K.E., Bottazzo, G.F., and Bingley, P.J. (1999). Progression to diabetes in relatives with islet autoantibodies. Is it inevitable? *Diabetes Care 22,* 2049–2054.

Gillett, P.M., Gillett, H.R., Israel, D.M., Metzger, D.L., Stewart, L., Chanoine, J.P., and Freeman, H.J. (2001). High prevalence of celiac disease in patients with type 1 diabetes detected by antibodies to endomysium and tissue transglutaminase. *Can. J. Gastroenterol. 15,* 297–301.

Goldbach-Mansky, R., Lee, J., McCoy, A., Hoxworth, J., Yarboro, C., Smolen, J.S., Steiner, G., Rosen, A., Zhang, C., Menard, H.A., Zhou, Z.J., Palosuo, T., van Venrooij, W.J., Wilder, R.L., Klippel, J.H., Schumacher, H.R., Jr., and El Gabalawy, H.S. (2000). Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. *Arthritis Res. 2,* 236–243.

Greenbaum, C.J., Palmer, J.P., Kuglin, B., Kolb, H., and Participating Laboratories. (1992). Insulin autoantibodies measured by radioimmunoassay methodology are more related to insulin dependent diabetes mellitus than those measured by enzyme-linked immunosorbent assay: results of the fourth international workshop on the standardization of insulin autoantibody measurement. *J. Clin. Endocrinol. Metab. 74,* 1040–1044.

Hall, M.A., Mazzilli, M.C., Satz, M.L., Barboni, F., Bartova, A., Brunnler, G., Ciclitira, P.J., Corazza, G.R., Ferrante, P., Gerok, W., Herrara, M., Kollek, A., Keller, E., Lanchbury, J.S., Mantovani, V., Muser, K., Petronzelli, F., Roschmann, E., Theiler, G., Volk, B.A., Welsh, K.I., Wienker, T., and Albert, E. (1991). Coeliac disease study. *Eleventh International Histocompatibility Workshop 6.5,* 722–729.

Halldorsdottir, H.D., Jonsson, T., Thorsteinsson, J., and Valdimarsson, H. (2000). A prospective study on the incidence of rheumatoid arthritis among people with persistent increase of rheumatoid factor. *Ann. Rheum. Dis. 59,* 149–151.

Hill, J.A., Southwood, S., Sette, A., Jevnikar, A.M., Bell, D.A., and Cairns, E. (2003). Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J. Immunol. 171,* 538–541.

Hoffenberg, E.J., McKenzie, T.L., Barriga, K.J., Eisenbarth, G.S., Bao, F., Haas, J.E., Erlich, H.A., Sokol, R.J., Taki, I., and Rewers, M.J. (2003). A prospective study of the incidence of childhood celiac disease. *J. Paediatr. 143,* 308–314.

Hollowell, J.G., Staehling, N.W., Flanders, W.D., Hannon, W.H., Gunter, E.W., Spencer, C.A., and Braverman, L.E. (2002). Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J. Clin. Endocrinol. Metab. 87,* 489–499.

Hue, S., Monteiro, R.C., Berrih-Aknin, S., and Caillat-Zucman, S. (2003). Potential role of NKG2D/MHC class I-related chain A interaction in intrathymic maturation of single-positive CD8 T cells. *J. Immunol. 171,* 1909–1917.

Ignat, G.P., Rat, A.C., Sychra, J.J., Vo, J., Varga, J., and Teodorescu, M. (2003). Information on diagnosis and management of systemic lupus erythematosus derived from the routine measurement of 8 nuclear autoantibodies. *J. Rheumatol. 30,* 1761–1769.

Jackson, R.A., Vardi, P., Herskowitz, R.D., Soeldner, J.S., and Eisenbarth, G.S. (1988). Dual parameter linear model for prediction of onset of type I diabetes in islet cell antibody positive relatives. *Clin. Res. 36,* 585A.

Kasperlik-Zaluska, A.A., Czarnocka, B., Czech, W., Walecki, J., Makowska, A.M., Brzezinski, J., and Aniszewski, J. (1998). Secondary adrenal insufficiency associated with autoimmune disorders: a report of twenty-five cases. *Clin. Endocrinol. (Oxf) 49,* 779–783.

Keskinen, P., Korhonen, S., Kupila, A., Veijola, R., Erkkila, S., Savolainen, H., Arvilommi, P., Simell, T., Ilonen, J., Knip, M., and Simell, O. (2002). First-phase insulin response in young healthy children at genetic and immunological risk for Type I diabetes. *Diabetologia 45,* 1639–1648.

Kim, E.Y., Shin, C.H., and Yang, S.W. (2003). Polymorphisms of HLA class II predispose children and adolescents with type 1 diabetes mellitus to autoimmune thyroid disease. *Autoimmunity 36,* 177–181.

Kimpimaki, T., Kupila, A., Hamalainen, A.M., Kukko, M., Kulmala, P., Savola, K., Simell, T., Keskinen, P., Ilonen, J., Simell, O., and Knip, M. (2001). The first signs of β-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish Type 1 Diabetes Prediction and Prevention Study. *J. Clin. Endocrinol. Metab. 86,* 4782–4788.

Kupila, A., Muona, P., Simell, T., Arvilommi, P., Savolainen, H., Hamalainen, A.M., Korhonen, S., Kimpimaki, T., Sjoroos, M., Ilonen, J., Knip, M., and Simell, O. (2001). Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. *Diabetologia 44,* 290–297.

Laaksonen, M., Pastinen, T., Sjoroos, M., Kuokkanen, S., Ruutiainen, J., Sumelahti, M.L., Reijonen, H., Salonen, R., Wikstrom, J., Panelius, M., Partanen, J., Tienari, P.J., and Ilonen, J. (2002). HLA class II associated risk and protection against multiple sclerosis—a Finnish family study. *J. Neuroimmunol. 122,* 140–145.

LaGasse, J.M., Brantley, M.S., Leech, N.J., Rowe, R.E., Monks, S., Palmer, J.P., Nepom, G.T., McCulloch, D.K., and Hagopian, W.A. (2002). Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. *Diabetes Care 25,* 505–511.

Landewe, R.B., Boers, M., Verhoeven, A.C., Westhovens, R., van de Laar, M.A., Markusse, H.M., van Denderen, J.C., Westedt, M.L., Peeters, A.J., Dijkmans, B.A., Jacobs, P., Boonen, A., van der Heijde, D.M., and van der, Linden, S. (2002). COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. *Arthritis Rheum. 46,* 347–356.

Lendrum, R., Walker, G., and Gamble, D.R. (1975). Islet-cell antibodies in juvenile diabetes mellitus of recent onset. *Lancet 7912,* 880–883.

Liu, E., Bao, F., Barriga, K., Miao, D., Yu, L., Erlich, H.A., Haas, J.E., Eisenbarth, G.S., Rewers, M.J., and Hoffenberg, E.J. (2003).

Fluctuating transglutaminase autoantibodies are related to histologic features of celiac disease. *Clin. Gastroenterol. Hepatol. 1,* 356–362.

Lowe, R.M., Graham, J., Sund, G., Kockum, I., Landin-Olsson, M., Schaefer, J.B., Torn, C., Lernmark, A., and Dahlquist, G. (2000). The length of the CTLA-4 microsatellite (AT)N-repeat affects the risk for type 1 diabetes. Diabetes Incidence in Sweden Study Group. *Autoimmunity 32,* 173–180.

Maclaren, N., Lan, M., Coutant, R., Schatz, D., Silverstein, J., Muir, A., Clare-Salzer, M., She, J.X., Malone, J., Crockett, S., Schwartz, S., Quattrin, T., DeSilva, M., Vander, V.P., Notkins, A., and Krischer J. (1999). Only multiple autoantibodies to islet cells (ICA), insulin, GAD65, IA-2 and IA-2beta predict immune-mediated (Type 1) diabetes in relatives. *J. Autoimmun. 12,* 279–287.

Majka, D.S. and Holers, V.M. (2003). Can we accurately predict the development of rheumatoid arthritis in the preclinical phase? *Arthritis Rheum. 48,* 2701–2705.

Nerup, J., Platz, P., Anderson, O.O., Christy, M., Lyngsoe, J., Poulsen, J.E., Ryder, L.P., Nielsen, L.S., Thomsen, M., and Svejgaard, A. (1974). HLA antigens and diabetes mellitus. *Lancet 7885,* 864–866.

Nielen, M.M., van Schaardenburg, D., Reesink, H.W., van de Stadt, R.J., van der Horst-Bruinsma, I.E., de Koning, M.H., Habibuw, M.R., Vandenbroucke, J.P., and Dijkmans, B.A. (2004). Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum. 50,* 380–386.

Nistico, L., Buzzetti, R., Pritchard, L.E., van der Auwera, B., Giovannini, C., Bosi, E., Larrad, M.T., Rios, M.S., Chow, C.C., Cockram, C.S., Jacobs, K., Mijovic, C., Bain, S.C., Barnett, A.H., Vandewalle, C.L., Schuit, F., Gorus, F.K., Tosi, R., Pozzilli, P., and Todd, J.A. (1996). The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum. Mol. Genet. 5,* 1075–1080.

Noble, J.A., Valdes, A.M., Cook, M., Klitz, W., Thomson, G., and Erlich, H.A. (1996). The role of HLA class II genes in insulin-dependent diabetes mellitus: Molecular analysis of 180 Caucasian, multiplex families. *Am. J. Hum. Genet. 59,* 1134–1148.

Panagiotopoulos, C., Qin, H., Tan, R., and Verchere, C.B. (2003). Identification of a β-cell-specific HLA class I restricted epitope in type 1 diabetes. *Diabetes 52,* 2647–2651.

Park, Y.S., Sanjeevi, C.B., Robles, D., Yu, L., Rewers, M., Gottlieb, P.A., Fain, P., and Eisenbarth, G.S. (2002). Additional association of intra-MHC genes, MICA and D6S273, with Addison's disease. *Tissue Antigens 60,* 155–163.

Pearce, S.H. and Leech, N.J. (2004). Toward precise forecasting of autoimmune endocrinopathy. *J. Clin. Endocrinol. Metab. 89,* 544–547.

Peterson, P., Salmi, H., Hyoty, H., Miettinen, A., Ilonen, J., Reijonen, H., Knip, M., Åkerblom, H.K., and Krohn, K. (1997). Steroid 21-hydroxylase autoantibodies in insulin-dependent diabetes mellitus. Childhood Diabete sin Finland (DiMe) Study Group. *Clin. Immunol. Immunopathol. 82,* 37–42.

Pietropaolo, M., Becker, D.J., LaPorte, R.E., Dorman, J.S., Riboni, S., Rudert, W.A., Mazumdar, S., and Trucco, M. (2002). Progression to insulin-requiring diabetes in seronegative prediabetic subjects: the role of two HLA-DQ high-risk haplotypes. *Diabetologia 45,* 66–76.

Pugliese, A., Gianani, R., Moromisato, R., Awdeh, Z.L., Alper, C.A., Erlich, H.A., Jackson, R.A., and Eisenbarth, G.S. (1995). HLA-DQB1*0602 is associated with dominant protection from diabetes even among islet cell antibody-positive first-degree relatives of patients with IDDM *Diabetes 44,* 608–613.

Rantapaa-Dahlqvist, S., de Jong, B.A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., and van Venrooij, W.J. (2003). Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum. 48,* 2741–2749.

Redondo, M.J., Fain, P.R., and Eisenbarth, G.S. (2001). Genetics of type 1A diabetes. *Recent Prog. Horm. Res. 56,* 69–89.

Reijonen, H., Novak, E.J., Kochik, S., Heninger, A., Liu, A.W., Kwok, W.W., and Nepom, G.T. (2002). Detection of GAD65-specific T-cells by major histocompatibility complex class II tetramers in type 1 diabetic patients and at-risk subjects. *Diabetes 51,* 1375–1382.

Reijonen, H., Kwok, W.W., and Nepom, G.T. (2003) Detection of CD4+ autoreactive T cells in T1D using HLA class II tetramers. *Ann. N. Y. Acad. Sci. 1005,* 82–87.

Rewers, M., Bugawan, T.L., Norris, J.M., Blair, A., Beaty, B., Hoffman, M., McDuffie, R.S., Jr, Hamman, R.F., Klingensmith, G., Eisenbarth, G.S., and Erlich, H.A. (1996). Newborn screening for HLA markers associated with IDDM: diabetes autoimmunity study in the young (DAISY). *Diabetologia 39,* 807–812.

Rewers, M., Norris, J.M., Eisenbarth, G.S., Erlich, H.A., Beaty, B., Klingensmith, G., Hoffman, M., Yu, L., Bugawan, T.L., Blair, A., Hamman, R.F., Groshek, M., and McDuffie, R.S., Jr. (1996). β-cell autoantibodies in infants and toddlers without IDDM relatives: diabetes autoimmunity study in the young (DAISY). *J. Autoimmun. 9,* 405–410.

Santamaria, P., Barbosa, J.J., Lindstrom, A.L., Lemke, T.A., Goetz, F.C., and Rich, S.S. (1994). HLA-DQB1-associated susceptibility that distinguishes Hashimotos thyroiditis from Graves disease in type I diabetic patients. *J. Clin. Endocrinol. Metab. 78,* 878–883.

Silman, A.J. and Pearson, J.E. (2002). Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res. 4*(Suppl 3), S265–S272.

Smith, C.P., Tarn, A.C., Thomas, J.M., Overkamp, D., Corakci, A., Savage, M.O., and Gale, E.A. (1988). Between and within subject variation of the first phase insulin response to intravenous glucose. *Diabetologia 31,* 123–125.

Sumnik, Z., Drevinek, P., Snajderova, M., Kolouskova, S., Sedlakova, P., Pechova, M., Vavrinec, J., and Cinek, O. (2003). HLA-DQ polymorphisms modify the risk of thyroid autoimmunity in children with type 1 diabetes mellitus. *J. Pediatr. Endocrinol. Metab. 16,* 851–858.

Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y., and Yamamoto, K. (2003). Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet. 34,* 395–402.

Tillil, H. and Kobberling, J. (1987). Age-corrected empirical genetic risk estimates for first-degree relatives of IDDM patients. *Diabetes 36,* 93–99.

Turner, R., Stratton, I., Horton, V., Manley, S., Zimmet, P., Mackay, I.R., Shattock, M., Bottazzo, G.F., and Holman, R. (1997). UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. *Lancet 350,* 1288–1293.

Tursi, A., Brandimarte, G., and Giorgetti, G.M. (2003). Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease. *J. Clin. Gastroenterol. 36,* 219–221.

Umpierrez, G.E., Latif, K.A., Murphy, M.B., Lambeth, H.C., Stentz, F., Bush, A., and Kitabchi, A.E. (2003). Thyroid dysfunction in patients with type 1 diabetes: A longitudinal study. *Diabetes Care 26,* 1181–1185.

Vakeva, A., Kontiainen, S., Miettinen, A., Schlenzka, A., and Maenpaa, J. (1992). Thyroid peroxidase antibodies in children with autoimmune thyroiditis. *J. Clin. Pathol. 45,* 106–109.

Vanderpump, M.P., Tunbridge, W.M., French, J.M., Appleton, D., Bates, D., Clark, F., Grimley, E.J., Hasan, D.M., Rodgers, H., and Tunbridge, F. (1995). The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clin. Endocrinol. (Oxf) 43,* 55–68.

Verge, C.F., Gianani, R., Kawasaki, E., Yu, L., Pietropaolo, M., Jackson, R.A., Chase, H.P., and Eisenbarth, G.S. (1996). Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA–2 autoantibodies. *Diabetes 45,* 926–933.

Vossenaar, E.R., Zendman, A.J., and van Venrooij, W.J. (2004). Citrullination, a possible functional link between susceptibility genes and rheumatoid arthritis. *Arthritis Res. Ther. 6,* 1–5.

Vossenaar, E.R., Zendman, A.J., van Venrooij, W.J., and Pruijn, G.J. (2003). PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioessays 25,* 1106–1118.

Winqvist, O., Karlsson, F.A., and Kampe, O. (1992). 21-hydroxylase, a major autoantigen in idiopathic Addison's disease. *Lancet 339,* 1559–1562.

Yamamoto, A.M., Amoura, Z., Johannet, C., Jeronimo, A.L., Campos, H., Koutouzov, S., Piette, J.C., and Bach, J.F. (2000). Quantitative radioligand assays using de novo-synthesized recombinant autoantigens in connective tissue diseases: new tools to approach the pathogenic significance of anti-RNP antibodies in rheumatic diseases. *Arthritis Rheum. 43,* 689–698.

Yu, L., Brewer, K.W., Gates, S., Wang, T., Babu, S., Gottlieb, P.A., Freed, B.M., Noble, J., Erlich, H.A., and Eisenbarth, G.S. (1999). DRB1*04 and DQ alleles: expression of 21-hydroxylase autoantibodies and risk of progression to Addison's disease. *J. Clin. Endocrinol. Metab. 84,* 328–335.

Yu, J., Yu, L., Bugawan, T.L., Erlich, H.A., Barriga, K., Hoffman, M., Rewers, M., and Eisenbarth, G.S. (2000). Transient anti-islet autoantibodies: infrequent occurrence and lack of association with genetic risk factors. *J. Clin. Endocrinol. Metab. 85,* 2421–2428.

Yu, L., Cuthbertson, D.D., Maclaren, N., Jackson, R., Palmer, J., Orban, T., Eisenbarth, G.S., and Krischer, J. (2001). Expression of GAD65 and islet cell antibody (ICA512) autoantibodies amongst cytoplasmic ICA+ relatives is associated with eligibility for Diabetes Prevention Trial Type-1. *Diabetes 50,* 1735–1740.

Zelissen, P.M., Bast, E.J.E.G., and Croughs, R.J.M. (1995). Associated autoimmunity in Addison's disease. *J. Autoimmun. 8,* 121–130.

Ziegler, R., Alper, C.A., Awdeh, Z.L., Castano, L., Brink, S.J., Soeldner, J.S., Jackson, R.A., and Eisenbarth, G.S. (1991). Specific association of HLA-DR4 with increased prevalence and level of insulin autoantibodies in first-degree relatives of patients with type I diabetes. *Diabetes 40,* 709–714.

CHAPTER

75

Prevention of Autoimmune Disease: Type 1 Diabetes as a Paradigm

LEONARD C. HARRISON

Autoimmunity and Transplantation Division, The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville 3050, Victoria, Australia

Insulin-dependent or type 1 diabetes (T1D) is an autoimmune disease in which cell-mediated immunity destroys the insulin-secreting beta cells in the pancreatic islets (see Chapter 6). Type 1 diabetes is genetically-determined but its expression is modified by environment. In most cases the disease process begins months to years before major loss of beta-cell function heralds symptoms of hyperglycemia, a natural history that has clear implications for prevention (see Chapter 36). Increasing knowledge of the mechanisms of beta-cell destruction and the ability to identify individuals at risk for T1D, together with proof-of-principle for therapeutic intervention in the non-obese diabetic (NOD) mouse model, are platforms for T1D prevention in humans. Indeed, T1D can be seen as a paradigm for the preclinical diagnosis and prevention of autoimmune disease in general. Preventing T1D applies not only to people at risk but also to those with clinical diabetes, in order to preserve residual beta-cell function, allow possible beta-cell regeneration and prevent recurrent autoimmune disease after therapeutic beta-cell replacement or regeneration.

Ideally, T1D would be eradicated by primary prevention aimed at avoiding or averting the environmental factors that are thought to precipitate disease in genetically at-risk individuals, but these factors have not been clearly identified and may be ubiquitous. Without knowing these environmental factors (and not being able to modify genetic susceptibility), the prospects for primary prevention are uncertain. Secondary prevention, after the disease process has started, has received considerable attention for over two decades, with a range of candidate therapeutic agents being evaluated, predominantly in people with recent-onset clinical diabetes. Prevention is, however, mostly applicable to subclinical disease, rather than to clinical disease when beta-cell destruction is end-stage.

Prerequisites for a human therapeutic are efficacy and safety in animals. The most widely used animal model of T1D is the NOD mouse, which has contributed substantially to our understanding of the disease process and the expectation that T1D should be preventable (Leiter et al., 1987). The NOD mouse shares features with human T1D, including polygenic inheritance dominated by genes for antigen-presenting molecules in the major histocompatibility complex (MHC), autoimmunity to (pro)insulin and glutamic acid decarboxylase (GAD), transfer of disease by bone marrow and a protracted subclinical stage (Adorini et al., 2002). Many immune and other interventions modify the incidence of diabetes in NOD mice (Atkinson and Leiter, 1999); indeed, some would say too many, questioning whether this inbred mouse is in fact a good surrogate for the evaluation of candidate therapeutic agents in humans. However, most interventions prevent disease in only a proportion of NOD mice, some merely retard disease and others have no effect (and, therefore, are not reported), and many of the reported disease modifiers have not been tested in humans.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Secondary prevention strategies are summarized in Table 75.1. The emphasis has recently shifted from nonspecific modalities to more disease-specific immunoregulatory agents. Apparent preservation of C-peptide secretion in the first year after diagnosis has been reported in many randomized trials (e.g., Harrison et al., 1985; Feutren et al., 1986; Canadian-European Randomized Control Trial Group, 1988; Cook et al., 1989; Assan et al., 1990; Goday et al., 1993; Bjork et al., 2001; Raz et al., 2001; Herold et al., 2002), but has not been sustained. Autoantigen-specific immunotherapy—the "Holy Grail"—is safe and effective in animal models of autoimmune disease but has still not been shown to be clinically effective in at-risk humans. Prevention trials would be greatly facilitated by better assays for mechanistic immune and metabolic markers, especially for islet autoantigen-reactive T cells, and by non-invasive means of assessing islet pathology.

While the focus has been on beta-cell immunopathology leading to insulin deficiency, the level of blood glucose reflects a carefully-regulated balance between both insulin secretion and insulin action. Recently, insulin resistance has been shown to be a major, independent risk factor for progression to diabetes in individuals with islet autoantibodies (Fourlanos et al., 2004). Hence, promotion of insulin action must also be considered for the secondary prevention of T1D. The prevention of T1D will occur incrementally, with targeted agents being evaluated, often in combination, according to disease stage and risk, analogous to the evolving treatment regimens for cancer or HIV infection.

PREVENTION—WHY?

Complications of T1D can be reduced but not prevented by intensive blood glucose control with injectable insulin. In the Diabetes Control and Complications Trial (DCCT), even intensively-treated, motivated subjects in a study situation developed significant complications over a 10-year follow-up: neuropathy in 5%, retinopathy in 10%, and nephropathy in 15%. Moreover, intensive treatment was accompanied by 2–3-fold increase in severe, symptomatic hypoglycemic episodes (Diabetes Control and Complications Trial Research Group, 1993). Hence, retaining some beta-cell function or delaying the onset of clinical disease would significantly reduce the burden of T1D, because patients with residual beta-cell function require lower doses of insulin and have better blood-glucose control. In our experience of screening first-degree relatives, approximately 3% of whom are positive for one or more islet autoantibodies (Colman et al., 1998), the cost of detecting one relative with a 25–50% risk of diabetes within 5 years is approximately twice the annual cost of treating someone with T1D. Thus, a relatively inexpensive means of prevention would need only to delay the onset of diabetes by 2–3 years for screening to be cost-effective. The Finnish IDDM Prediction and Prevention Project (DIPP) found that neonatal screening for HLA genetic risk was cost-effective compared with autoantibody screening of the whole population (Hahl et al., 1998). If a safe and effective intervention were to be available, genetic risk-based screening at birth would be economic in populations with a high incidence of T1D.

PREVENTION—WHO?

Candidates for prevention are those with genetic and/or immune markers of risk and, less strictly, those with recent-onset diabetes in whom residual beta-cell function might be preserved or even regenerated. Most studies of people at risk have centered on first-degree relatives of a T1D proband identified as positive for autoantibodies to one or more of the islet autoantigens (pro)insulin, GAD and tyrosine phosphatase-like insulinoma antigen 2 (IA2) (Verge et al., 1996; Bingley et al., 1999; Colman et al., 2000; Harrison, 2001). A critical prerequisite for intervention in asymptomatic individuals is the ability to predict development of clinical disease. This can be achieved by measuring immune and metabolic markers of T1D (Box 75.1). In young, first-degree relatives the 5-year risk of diabetes is of the order <25%, 25–50%, and >50% if they have autoantibodies to one, two, and three islet auto-antigens, respectively; for single autoantibodies, those to insulin (IAA) are the most predictive, and to GAD (GAD Ab) the least predictive. The addition of measures of insulin secretion and insulin action further refine risk. Thus, first-phase insulin response (FPIR) to intravenous glucose at or below the first percentile signifies end-stage preclinical disease. More importantly, among autoantibody-positive relatives with a normal FPIR years before clinical onset, the highest risk is conferred by insulin resistance (Fourlanos et al., 2004). This finding has impor-

Box 75.1
Markers of risk for diabetes in an islet autoantibody-positive relative

- Number of antigen specificities of islet autoantibodies
- Antigen specificity of islet autoantibody
- Level of islet autoantibody
- Age at detection of islet autoantibody
- First-phase insulin response (FPIR) to I.V. glucose
- Insulin resistance, e.g., estimated as HOMA-R
- HLA alleles for risk or protection
- HLA haplotype sharing with proband
- Kinship with proband

Table 75.1 Trials for prevention of type 1 diabetes

	Subjects (n)	Follow-up (months)	Outcome	Reference
Primary prevention				
Non-antigen-specific immune modulation				
Cow's milk elimination (TRIGR)	HLA at-risk infants (2032)	120	Ongoing	Akerblom H.
Gluten elimination	AR (7)	24	No effect on islet antibody levels	Hummel et al., 2002
Secondary prevention				
Nonspecific immune suppression				
Azathioprine (2 mg/kg/day)	RD (24)	12	Higher basal and glucagon-stimulated C-peptide, and more remissions	Harrison et al., 1985
Cyclosporine (7.5 mg/kg/day)	RD (122)	9	More remissions	Feutren et al., 1986
Azathioprine (2 mg/kg/day) + prednisolone (reducing dose)	RD (46)	12	Higher meal-stimulated C-peptide and lower insulin dose	Silverstein et al., 1988
Cyclosporine (20 mg/kg/day)	RD (188)	12	Higher glucagon C-peptide and more remissions. Greater benefit in most recently diagnosed	Canadian-European Randomized Control Trial Group 1988
Azathioprine (2 mg/kg/day)	RD (49)	12	Higher meal-stimulated C-peptide	Cook et al., 1989
Azathioprine & thymostimulin	RD (45)	12	Higher glucagon-stimulated C-peptide and more remissions	Moncada et al., 1990
Cyclosporine	RD (219)	24	Higher meal-stimulated C-peptide and more remissions	Assan et al., 1990
Cyclosporine (10 mg/kg/day, 4 months)	RD (43)	36	No difference in glucagon-stimulated C-peptide, HbA1C, insulin dose	Chase et al., 1990b
Prednisolone (15 mg/day, 8 months); Indomethacin (100 mg/day, 8 months)	RD (25)	24	Lower insulin dose and higher urine C-peptide in prednisolone group	Secchi et al., 1990
Cyclosporine (10 mg/kg/day)	RD (23)	12	Higher meal- but not glucagon- or glucose-stimulated C-peptide. No difference in insulin dose.	Skyler and Rabinovitch, 1992
Anti-CD5/ricin A chain (unblinded)	RD (15)	12	Higher meal-stimulated C-peptide	Skyler et al., 1993
Glucocorticoid	RD (32)	12	Higher glucagon-stimulated C-peptide, but no remissions	Goday et al., 1993
Anti-CD4 + glucocorticoid	RD (12)	12	No difference in insulin dose, or islet antibody titers.	Kohnert et al., 1996
Methotrexate (unblinded)	RD (10)	36	No effect on basal or meal-stimulated C-peptide. Insulin dose higher.	Buckingham and Sandborg 2000
Anti-CD3 (hOKT3) (unblinded)	RD (18)	12	Increase in meal-stimulated C-peptide in first year. IL-10 detected in serum.	Herold et al., 2002
Anti-CD3	RD (80)	48	Ongoing	Gorus F. and Chatenoud L.
Mycophenolate mofetil ± daclizumab (anti-IL-2 receptor) (MMF/DZB)	RD (180)	48	Ongoing	Gottlieb P.A.

Table 75.1 (Continued)

	Subjects (n)	Follow-up (months)	Outcome	Reference
Daclizumab	RD (40)	24	Ongoing	Rodriguez H.
Rituximab (anti-CD20)	RD (?)	?	Pending	Pescovitz M.
IL-2 + rapamycin	RD (?)	?	Pending	Rabinovitch A.
Nonspecific immune stimulation				
BCG vaccine	RD (26)	18	No effect on glucagon-stimulated C-peptide, insulin dose or HbA1C	Elliott et al., 1998
BCG vaccine	RD (94)	24	No effect on mixed meal-stimulated C-peptide, insulin dose or HbA1C	Allen et al., 1999
Q fever vaccine	RD (39)	12	No effect on glucagon-stimulated C-peptide or insulin dose	Schmidli R.
Oral IFN-α	RD (120)	12+	Ongoing	Brod S.A.
Nonspecific immune regulation				
Thymopoietin	RD (32)	6	Lower insulin antibodies and insulin dose. More remissions. No difference in C-peptide or HbA1c.	Giordano et al., 1990
Gammaglobulin	RD (16)	6	Higher basal C-peptide Lower insulin dose, unchanged HbA1c	Panto et al., 1990
Linomide	RD (63)	12	Lower HbA1c, insulin dose. No difference in glucagon-stimulated C-peptide.	Coutant et al., 1998
HSP60 p277 peptide (DiaPep)	RD (35)	10	Decrease in glucagon-stimulated C-peptide and insulin dose in placebo but not treated group.	Raz et al., 2001
1,25-dihydroxy vitamin D3	RD (40)	18	Ongoing open	Walter M. and Ziegler A.
1,25-dihydroxy vitamin D3	RD (20)	18	Ongoing placebo-controlled	Walter M. and Ziegler A.
Antigen-specific immune regulation				
Parenteral insulin (IV vs. SC 2 weeks)	RD (26)	12	Higher meal-stimulated C-peptide, lower HbA1c	Shah et al., 1989
Parenteral insulin	RD (49)	60	Higher glucagon-stimulated C-peptide and improved insulin sensitivity and glycemic control.	Linn et al., 1996
Parenteral insulin (IV vs. SC 2 weeks)	RD (19)	12	Higher meal- and glucagon-stimulated C-peptide and lower HbA1c	Schnell et al., 1997
Parenteral insulin	RD (10)		Higher C-peptide response to oral glucose, HbA1C unchanged	Kobayashi et al., 1996
Parenteral insulin and sulphonylurea (glipizide)	RD (27)	12	Higher basal and glucagon-stimulated C-peptide, more remissions	Selam et al., 1993
Oral insulin	RD (80)	12	No effect on basal C-peptide, HbAIC, insulin dose or insulin antibodies	Pozzilli et al., 2000

Oral insulin	RD (131)	12	No effect on basal, glucagon- or meal-stimulated C-peptide, HbAIC, insulin dose or islet antibody levels	Chaillous et al., 2000
Parenteral insulin	AR (14)	84	Delay in onset of diabetes. No effect on islet antibody levels.	Füchtenbusch et al., 1998
Parenteral insulin (DPT-1)	AR (339)	44	No effect on diabetes development.	Diabetes Prevention Trial-Type 1 Diabetes Study Group, 2002
Intranasal insulin (INIT I)	AR (38)	48	Increased antibody and decreased T-cell responses to insulin. Stable first-phase insulin response to I.V. glucose.	Harrison et al., 2004
Oral insulin (DPT-1)	AR (372)	52	No effect on diabetes development.	www.nih.gov/news/pr/jun2003/niddk-15.htm
Parenteral insulin B chain in incomplete Freund's adjuvant	RD (12)	24	Ongoing	Orban T.
Parenteral insulin B chain 9-23 "altered peptide ligand" NBI-6024-0101 ("Neurocrine")	RD (188)	25	Ongoing	Gottlieb P.A.
Intranasal insulin (DIPP)	AR (200+)	?	Ongoing	Simell O.
Parenteral GAD65 (Diamyd™)	RD (160)	?	Pending	www.diamyd.com
β cell protection				
Nicotinamide	RD (20)	12	Higher glucagon-stimulated C-peptide at 45 days, then decline. No difference in remissions.	Mendola et al., 1989
Nicotinamide	RD (23)	9	Higher basal and glucagon-stimulated C-peptide	Vague et al., 1989
Nicotinamide	RD (35)	12	No difference in basal or glucagon-stimulated C-peptide	Chase et al., 1990a
Nicotinamide	RD (56)	12	Higher glucagon-stimulated C-peptide in subjects >15 years old	Pozzilli et al., 1995
Nicotinamide ± cyclosporine	RD (90)	12	Lower insulin dose. No difference in remissions	Pozzilli et al., 1994
Nicotinamide ± parenteral insulin	RD (34)	12	No difference in glucagon-stimulated C-peptide	Vidal et al., 2000
Nicotinamide versus vitamin E (no control group)	RD (84)	12	No difference in basal or glucagon-stimulated C-peptide, HbA1c or insulin dose	Pozzilli et al., 1997
Nicotinamide (DENIS)	AR (55)	36	No effect on diabetes development	Lampeter et al., 1998
Nicotinamide (ENDIT)	AR (552)		No effect on diabetes development	Philips et al., 2002
Octreotide	RD (20)	12	Higher glucagon-stimulated C-peptide at 6 and 12 months; no difference in HbA1c or insulin dose	Grunt et al., 1994
Diazoxide	RD adults (40)	18	Higher basal C-peptide	Bjork et al., 1996
Diazoxide	RD children (56)		Higher(?) stimulated C-peptide at 12, not 24, months	Bjork et al., 2001
Antioxidants	RD (46)	30	No difference in meal-stimulated C-peptide, insulin dose or HbA1c	Ludvigsson et al., 2001

AR, islet autoantibody-positive first-degree relative; DENIS, Deutsche Nicotinamide Intervention Study; DIPP, Diabetes Prediction and Prevention Project; DPT-1, Diabetes Prevention Trial Type 1; ENDIT, European Nicotinamide Diabetes Intervention Trial; IFN-α, interferon-α; IL-10, interleukin 10; INIT I, Melbourne Intranasal Insulin Trial; RD recently-diagnosed diabetic; TRIGR, trial to reduce IDDM in the genetically at-risk.

tant implications for new preventative approaches (to improve insulin action), as well as for the selection of autoantibody-positive subjects for prevention trials.

Several caveats apply. Approximately 10% of patients have no detectable antibodies at diagnosis. In addition, while first-degree relatives, with shared susceptibility genes and environmental risk factors, have at least a 10-fold higher prevalence of T1D than the background population, they still represent no more than 15% of people diagnosed with T1D. The risks in first-degree relatives cannot be directly extrapolated to the general population because the predictive value of a risk marker reflects the population prevalence of disease, according to Bayes' theorem. The prevalence and predictive value of islet autoantibodies in the general population has not been widely investigated but will be an important issue once effective means of secondary prevention are found. In Finland, which has the highest incidence of T1D in the world, neonatal screening for high-risk HLA class II susceptibility genes can identify over half of those destined to develop T1D (Kimpimäki et al., 2001). Neonatal screening to identify genetically at-risk populations is a basis for primary prevention, but its modest predictive value would only justify an intervention that was simple and safe, for example, diet modification or vaccination.

The case for intervention in asymptomatic, at-risk individuals, earlier when prediction is less accurate as against later or just after the onset of diabetes, hinges on considerations of safety and likely efficacy (Figure 75.1). Some agents, such as potentially toxic immunosuppressive drugs, cannot be given rationally in asymptomatic individuals, many of whom are children, whereas others such as agents that might promote protective immune homeostasis, while relatively safe, are likely to be ineffective in end-stage disease. Prevention should be more effective early in the natural history of preclinical disease, yet at the same time, prediction is less exact. In individuals with recent-onset diabetes the consideration of safety versus efficacy would appear to be less of a dilemma, but the major loss of beta-cell function at this time militates against efficacy. Nevertheless, in NOD mice treatment with anti-T-cell receptor (CD3) monoclonal antibody (mAb) at the onset of diabetes surprisingly resulted in reversal of diabetes (Chatenoud, 2003; see Chapter 76). A subsequent pilot trial of anti-CD3 monoclonal antibody in humans with recent-onset diabetes revealed preservation of residual β-cell function for at least a year but not reversal of diabetes (Herold et al., 2002). Removing the burden of pathogenic, effector T cells at this stage is an important strategy to allow the emergence or induction of protective, regulatory T cells, with potential for β-cell recovery and possible regeneration. Finally, it is important to note that about 10% of adults with diabetes have what appears to be a slowly-progressive form of T1D, associated mainly with GAD Ab, that initially is non-insulin-requiring (Hagopian et al., 1993; Tuomi et al., 1993; Gottsater et al., 1995; Turner et al., 1997). These patients have, on average, higher residual β-cell function at diagnosis than younger patients with classical T1D (Gottsater et al., 1993), implying a wider therapeutic window for secondary prevention spanning the onset of symptoms.

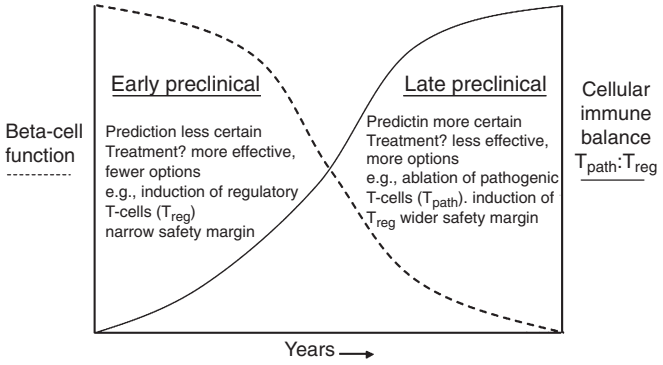


Figure 75.1 Preventing type 1 diabetes: early versus late intervention.

PREVENTION—HOW?

The number of candidate agents that meet scientific and ethical criteria for use in at-risk individuals is limited, and the identification of these individuals is a major logistic exercise. Consequently, the majority of the more than 60 clinical trials for "prevention" of T1D (Table 75.1) have been conducted in people with recent-onset diabetes, beginning with trials of glucocorticoids, azathioprine, and cyclosporine in the 1980s. No intervention, however, has yet demonstrated efficacy in the longer-term. Trials in recent-onset patients (see Table 75.1), including earlier trials of azathioprine and cyclosporine and more recent trials of heat shock protein (HSP) 60 peptide p277 ("DiaPep") vaccine and humanized anti-CD3 mAb, yielded a beneficial effect as judged by clinical remission and/or preservation of β-cell function, but significant differences between control and treatment groups have generally been restricted to the first year after diagnosis. Residual β-cell function is usually measured by the glucagon- or mixed meal-stimulated increase in plasma C-peptide, which is secreted in equimolar proportions to insulin following cleavage of proinsulin in the β cell and is a surrogate for insulin when exogenous insulin or insulin antibodies could interfere with the measurement of endogenous insulin levels. Failure of the "first-year effect" to be sustained could be due to only a transient effect of the agent, or to bias in participant selection. Individuals with recent-onset diabetes must be carefully stratified according to age, HLA gene status, autoimmune

status including number and level of islet autoantibodies, residual β-cell function, and insulin resistance, any of which may influence the natural history of declining beta-cell function after diagnosis (Greenbaum and Harrison, 2003). Greenbaum (2002) noted that the mean age of subjects (22 vs. 15 years, $p < 0.01$) was higher in nine trials yielding an initial positive outcome than in another 10 trials yielding a negative outcome. Furthermore, the proportion of trials reporting a positive outcome has decreased from the 1980s, along with a shift in the primary outcome measure away from mere clinical remission to the more rigorous measure of residual insulin (stimulated C-peptide) secretion. Standardization of trial design is critical and would facilitate comparative analysis of trials. To this end, the Immunology of Diabetes Society (IDS) has published guidelines for prevention trials in recent-onset T1D (Greenbaum and Harrison, 2003).

Not all trials have been based on animal data for efficacy or safety or have incorporated surrogate disease markers. Interventions, particularly in asymptomatic, at-risk individuals, must be safe and should ideally be based on proof-of-concept in animal studies. The primary outcome of trials is to attenuate or prevent diabetes. However, measures of beta-cell function such as the oral glucose tolerance test (OGTT) or the mixed meal tolerance test in subjects with diabetes, or the intravenous glucose tolerance test (IVGTT) to measure FPIR in at-risk subjects, may provide critical interim information on efficacy. How well immune markers, islet autoantibodies, and T-cells reactive to islet autoantigens inform on the nature and outcome of islet pathology is unknown, but these markers should be incorporated into trials in order to find out.

Although humans at high risk for T1D have a relatively restricted set of HLA susceptibility genes they are, in contrast to inbred NOD mice, likely to display significant heterogeneity in response to any therapeutic intervention. Prevention trials in T1D provide an opportunity to gain insight into pharmacogenomics. An example pertinent to the therapeutic application of (pro)insulin as an immune tolerizing agent, e.g., administered via mucosal routes (Harrison et al., 2004) is the *IDDM2* susceptibility locus for human T1D. *IDDM2* maps to a variable number of tandem repeats (VNTR) upstream of the proinsulin gene; long (class III) and short (class I) *VNTR* alleles are associated, respectively, with lower and higher susceptibility to T1D (Bennett et al., 1995). The length of the VNTR correlates with the level of proinsulin gene transcription in the thymus (Pugliese et al., 1997) and in a peripheral population of myeloid cells (Narendran et al., 2004), which in turn could determine the extent of deletion of proinsulin-specific T-cells during their intrathymic development. Thus, the immune response to (pro)insulin, which is likely to be key to understanding beta-cell destruction (Narendran et al., 2003), may be predetermined by *IDDM2*.

PRIMARY PREVENTION

Primary prevention can be targeted at environmental factors that could precipitate islet autoimmunity. The evidence for an etiological role of environment in T1D is persuasive: in discordant identical twin pairs the lifetime risk of diabetes for the nondiabetic twin is 50–70% (Gale et al., 2001; Redondo et al., 2001); the incidence of T1D has increased over the last 15 years in many countries, particularly in the very young (Green and Patterson, 2001; Gale, 2002); in both hemispheres of the world an effect for infectious agents is suggested by a peak in spring–summer births of children who will develop T1D (Laron, 2000; Willis et al., 2002) and a peak in winter in diagnosis of T1D (Douglas et al., 1999; Green and Patterson, 2001; Willis et al., 2002); Type 1 diabetes occurred after exposure to rubella virus *in utero* (Forrest et al., 1971) and to the toxin "Vacor" (Karam et al., 1980). Interest in specific environmental agents is centered mostly on dietary components and viruses.

Diet and the Role of the Intestinal Mucosa

The hypothesis that early exposure of the infant to cow's milk and/or the lack of breastfeeding predisposes to T1D dates from the 1980s. It has important implications but remains controversial because the evidence on which it is based is indirect and open to criticism (Harrison and Honeyman, 1999). Two meta-analyses of multiple studies in which diabetes prevalence was associated retrospectively with infant feeding revealed only a marginal increase in relative risk (Gerstein, 1994; Norris and Scott, 1996). In the Denver-based Diabetes Autoimmunity Study in the Young (DAISY), infant feeding patterns retrospectively analyzed up to 6 months of age were not found to be related to the development of islet autoantibodies up to 7 years of age (Norris et al., 1996). In the Australian BabyDiab Study (Couper et al., 1999) and the German BabyDiab Study (Hummel et al., 2000), there was no association between infant feeding patterns and the development of islet autoantibodies. Nevertheless, to answer whether cow's milk exposure is a risk, the Trial to Reduce IDDM in the Genetically at-Risk (TRIGR), a multicountry study has been initiated. Newborns with a T1D first-degree relative and with HLA risk alleles, initially exclusively breastfed, are being randomized to either a casein hydrolysate formula ("Neutramigen") or a conventional cow's milk-based formula until at least 6 months of age, and will be followed for up to 10 years. The "cow's milk hypothesis" depended in part on reports that children with T1D have an increased immune response to cow's milk proteins (reviewed in Harrison and Honeyman, 1999). However, scrutiny of the literature indicates that, rather than being disease-specific, this reflects genetic predisposition to increased immunity to dietary proteins in general, associated with the HLA haplotype A1-B8-

DR3-DQ2, which also predisposes to celiac disease and selective IgA deficiency (Harrison and Honeyman, 1999). In the two rodent models of T1D, the NOD mouse and the BioBreeding (BB) rat, evidence has emerged that plant rather than milk proteins promote the development of islet autoimmunity (Beales et al., 2002). Antibodies to wheat gluten proteins are found in a proportion of T1D patients at the time of diagnosis (MacFarlane et al., 2002) and in one report the prevalence of autoimmune diseases, including T1D, in individuals with celiac disease was related to the duration of exposure to gluten (Ventura et al., 1999). However, in a small, unblinded pilot study 1 year of gluten-free diet had no effect on the natural history of islet autoimmunity in offspring of parents with T1D (Hummel et al., 2002).

We sought to reframe the cow's milk hypothesis around mucosal immune function in T1D (Harrison and Honeyman, 1999). The essential role of a normal mucosal immune system in maintaining immune homeostasis is illustrated by the effect of a germ-free versus conventional "dirty" environment on diabetes incidence in NOD mice. The incidence of spontaneous diabetes in NOD mice differs greatly between colonies around the world and appears to be inversely correlated with exposure to microbial infection (Pozzilli et al., 1993). The high incidence of diabetes in NOD mice housed under pathogen-free conditions is reduced by conventional conditions of housing and feeding (Suzuki, 1987; Funda et al., 2005). Under such conditions, bacterial colonization of the intestine is accompanied by an increase in number of intra-epithelial lymphocytes (Imaoka et al., 1996) and maturation of mucosal immune function (Kawaguchi-Miyashita et al., 1996). The increase in recent years in the prevalence of autoimmune and allergic disorders in the first world has been attributed to "clean living" conditions—the "hygiene hypothesis" (Strachan, 1989; Holt, 1994). Supplementary to the effects of microbial environment, breast milk contains growth factors, cytokines, and other immunomodulatory agents that promote functional maturation of the intestinal mucosa and mucosal immune system. Breast milk also contains endogenous insulin (Shehadeh et al., 2001), which could induce "oral tolerance" to insulin and so protect against the development of T1D, as described below. Thus, rather than cow's milk promoting T1D, human milk could be protective, by immune maturational effects and/or by delivering human insulin to the "tolerogenic" mucosal immune system.

Epidemiologic evidence has emerged for a protective role of vitamin D, a steroid with pleiotropic immune and non-immune effects. Vitamin D is synthesized in the skin after ultraviolet radiation exposure and the only other major source is diet supplementation. The recommended daily intake of vitamin D has fallen and increased awareness of skin cancer has reduced sun exposure. Children in Finland who received vitamin D supplementation had a lower incidence of T1D (Hypponen et al., 2001), and children in Norway had a lower incidence of T1D if their mothers took cod liver oil (a source of vitamin D) during pregnancy (Stene et al., 2000). In a multinational European case–control study, the odds ratio for T1D was significantly reduced in children given vitamin D (EURODIAB Substudy 2 Study Group, 1999). Trials of vitamin D supplementation are in progress in subjects with recent-onset T1D.

Viruses

Viral mechanisms could include direct infection of β cells, infection of the exocrine pancreas with bystander death of β cells, molecular mimicry between T-cell epitopes in a virus and those in β-cell autoantigens, superantigen stimulation of T cells, or combinations of these. If a particular virus was clearly implicated, vaccination of children early in life, if generally safe, would be protective, but with disease induction by molecular mimicry being a possible caveat.

The first virus to be associated with T1D was rubella (Forrest et al., 1971). Children with congenital rubella born to mothers who contracted rubella early in pregnancy had evidence of infection in the brain, pancreas, and other tissues, and 20% developed insulin-dependent diabetes (Menser et al., 1978). Subsequently, almost twice this proportion of such children were reported to develop islet-cell antibodies (Ginsberg-Fellner et al., 1985). Children with congenital rubella and ensuing diabetes were noted to have a higher frequency of the T1D susceptibility haplotype HLA-A1-B8-(DR3-DQ2) (Menser et al., 1974). More recently, evidence for molecular mimicry between rubella and GAD has been reported (Ou et al., 2000). Rubella vaccine has virtually eliminated congenital rubella and may represent the first example of primary prevention of T1D. Clearly, many other environmental factors are involved, because the incidence of T1D has continued to increase in "first world" countries. Despite the report of rubella-GAD mimicry (Ou et al., 2000), strengthened by the finding of islet autoantibodies in children infected with rubella virus (Lindberg et al., 1999), there is no evidence from multiple studies (Hummel et al., 2000; DeStefano et al., 2001) that the vaccination with attenuated rubella virus is associated with islet autoimmunity (see Chapter 24). Only 4% of girls receiving live attenuated rubella vaccine developed islet-cell antibodies, and these were transient and of low titer (Bodansky et al., 1990).

Mothers infected during pregnancy with some enteroviruses, including coxsackie and echoviruses were more likely to deliver children who developed T1D early in life (Dahlquist et al., 1995; Hyoty et al., 1995), but more recent studies have negated this (Füchtenbusch et al., 2001; Viskari et al., 2002). Enterovirus RNA was isolated from peripheral blood mononuclear cells of 50% of recent-onset

T1D cases in Scandinavia, and 26% of siblings and 0% of age- and sex-matched controls (Yin et al., 2002). In Germany and Australia, coxsackie B virus infections were noted to be more common in infants and children with preclinical T1D than in controls but were not coincident with the detection of islet autoantibodies (Füchtenbusch et al., 2001; Honeyman M.C., in preparation). Evidence for induction of T1D by mimicry by enteroviruses remains unconvincing (Marttila et al., 2001; Roep et al., 2002). The circumstantial nature of their association with T1D makes enteroviruses less promising as a vaccine target. Furthermore, there are 72 recognized serotypes and many thousands of enterovirus strain variants, and no vaccines except for polio virus yet exist. However, if diabetes-associated strains such as coxsackie B3, B4, or echo 6 were to be clearly implicated, they would become candidate vaccines for genetically at-risk children.

Mumps virus epidemics have been associated with onset of T1D 2–4 years later (Khakpour and Nik-Akhtar, 1975; Hyoty et al., 1988). Intriguingly, the introduction of a mumps vaccine was associated with a plateau in the rising incidence of T1D in Finland (Hyoty et al., 1993), but this was temporary, and mumps vaccination is unlikely to be of value for preventing T1D. Cytomegalovirus (CMV) can damage β cells (Jenson et al., 1980) and contains a peptide sequence mimic of a T-cell epitope in GAD recognized by a T-cell clone from a subject with stiff person syndrome (Roep et al., 2002). However, evidence for CMV infection in T1D is weak (Hiltunen et al., 1995). Furthermore, although CMV is now a major cause of congenital defects, development of a vaccine is problematic because of latency and possible carcinogenicity of an attenuated virus.

The discovery of strong sequence similarities between T-cell epitopes in IA2 and GAD and the VP7 protein of rotavirus in islet antibody-positive relatives (Honeyman et al., 1998) suggested that molecular mimicry with rotavirus might precipitate islet autoimmunity. Rotavirus epidemics occur each winter particularly in kindergartens and are the most common cause of gastroenteritis in children. Rotavirus provides a profound inflammatory stimulus to the gut until sufficient IgA develops, by about 5 years of age. In the Australian BabyDiab Study, rotavirus infections were temporally associated with increases in islet autoantibodies in 24 children before they developed diabetes (Honeyman et al., 2000). It was then shown that rotavirus could infect β cells in islets from mice, pigs, and monkeys (Coulson et al., 2002). Recently, it has been shown (Honeyman M.C., in preparation) that both a majority of children at-risk for T1D, and HLA-matched controls, have T-cell responses to the similar peptide sequences in rotavirus VP7, and IA2, and GAD. This indicates that while ubiquitous rotavirus infections may drive cross-reactive immunity to islet autoantigens, this alone is not diabetogenic. Mimicry could, however, complement and sustain the immune response to direct infection of β cells. Rotavirus vaccines have been developed, but were withdrawn because of safety concerns following cases of intestinal intussusception. Whether a rotavirus vaccine would alter the incidence of T1D is unanswerable without vaccination of the population. The outcomes of infections in infants may be complex. Infection by rotavirus and other diabetogenic viruses in a non-inflammatory context, such as during breastfeeding, could conceivably be protective against the development of T1D.

SECONDARY PREVENTION

The approach to prevention that comes closest to the ideal for early preclinical disease, or even primary prevention, is autoantigen-specific immune tolerance induction. This involves, paradoxically, administration of an autoantigen to induce protective autoimmunity (also referred to as "negative vaccination"). The concept is that self-antigen-specific immunoregulatory mechanisms are physiological and can be boosted or restored to prevent pathological autoimmunity. Strategies to achieve this include the delivery of autoantigen by a "tolerogenic" route (e.g., mucosal), cell type (e.g., resting dendritic cell), mode (e.g., with blockade of costimulation molecules), or form (e.g., as an "altered peptide ligand"), all of which can prevent or suppress experimental autoimmune diseases in rodents (Faria and Weiner, 1999; Harrison and Hafler, 2000; Krause et al., 2000). They operate either by deleting and/or inducing anergy in potentially pathogenic effector T cells or by inducing regulatory T cells (Treg). Autoreactive T cells that are driven strongly by antigen may undergo apoptotic cell death and deletion, while those that survive or respond "partially" may become anergic (von Herrath and Harrison, 2003). Of potential importance clinically is the ability of Treg to exert antigen-nonspecific "bystander suppression" in response to specific antigen (Faria and Weiner, 1999; Harrison and Hafler, 2000; von Herrath and Harrison, 2003). Thus, some Treg respond to antigen by secreting anti-inflammatory cytokines such as interleukin 10 (IL-10) or transforming growth factor-β (TGF-β) that may then impair the ability of dendritic cells to elicit T helper 1 (Th1) T-cell or cytotoxic T-cell responses to any antigen locally at the site of the lesion or in the draining lymph nodes. Bystander suppression obviates the need to know if the autoantigen used to induce tolerance is necessarily the major or primary pathogenic autoantigen.

Mucosa-Mediated Antigen-Specific Therapy

Numerous experiments have shown that it is possible to partially protect NOD mice from diabetes by the mucosal administration of islet autoantigen. Zhang et al. (1991) initially reported protection after oral porcine insulin. Bergerot

et al. (1994) then showed that human insulin induced $CD4^+$ Treg that could transfer protection to naïve mice. Protection following oral insulin was later found to be associated with decreased expression of interferon-γ (IFN-γ)-secreting Th1 T cells in the pancreas and pancreatic lymph nodes (Hancock et al., 1995; Ploix et al., 1998). Oral insulin-induced CD4 Treg have also been shown to prevent immune-mediated diabetes induced by lymphocytic choriomeningitis virus (LCMV) infection of mice expressing the viral nucleoprotein of LCMV under control of the rat insulin promoter in their β cells (Homann et al., 1999). The majority of T cells in the islets of oral insulin-treated mice without diabetes secreted the Th2 (IL-4, IL-10) and Th3 (TGF-β) cytokines, in contrast to IFN-γ-secreting Th1 cells in islets of mice developing diabetes. The protective effect of oral insulin was enhanced by simultaneous feeding with IL-10 (Slavin et al., 2001), bacterial component OM-89 (Bellmann et al., 1997; Hartmann et al., 1997) or schistosome egg antigen (Maron et al., 1998), all of which promote Th2 responses. Fusion of insulin to cholera toxin B-subunit (CTB) significantly improved the ability of oral insulin to prevent diabetes (Bergerot et al., 1997). Oral CTB-insulin conjugates in NOD mice induced a shift from a Th1 to a Th2 immunity associated with the induction of regulatory $CD4^+$ T cells (Ploix et al., 1999). NOD mice were protected from diabetes by feeding potatoes that transgenically express CTB-insulin conjugates (Arakawa et al., 1998). Oral GAD has also been shown to suppress diabetes development in NOD mice (Ma et al., 1997). Although it is generally believed that neonates are less susceptible to mucosal tolerance induction, oral administration of insulin, insulin B-chain, or GAD peptide during the neonatal period still suppressed diabetes development in NOD mice (Maron et al., 2001). This suggests that mucosal delivery of islet autoantigen (e.g., in milk) could be used to treat very young infants at risk of developing T1D.

Protection against diabetes in NOD mice can also be achieved by nasorespiratory administration of islet autoantigens. This route of direct delivery to the mucosa avoids antigen degradation. When insulin was administered as an aerosol to NOD mice at 8 weeks of age, after the onset of subclinical disease, insulitis and diabetes incidence were both significantly reduced (Harrison et al., 1996). Aerosol insulin induced novel anti-diabetic CD8 γδ T cells that suppressed the adoptive transfer of diabetes to nondiabetic mice by T cells of diabetic mice. The type of Treg induced by (pro)insulin depends on the route and form of antigen. Nasorespiratory insulin, nondegraded and conformationally intact, induces CD8 γδ Treg, whereas oral insulin degraded to peptides, or intranasal or oral (pro)insulin peptides, induce CD4 Treg (Hänninen and Harrison, 2000; Martinez et al., 2003). Intranasal administration of the insulin B-chain peptide (amino acids 9–23), an epitope recognized islet-infiltrating $CD4^+$ T-cell clones capable of adoptively transferring diabetes to naïve mice, induced $CD4^+$ Treg and protected NOD mice from diabetes (Daniel and Wegmann, 1996). A peptide that spans the B–C chain junction in proinsulin also induces $CD4^+$ Treg after intranasal administration (Martinez et al., 2003). This peptide, like insulin B9-23, binds to the NOD mouse class II MHC, I-A^{g7} (Harrison et al., 1997) and is a T-cell epitope in NOD mice (Chen et al., 2001) and humans at risk for T1D (Rudy et al., 1995). T-cell epitope peptides from GAD administered intranasally were also protective, and associated with the induction of regulatory $CD4^+$ Treg and with reduced IFN-γ responses to GAD (Tian et al., 1996). Islet autoantigen proteins or peptides that induce Treg are potential "vaccines" for intranasal delivery to prevent T1D in humans, but this promise remains to be fulfilled (see below).

Hematopoietic Stem Cell-Mediated Antigen-Specific Therapy

Autoimmune diabetes in the NOD mouse can be transferred to non-susceptible NOD F1 recipients by allogeneic bone marrow transplantation (BMT) (Serreze et al., 1988; Wicker et al., 1988; LaFace and Peck, 1989). This demonstrates that the mechanisms of disease development operate within the hematopoietic compartment and implies that autoimmune diabetes may be prevented by manipulating hematopoietic cells. Indeed, replacement of the NOD mouse hematopoietic compartment with that from diabetes-resistant mouse strains by BMT blocks development of diabetes (Ikehara et al., 1985; LaFace and Peck, 1989; Beilhack et al., 2003). However, complete replacement is not required as generation of mixed-allogeneic hematopoietic chimeras is equally effective (Li et al., 1996; Kaufman et al., 1997; Mathieu et al., 1997). Autoimmune diabetes is prevented in mixed chimeras most likely by the introduction of thymic and/or peripheral tolerance-inducing antigen-presenting cells that express diabetes-nonsusceptible MHC molecules. This would be analogous to the protection afforded by transgenic introduction into NOD mice of diabetes-resistant MHC molecules such as I-E and alternative I-A molecules (Nishimoto et al., 1987; Lund et al., 1990; Singer et al., 1993; 1998), which may act by enhancing thymic selection of T cells (Schmidt et al., 1997; Luhder et al., 1998) or by inducing Treg (Singer et al., 1993). Induction of Treg, however, does not appear to account for diabetes prevention in mixed chimeras (Mathieu et al., 1997). Introduction of other genes (non-MHC for diabetes resistance) into the hematopoietic compartment could also contribute.

Allogeneic or mixed-allogeneic BMT strategies are being trialled for severe autoimmune diseases (Burt et al., 2002). However, they are unlikely to be suitable for T1D due to the requirement for cytotoxic conditioning of the host, and the risks of graft rejection (Castro-Malaspina et al., 2002) and

graft-versus-host disease (Ratanatharathorn et al., 2001). A much safer approach would be to use autologous, genetically-engineered hematopoietic stem cells to introduce molecules into the hematopoietic compartment that could prevent autoimmune disease. Adopting this approach, we found that transfer to young, irradiated NOD mice of 10^3 syngeneic hematopoietic stem cells that encoded proinsulin expression in antigen-presenting cell progeny totally prevented diabetes (Steptoe et al., 2003). This dramatic effect appears to depend on proinsulin expression by "resting" immature dendritic cells (Steptoe et al., 2004). The application of this "cell therapy" to humans faces two obstacles related to safety—introducing genes into stem cells without the risk of oncogenesis and avoiding toxic conditioning regimens in the host.

Trials of Islet Autoantigen-Specific Therapy in Humans

The large multicenter Diabetes Prevention Trial 1 (DPT-1) was launched in the United States in 1994 to determine whether antigen-specific therapy with either systemic or oral insulin would delay or prevent the onset of diabetes in at-risk relatives. Previously, intensive systemic insulin therapy had been reported to prolong the "honeymoon phase" after diagnosis (Shah et al., 1989), and a pilot study of prophylactic systemic insulin had suggested that this approach might be of benefit in at-risk relatives (Keller et al., 1993). In DPT-1, low-dose systemic insulin (annual intravenous insulin infusions and daily subcutaneous injections) was given to a high-risk group of relatives (>50% risk of diabetes over 5 years), matched with an untreated but closely monitored control group. Unfortunately, this treatment had no effect on diabetes incidence (Diabetes Prevention Trial-Type 1 Diabetes Study Group, 2002). The more recent randomized control DPT-1 of oral insulin recruited relatives with a 25–50% 5-year risk of developing diabetes, but again the primary analysis revealed no effect (*http://www.nih.gov/news/pr/jun2003/niddk-15.htm*). Two trials of oral insulin (up to 7.5 mg daily for 12 months) in recently-diagnosed patients showed no protective effect on residual β-cell function (Chaillous et al., 2000; Pozzilli et al., 2000).

Why have these trials failed? There are probably several answers: selection of subjects with end-stage disease; inadequate dose or bioavailability of the agent, possibly related to the route of administration; and co-induction of pathogenic T-cells. Regarding stage of disease, induction of antigen-specific immunoregulation, without concomitant inactivation or deletion of pathogenic effector cells, is likely to be relatively ineffective in end-stage disease. If a balance between pathogenic and protective T cells determines clinical outcome, then antigen-specific immunoregulation should be most effective in early preclinical disease; later, it might be complementary to a limited course of treatment aimed at reducing the burden of pathogenic effector T cells. Regarding route of administration, oral delivery may not be optimal for mucosa-mediated tolerance, because proteins are generally degraded after ingestion, whereas, even in the case of a small peptide, responses are seen after nasorespiratory but not oral delivery (Metzler and Wraith, 1993). In the mouse, nasal delivery of the model antigen, ovalbumin, elicited antigen-specific T-cell responses in cervical, mediastinal, and mesenteric mucosal lymph nodes, whereas oral delivery elicited responses only in the mesenteric nodes (Hänninen et al., 2001). Moreover, irrespective of route, mucosal delivery of ovalbumin was a "double-edged sword," inducing both tolerance and pathogenic $CD8^+$ cytotoxic T cells (Hänninen et al., 2002). To achieve a clinical effect from tolerance induction, it was necessary to block induction of pathogenic T cells by transient co-administration of systemic anti-CD40 ligand monoclonal antibody (Hänninen et al., 2002). Whether mucosal insulin is also a double-edged sword is unknown. However, the proinsulin B–C chain peptide that induces $CD4^+$ Treg in NOD mice is a "combitope" of $CD4^+$ ($I\text{-}A^{g7}$-restricted) and $CD8^+$ (K^d-restricted) T-cell epitopes, and is significantly more protective after intranasal administration if the C-terminal p9 anchor residue for binding to K^d is either deleted or mutated (Martinez et al., 2003). These findings underscore the necessity to evaluate immune responses to mucosal autoantigens in human trials.

The rationale for the DPT-1 trial of oral insulin was the induction of "oral tolerance" in the NOD mouse. In the mouse, milligrams of gavaged insulin were required to induce "regulatory" anti-diabetogenic $CD4^+$ T cells and partially suppressed development of diabetes (Zhang et al., 1991; Bergerot et al., 1994). Yet, the daily dose of insulin given orally in the human trials was only 7.5 mg, which, on a body-weight basis, equates to only a few micrograms in the mouse. Oral insulin is degraded and its bioavailability to induce mucosa-mediated immune tolerance in the upper small intestine is unpredictable. Despite this, dose-ranging studies in humans to determine a bioavailable dose of oral insulin were not undertaken, raising the question whether such a major trial was judicious without reassurance that the dose of insulin used would have had an immune effect. Insulin antibodies are a readout of bioavailability observed after aerosol insulin in NOD mice (Harrison et al., 1996) and intranasal insulin in humans (Harrison et al., 2004). Irrespective of whether such antibodies are a marker of immunoprotection, their induction demonstrates that the dose used was bioactive. Because insulin autoantibodies are a risk marker for T1D, the increase in insulin antibodies after administration of nasorespiratory insulin would seem to conflict with immunoprotection. However, this observation, together with the concomitant decrease in the T-cell-proliferative response to insulin after intranasal insulin

(Harrison et al., 2004), is entirely consistent with mucosal tolerance. Reciprocal cellular and humoral immune responses, originally termed "immune deviation" by Parish (1996), and later popularized in terms of the Th1/Th2 paradigm, are a feature of the earliest descriptions of mucosal tolerance. This was confirmed in landmark but overlooked human studies with the experimental antigen keyhole limpet hemocyanin (KLH). When KLH was administered orally (Husby et al., 1994) or nasally (Waldo et al., 1994) to human volunteers, subsequent antibody and T-cell responses to systemic immunization with KLH increased and decreased, respectively.

Enthusiasm to translate mucosal tolerance from rodents to humans has been tempered by failure to show clinical benefit not only of oral insulin but also of oral myelin basic protein in multiple sclerosis (Weiner et al., 1993) and oral collagen in rheumatoid arthritis (Trentham et al., 1993; McKown et al., 1999). As suggested, these failures could be due to an inability of mucosal tolerance to counteract pathogenic T cells in end-stage autoimmune disease or to the coinduction of a pathogenic immune response. In addition, as with the oral insulin trials, none of these trials reported an immune effect of the oral antigen. Without this evidence, it is not clear that the dose used was bioavailable and immunoactive. Therefore, major trials of mucosal antigens in future should not be undertaken without fore-knowledge that the dose is at least bioactive. At the same time, there is an urgent need for the measurement of potentially relevant biomarkers, particularly pro-inflammatory (potentially pathogenic) and anti-inflammatory (regulatory) T cells, to be rigorously standardized and incorporated into trials.

The Melbourne intranasal insulin trial I (INIT I) (Harrison et al., 2004) was a randomized controlled crossover pilot trial of intranasal insulin vaccine in young T1D relatives (median age 10.8 years; n = 38) with islet autoantibodies. Two 400 μg doses of insulin per nostril were self-administered daily for 10 days, then on two consecutive days each weekend, for 6 months. The aim was to determine if intranasal insulin was safe and would induce changes in surrogate immune and metabolic markers consistent with an immunoprotective effect. No local or systemic adverse effects were observed. Diabetes developed in 12 subjects who had negligible β-cell function at entry, after a median of 1.1 years. β-cell function in the remaining 26, the majority of whom had antibodies to 2 or 3 islet autoantigens and FPIR outside the first percentile at entry, generally remained stable over a median follow-up of 3.0 years. Intranasal insulin was associated with an increase in anti-insulin antibody and a decrease in T-cell responses to (denatured) insulin. This trial identified a dose of intranasal insulin that was safe and which induced changes in immunity to insulin as previously reported in NOD mice. Because INIT I was a crossover trial in which all subjects received treatment with intranasal insulin for 6 months, it could not determine if intranasal insulin prevents loss of β-cell function and diabetes. This will be answered in a follow-up trial.

In adults with autoimmune diabetes there has been only one published clinical trial of prophylactic insulin. This small study by Kobayashi et al. (1996) demonstrated that low doses of subcutaneous insulin improved the C-peptide response to oral glucose and decreased HbA1c at 6 and 12 months after diagnosis, compared to treatment with oral sulfonylurea drugs. Whether the beneficial effect of insulin treatment was due to metabolic or immunoregulatory mechanisms is not known. A randomized controlled-phase 2 trial of intranasal insulin (INIT III) in adults with recent-onset autoimmune diabetes is nearing completion in Melbourne. A phase 2 trial of subcutaneous GAD, sponsored by Diamyd Medical AB, is also underway in adults with recent-onset T1D.

PREVENTION OF TYPE 1 DIABETES IN THE THIRD MILLENNIUM

The rate at which knowledge about T1D accumulated at the end of the second millennium provides strong hope that the disease will be preventable early in the third. This will occur only incrementally and will involve a combination of interventions suited to genotype, risk level and disease stage. Environmental agents that precipitate or exacerbate islet autoimmunity are likely to be ubiquitous and, therefore, a single intervention (e.g., vaccination against a virus) is unlikely to be the ultimate solution. Antigen-specific immunotherapy applied rationally to enhance natural mechanisms of protective immune homeostasis promises to be a safe and widely-applicable intervention in early subclinical disease. In end-stage disease, combinatorial treatment that not only enhances immune homeostasis but suppresses pathogenic immunity is likely to be required. Advances in manipulating gene expression safely in stem cells will open new avenues for antigen-specific tolerogenic therapy. T1D is likely to be the first preventable autoimmune disease. This will facilitate replacement or regeneration of pancreatic islet β cells in people with established diabetes. The lessons learnt from the preclinical diagnosis, prediction and prevention of T1D should be applicable to the prevention of other autoimmune diseases.

Acknowledgments

The author is supported by the National Health and Medical Research Council of Australia and by a Center Program Grant from the Juvenile Diabetes Research Foundation. He is indebted to his colleagues and students in clinical medicine and laboratory research, and to people and families with diabetes, for much support and inspiration. Catherine McLean provided excellent administrative assistance.

References

Adorini, L., Gregori, S., and Harrison, L.C. (2002). Understanding autoimmune diabetes: insights from mouse models. *Trends Mol. Med. 8,* 31–38.

Allen, H.F., Klingensmith, G.J., Jensen, P., Simoes, E., Hayward, A., and Chase, H.P. (1999). Effect of Bacillus Calmette-Guerin vaccination on new-onset type 1 diabetes. A randomized clinical study. *Diabetes Care 22,* 1703–1707.

Arakawa, T., Yu, J., Chong, D.K., Hough, J., Engen, P.C., and Langridge, W.H. (1998). A plant-based cholera toxin B subunit-insulin fusion protein protects against the development of autoimmune diabetes. *Nat. Biotechnol. 16,* 934–938.

Assan, R., Feutren, G., Sirmai, J., Laborie, C., Boitard, C., Vexiau, P., Du Rostu, H., Rodier, M., Figoni, M., and Vague, P. (1990). Plasma C-peptide levels and clinical remissions in recent-onset type I diabetic patients treated with cyclosporin A and insulin. *Diabetes 39,* 768–774.

Atkinson, M.A. and Leiter, E.H. (1999). The NOD mouse model of type 1 diabetes: as good as it gets? *Nat. Med. 5,* 601–604.

Beales, P.E., Elliott, R.B., Flohe, S., Hill, J.P., Kolb, H., Pozzilli, P., Wang, G.S., Wasmuth, H., and Scott, F.W. (2002). A multi-centre, blinded international trial of the effect of A(1) and A(2) beta-casein variants on diabetes incidence in two rodent models of spontaneous Type I diabetes. *Diabetologia 45,* 1240–1246.

Beilhack, G.F., Scheffold, Y.C., Weissman, I.L., Taylor, C., Jerabek, L., Burge, M.J., Masek, M.A., and Shizuru, J.A. (2003). Purified allogeneic hematopoietic stem cell transplantation blocks diabetes pathogenesis in NOD mice. *Diabetes 52,* 59–68.

Bellmann, K., Kolb, H., Hartmann, B., Rothe, H., Rowsell, P., Rastegar, S., Burghardt, K., and Scott, F.W. (1997). Intervention in autoimmune diabetes by targeting the gut immune system. *Int. J. Immunopharmacol. 19,* 573–577.

Bennett, S.T., Lucassen, A.M., Gough, S.C., Powell, E.E., Undlien, D.E., Pritchard, L.E., Merriman, M.E., Kawaguchi, Y., Dronsfield, M.J., and Pociot, F. (1995). Susceptibility to human type 1 diabetes at IDDM2 is determined by tandem repeat variation at the insulin gene minisatellite locus. *Nat. Genet. 9,* 284–292.

Bergerot, I., Fabien, N., Maguer, V., and Thivolet, C. (1994). Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes. *J. Autoimmunity 7,* 655–663.

Bergerot, I., Ploix, C., Petersen, J., Moulin, V., Rask, C., Fabien, N., Lindblad, M., Mayer, A., Czerkinsky, C., Holmgren, J., and Thivolet, C. (1997). A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc. Natl. Acad. Sci. U. S. A. 94,* 4610–4614.

Bingley, P.J., Williams, A.J., and Gale, E.A. (1999). Optimized autoantibody-based risk assessment in family members. Implications for future intervention trials. *Diabetes Care 22,* 1796–1801.

Bjork, E., Berne, C., Kampe, O., Wibell, L., Oskarsson, P., and Karlsson, F.A. (1996). Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. *Diabetes 45,* 1427–1430.

Bjork, E., Ortquist, E., Wallensteen, M., Ludvigsson, J., Aman, J., Johansson, C., Forsander, G., Blom, L., Berglund, L., Persson, B., Berne, C., and Karlsson, A.F. (2001). Diazoxide treatment at onset in childhood type 1 diabetes. *Diabetes 50,* A90.

Bodansky, H.J., Dean, B.M., Grant, P.J., McNally, J., Schweiger, M.S., Hambling, M.H., Bottazzo, G.F. and Wales, J.K. (1990). Does exposure to rubella virus generate endocrine autoimmunity? *Diabet. Med. 7,* 611–614.

Buckingham, B.A. and Sandborg, C.I. (2000). A randomized trial of methotrexate in newly diagnosed patients with type 1 diabetes mellitus. *Clin. Immunol. 96,* 86–90.

Burt, R.K., Slavin, S., Burns, W.H., and Marmont, A.M. (2002). Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: getting closer to a cure? *Blood 99,* 768–784.

Canadian-European Randomized Control Trial Group (1988). Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes 37,* 1574–1582.

Castro-Malaspina, H., Harris, R.E., Gajewski, J., Ramsay, N., Collins, R., Dharan, B., King, R., and Deeg, H.J. (2002). Unrelated donor marrow transplantation for myelodysplastic syndromes: outcome analysis in 510 transplants facilitated by the National Marrow Donor Program. *Blood 99,* 1943–1951.

Chaillous, L., Lefevre, H., Thivolet, C., Boitard, C., Lahlou, N., Atlan-Gepner, C., Bouhanick, B., Mogenet, A., Nicolino, M., Carel, J.C., Lecomte, P., Marechaud, R., Bougneres, P., Charbonnel, B., and Sai, P. (2000). Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. Diabete Insuline Orale group. *Lancet 356,* 545–549.

Chase, H.P., Butler-Simon, N., Garg, S., McDuffie, M., Hoops, S.L., and O'Brien, D. (1990a). A trial of nicotinamide in newly diagnosed patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia 33,* 444–446.

Chase, H.P., Butler-Simon, N., Garg, S.K., Hayward, A., Klingensmith, G.J., Hamman, R.F., and O'Brien, D. (1990b). Cyclosporine A for the treatment of new-onset insulin-dependent diabetes mellitus. *Pediatrics 85,* 241–245.

Chatenoud, L. (2003). CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol. 3,* 123–132.

Chen, W., Bergerot, I., Elliott, J.F., Harrison, L.C., and Delovitch, T.L. (2001). Evidence that a peptide spanning the B-C junction of proinsulin is a primary autoantigen epitope in the pathogenesis of type 1 diabetes. *J. Immunol. 167,* 4926–4935.

Colman, P.G., McNair, P., Margetts, H., Schmidli, R., Werther, G., Alford, F., Ward, G., Tait, B., Honeyman, M.C., and Harrison, L.C. (1998). The Melbourne Pre-Diabetes Study: screening for pre-clinical insulin-dependent diabetes in first-degree relatives of probands. *Med. J. Aust. 169,* 81–84.

Colman, P.G., Steele, C., Couper, J.J., Beresford, S.J., Powell, T., Kewming, K., Pollard, A., Gellert, S., Tait, B., Honeyman, M., and Harrison, L.C. (2000). Islet autoimmunity in infants with a Type I diabetic relative is common but is frequently restricted to one autoantibody. *Diabetologia 43,* 203–209.

Cook, J.J., Hudson, I., Harrison, L.C., Dean, B., Colman, P.G., Werther, G.A., Warne, G.L., and Court, J.M. (1989). A double-blind controlled trial of azathioprine in children with newly-diagnosed type 1 diabetes. *Diabetes 38,* 779–783.

Coulson, B.S., Witterick, P.D., Tan, Y., Hewish, M.J., Mountford, J.N., Harrison, L.C., and Honeyman, M.C. (2002). Growth of rotaviruses in primary pancreatic cells. *J. Virol. 76,* 9537–9544.

Couper, J.J., Steele, C., Beresford, S., Powell, T., McCaul, K., Pollard, A., Gellert, S., Tait, B., Harrison, L.C., and Colman, P.G. (1999). Lack of association between duration of breast-feeding or introduction of cow's milk and development of islet autoimmunity. *Diabetes 48,* 2145–2149.

Coutant, R., Landais, P., Rosilio, M., Johnsen, C., Lahlou, N., Chatelain, P., Carel, J.C., Ludvigsson, J., Boitard, C., and Bougneres, P.F. (1998). Low dose linomide in Type I juvenile diabetes of recent onset: a randomised placebo-controlled double blind trial. *Diabetologia 41,* 1040–1046.

Dahlquist, G., Frisk, G., Ivarsson, S.A., Svanberg, L., Forsgren, M., and Diderholm, H. (1995). Indications that maternal coxsackie B virus infection during pregnancy is a risk factor for childhood-onset IDDM. *Diabetologia 38,* 1371–1373.

Daniel, D. and Wegmann, D. (1996). Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9–23). *Proc. Natl. Acad. Sci. U.S.A. 93,* 956–960.

DeStefano, F., Mullooly, J.P., Okoro, C.A., Chen, R.T., Marcy, S.M., Ward, J.I., Vadheim, C.M., Black, S.B., Shinefield, H.R., Davis, R.L., and Bohlke, K. (2001). Childhood vaccinations, vaccination timing, and risk of type 1 diabetes mellitus. *Pediatrics 108,* E112.

Diabetes Control and Complications Trial Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med. 329,* 977–986.

Diabetes Prevention Trial-Type 1 Diabetes Study Group (2002). Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N. Engl. J. Med. 346,* 1685–1691.

Douglas, S., McSporran, B., and Smail, P. (1999). Seasonality of presentation of type I diabetes mellitus in children. Scottish Study Group for the Care of Young Diabetics. *Scott. Med. J. 44,* 41–46.

Elliott, J.F., Marlin, K.L., and Couch, R.M. (1998). Effect of bacille Calmette-Guerin vaccination on C-peptide secretion in children newly diagnosed with IDDM. *Diabetes Care 21,* 1691–1693.

EURODIAB Substudy 2 Study Group (1999). Vitamin D supplement in early childhood and risk for Type I (insulin-dependent) diabetes mellitus. *Diabetologia 42,* 51–54.

Faria, A.M. and Weiner, H.L. (1999). Oral tolerance: mechanisms and therapeutic applications. *Adv. Immunol. 73,* 153–164.

Feutren, G., Papoz, L., Assan, R., Vialettes, B., Karsenty, G., Vexiau, P., Du Rostu, H., Rodier, M., Sirmai, J., and Lallemand, A. (1986). Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet 2,* 119–124.

Forrest, J.M., Menser, M.A., and Burgess, J.A. (1971). High frequency of diabetes mellitus in young adults with congenital rubella. *Lancet 2,* 332–334.

Fourlanos, S., Narendran, P., Byrnes, G., Colman, P., and Harrison, L.C. (2004). Insulin resistance is a risk factor for progression to type 1 diabetes. *Diabetologia 47,* 1661–1667.

Füchtenbusch, M., Rabl, W., Grassl, B., Bachmann, W., Standl, E., and Ziegler, A.G. (1998). Delay of type I diabetes in high risk, first degree relatives by parenteral antigen administration: the Schwabing Insulin Prophylaxis Pilot Trial. *Diabetologia 41,* 536–541.

Füchtenbusch, M., Irnstetter, A., Jager, G., and Ziegler, A.G. (2001). No evidence for an association of coxsackie virus infections during pregnancy and early childhood with development of islet autoantibodies in offspring of mothers or fathers with type 1 diabetes. *J. Autoimmun. 17,* 333–340.

Funda, D.P., Fundova, P., and Harrison, L.C. (2005). Environmental-mucosal interactions in the natural history of type 1 diabetes: the germ-free (GF) NOD mouse model. *Ann. N. Y. Acad. Sci.* (in press).

Gale, E.A. (2002). The rise of childhood type 1 diabetes in the 20th century. *Diabetes 51,* 3353–3361.

Gale, E.A., Bingley, P.J., Eisenbarth, G.S., Redondo, M.J., Kyvik, K.O., and Petersen, J.S. (2001). Reanalysis of twin studies suggests that diabetes is mainly genetic. *BMJ 323,* 997–998.

Gerstein, H. (1994). Cow's milk exposure and type 1 diabetes mellitus. *Diabetes Care 17,* 13–19.

Ginsberg-Fellner, F., Witt, M.E., Fedun, B., Taub, F., Dobersen, M.J., McEvoy, R.C., Cooper, L.Z., Notkins, A.L., and Rubinstein, P. (1985). Diabetes mellitus and autoimmunity in patients with the congenital rubella syndrome. *Rev. Infect. Dis. 7*(Suppl 1), S170–S176.

Giordano, C., Panto, F., Amato, M.P., Sapienza, N., Pugliese, A., and Galluzzo, A. (1990). Early administration of an immunomodulator and induction of remission in insulin-dependent diabetes mellitus. *J. Autoimmun. 3,* 611–617.

Goday, A., Pujol-Borrell, R., Fernandez, J., Casamitjana, R., Rios, M., Vilardell, E., and Gomis, R. (1993). Effects of a short prednisone regime at clinical onset of type 1 diabetes. *Diabetes Res. Clin. Pract. 20,* 39–46.

Gottsater, A., Landin-Olsson, M., Fernlund, P., Lernmark, A., and Sundkvist, G. (1993). Beta-cell function in relation to islet cell antibodies during the first 3 yr after clinical diagnosis of diabetes in type II diabetic patients. *Diabetes Care 16,* 902–910.

Gottsater, A., Landin-Olsson, M., Lernmark, A., Fernlund, P., Sundkvist, G., and Hagopian, W.A. (1995). Glutamic acid decarboxylase antibody levels predict role of B-cell decline in adult onset diabetes. *Diab. Res. Clin. Prac. 27,* 133–140.

Green, A. and Patterson, C.C. (2001). Trends in the incidence of childhood-onset diabetes in Europe 1989–1998. *Diabetologia 44,* B3–8.

Greenbaum, C. (2002). Type 1 diabetes intervention trials: what have we learned? A critical review of selected intervention trials. *Clin. Immunol. 104,* 97–104.

Greenbaum, C. and Harrison, L.C. (2003). Guidelines for intervention trials in subjects with newly-diagnosed type 1 diabetes. *Diabetes 52,* 1059–1065.

Grunt, J.A., al-Hakim, H., Willoughby, L., and Howard, C.P. (1994). A randomized trial of a somatostatin analog for preserving beta cell function in children with insulin dependent diabetes mellitus. *J. Pediatr. Endocrinol. 7,* 331–334.

Hagopian, W.A., Karlsen, A.E., Gottsater, A., Landin-Olsson, M., Grubin, C.E., Sundkvist, G., Petersen, J.S., Boel, E., Dyrberg, T., and Lernmark, A. (1993). Quantitative assay using recombinant human islet glutamic acid decarboxylase (GAD65) shows that 64K autoantibody positivity at onset predicts diabetes type. *J. Clin. Invest. 91,* 368–374.

Hahl, J., Simell, T., Ilonen, J., Knip, M., and Simell, O. (1998). Costs of predicting IDDM. *Diabetologia 41,* 79–85.

Hancock, W.W., Polanski, M., Zhang, J., Blogg, N., and Weiner, H.L. (1995). Suppression of insulitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta and prostaglandin-E. *Am. J. Pathol. 147,* 1193–1199.

Hänninen, A. and Harrison, L.C. (2000). Gamma delta T cells as mediators of mucosal tolerance: the autoimmune diabetes model. *Immunol. Rev. 173,* 109–119.

Hänninen, A., Braakhuis, A., Heath, W.R., and Harrison, L.C. (2001). Mucosal antigen primes diabetogenic cytotoxic T-lymphocytes regardless of dose or delivery route. *Diabetes 50,* 771–775.

Hänninen, A., Martinez, N.R., Davey, G.M., Heath, W.R., and Harrison, L.C. (2002). Transient blockade of CD40 ligand dissociates pathogenic from protective mucosal immunity. *J. Clin. Invest. 109,* 261–267.

Harrison, L.C. (2001). Risk assessment, prediction and prevention of type 1 diabetes. *Pediatric Diabetes 2,* 71–82.

Harrison, L.C. and Hafler, D.A. (2000). Antigen-specific therapy for autoimmune disease. *Curr. Opin. Immunol. 12,* 704–711.

Harrison, L.C. and Honeyman, M.C. (1999). Cow's milk and type 1 diabetes: the real debate is about mucosal immune function. *Diabetes 48,* 1501–1507.

Harrison, L.C., Colman, P.G., Dean, B., Baxter, R., and Martin, F.I. (1985). Increase in remission rate in newly diagnosed type I diabetic subjects treated with azathioprine. *Diabetes 34,* 1306–1308.

Harrison, L.C., Dempsey-Collier, M., Kramer, D.R., and Takahashi, K. (1996). Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. *J. Exp. Med. 184,* 2167–2174.

Harrison, L.C., Honeyman, M.C., Trembleau, S., Gregori, S., Gallazzi, F., Augstein, P., Brusic, V., Hammer, J., and Adorini, L. (1997). A peptide-binding motif for I-A(g7), the class II major histocompatibility complex (MHC) molecule of NOD and Biozzi AB/H mice. *J. Autoimmun. 10,* 165–173.

Harrison, L.C., Honeyman, M.C., Steele, C.E., Stone, N.L., Sarugeri, E., Bonifacio, E., Couper, J.J., and Colman, P.G. (2004). Pancreatic beta-cell function and immune responses to insulin after administration of intranasal insulin to humans at risk for type 1 diabetes. *Diabetes Care 27,* 2348–2355.

Hartmann, B., Bellmann, K., Ghiea, I., Kleemann, R., and Kolb, H. (1997). Oral insulin for diabetes prevention in NOD mice: potentiation by enhancing Th2 cytokine expression in the gut through bacterial adjuvant. *Diabetologia 40,* 902–909.

Herold, K.C., Hagopian, W., Auger, J.A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., Gitelman, S.E., Harlan, D.M., Xu, D., Zivin, R.A., and Bluestone, J.A. (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med. 346,* 1692–1698.

Hiltunen, M., Hyoty, H., Karjalainen, J., Leinikki, P., Knip, M., Lounamaa, R., and Akerblom, H.K. (1995). Serological evaluation of the role of cytomegalovirus in the pathogenesis of IDDM: a prospective study. The Childhood Diabetes in Finland Study Group. *Diabetologia 38,* 705–710.

Holt, P.G. (1994). A potential vaccine strategy for asthma and allied atopic disease during early childhood. *Lancet 344,* 456–458.

Homann, D., Dyrberg, T., Petersen, J., Oldstone, M.B., and von Herrath, M.G. (1999). Insulin in oral immune tolerance: a one-amino acid change in the B chain makes the difference. *J. Immunol. 163,* 1833–1838.

Honeyman, M.C., Stone, N.L., and Harrison, L.C. (1998). T-cell epitopes in type 1 diabetes autoantigen tyrosine phosphatase IA-2: potential for mimicry with rotavirus and other environmental agents. *Mol. Med. 4,* 231–239.

Honeyman, M.C., Coulson, B.S., Stone, N.L., Gellert, S.A., Goldwater, P.N., Steele, C.E., Couper, J.J., Tait, B.D., Colman, P.G., and Harrison, L.C. (2000). Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes 49,* 1319–1324.

Hummel, M., Fuchtenbusch, M., Schenker, M., and Ziegler, A.G. (2000). No major association of breast-feeding, vaccinations, and childhood viral diseases with early islet autoimmunity in the German BABYDIAB Study. *Diabetes Care 23,* 969–974.

Hummel, M., Bonifacio, E., Naserke, H.E., and Ziegler, A.G. (2002). Elimination of dietary gluten does not reduce titers of type 1 diabetes-associated autoantibodies in high-risk subjects. *Diabetes Care 25,* 1111–1116.

Husby, S., Mestecky, J., Moldoveanu, Z., Holland, S., and Elson, C.O. (1994). Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. *J. Immunol. 152,* 4663–4670.

Hyoty, H., Leinikki, P., Reunanen, A., Ilonen, J., Surcel, H.M., Rilva, A., Kaar, M.L., Huupponen, T., Hakulinen, A., and Makela, A.L. (1988). Mumps infections in the etiology of type 1 (insulin-dependent) diabetes. *Diabetes Res 9,* 111–116.

Hyoty, H., Hiltunen, M., Reunanen, A., Leinikki, P., Vesikari, T., Lounamaa, R., Tuomilehto, J., and Akerblom, H.K. (1993). Decline of mumps antibodies in type 1 (insulin-dependent) diabetic children and a plateau in the rising incidence of type 1 diabetes after introduction of the mumps-measles-rubella vaccine in Finland. Childhood Diabetes in Finland Study Group. *Diabetologia 36,* 1303–1308.

Hyoty, H., Hiltunen, M., Knip, M., Laakkonen, M., Vahasalo, P., Karjalainen, J., Koskela, P., Roivainen, M., Leinikki, P., and Hovi, T. (1995). A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Childhood Diabetes in Finland (DiMe) Study Group. *Diabetes 44,* 652–657.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R., and Virtanen, S.M. (2001). Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet 358,* 1500–1503.

Ikehara, S., Ohtsuki, H., Good, R.A., Asamoto, H., Nakamura, T., Sekita, K., Muso, E., Tochino, Y., Ida, T., and Kuzuya, H. (1985). Prevention of type I diabetes in nonobese diabetic mice by allogenic bone marrow transplantation. *Proc. Natl. Acad. Sci. U.S.A. 82,* 7743–7747.

Imaoka, A., Matsumoto, S., Setoyama, H., Okada, Y., and Umesaki, Y. (1996). Proliferative recruitment of intestinal intraepithelial lymphocytes after microbial colonization of germ-free mice. *Eur. J. Immunol. 26,* 945–948.

Jenson, A.B., Rosenberg, H.S., and Notkins, A.L. (1980). Pancreatic islet-cell damage in children with fatal viral infections. *Lancet 2,* 354–358.

Karam, J.H., Lewitt, P.A., Young, C.W., Nowlain, R.L., Frankel, B.J., Fujiya, H., Freedman, Z.R., and Grodsky, G.M. (1980). Insulinopenic diabetes after rodenticide (Vacor) ingestion: a unique model of acquired diabetes in man. *Diabetes 29,* 971–978.

Kaufman, C.L., Li, H., and Ildstad, S.T. (1997). Patterns of hemopoietic reconstitution in nonobese diabetic mice: dichotomy of allogeneic resistance versus competitive advantage of disease-resistant marrow. *J. Immunol. 158,* 2435–2442.

Kawaguchi-Miyashita, M., Shimizu, K., Nanno, M., Shimada, S., Watanabe, T., Koga, Y., Matsuoka, Y., Ishikawa, H., Hashimoto, K., and Ohwaki, M. (1996). Development and cytolytic function of intestinal intraepithelial T lymphocytes in antigen-minimized mice. *Immunology 89,* 268–273.

Keller, R.J., Eisenbarth, G.S., and Jackson, R.A. (1993). Insulin prophylaxis in individuals at high risk of type 1 diabetes. *Lancet 341,* 927–928.

Khakpour, M. and Nik-Akhtar, B. (1975). Diabetes mellitus following a mumps epidemic. *J. Trop. Med. Hyg. 78,* 262–263.

Kimpimäki, T., Kupila, A., Hämäläinen, A.-M., Kukko, M., Kulmala, P., Savola, K., Simell, T., Keskinen, P., Ilonen, J., Simell, O., and Knip, M. (2001). The first signs of B-cell autoimmunity appear in infancy in genetically susceptible children from the general population: The Finnish Type 1 Diabetes Prediction and Prevention Study. *J. Clin. Endocrinol. Metab. 86,* 4782–4788.

Kobayashi, T., Nakanishi, K., Murase, T., and Kosaka, K. (1996). Small doses of subcutaneous insulin as a strategy for preventing slowly progressive B-cell failure in islet cell antibody-positive patients with clinical features of NIDDM. *Diabetes 45,* 622–626.

Kohnert, K.D., Hehmke, B., Keilacker, H., Ziegler, M., Emmrich, F., Laube, F., and Michaelis, D. (1996). Antibody response to islet autoantigens in anti-CD4/prednisolone immune intervention of type 1 diabetes. *Int. J. Clin. Lab. Res. 26,* 55–59.

Krause, I., Blank, M., and Shoenfeld, Y. (2000). Immunomodulation of experimental autoimmune diseases via oral tolerance. *Crit. Rev. Immunol. 20,* 1–16.

LaFace, D.M. and Peck, A.B. (1989). Reciprocal allogeneic bone marrow transplantation between NOD mice and diabetes-nonsusceptible mice associated with transfer and prevention of autoimmune diabetes. *Diabetes 38,* 894–901.

Lampeter, E.F., Klinghammer, A., Scherbaum, W.A., Heinze, E., Haastert, B., Giani, G., and Kolb, H. (1998). The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. *Diabetes 47,* 980–984.

Laron, Z. (2000). Seasonality of birth in children with diabetes. Maternally transmitted infection might increase risk of diabetes. *BMJ 320,* 716.

Leiter, E.H., Prochazka, M., and Coleman, D.L. (1987). The non-obese diabetic (NOD) mouse. *Am. J. Pathol. 128,* 380–383.

Li, H., Kaufman, C.L., Boggs, S.S., Johnson, P.C., Patrene, K.D., and Ildstad, S.T. (1996). Mixed allogeneic chimerism induced by a sublethal approach prevents autoimmune diabetes and reverses insulitis in nonobese diabetic (NOD) mice. *J. Immunol. 156,* 380–388.

Lindberg, B., Ahlfors, K., Carlsson, A., Ericsson, U.B., Landin-Olsson, M., Lernmark, A., Ludvigsson, J., Sundkvist, G., and Ivarsson, S.A. (1999). Previous exposure to measles, mumps, and rubella—but not vaccination during adolescence—correlates to the prevalence of pancreatic and thyroid autoantibodies. *Pediatrics 104,* e12.

Linn, T., Ortac, K., Laube, H., and Federlin, K. (1996). Intensive therapy in adult insulin-dependent diabetes mellitus is associated with improved insulin sensitivity and reserve: a randomized, controlled, prospective study over 5 years in newly diagnosed patients. *Metabolism 45,* 1508–1513.

Ludvigsson, J., Samuelsson, U., Johansson, C., and Stenhammar, L. (2001). Treatment with antioxidants at onset of type 1 diabetes in children: a randomized, double-blind placebo-controlled study. *Diabetes Metab. Res. Rev. 17,* 131–136.

Luhder, F., Katz, J., Benoist, C., and Mathis, D. (1998). Major histocompatibility complex class II molecules can protect from diabetes by positively selecting T cells with additional specificities. *J. Exp. Med. 187,* 379–87.

Lund, T., O'Reilly, L., Hutchings, P., Kanagawa, O., Simpson, E., Gravely, R., Chandler, P., Dyson, J., Picard, J.K., and Edwards, A. (1990). Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A beta-chain or normal I-E alpha-chain. *Nature 345,* 727–729.

Ma, S.W., Zhao, D.L., Yin, Z.Q., Mukherjee, R., Singh, B., Qin, H.Y., Stiller, C.R., and Jevnikar, A.M. (1997). Transgenic plants expressing autoantigens fed to mice to induce oral immune tolerance. *Nat. Med. 3,* 793–796.

MacFarlane, A.J., Burghardt, K.M., Kelly, J., Simell, T., Simell, O., Altosaar, I., and Scott, F.W. (2002). A type 1 diabetes-related protein from wheat (*Triticum aestivum*): cDNA clone of a wheat storage globulin, Glb1, linked to islet damage. *J. Biol. Chem. 278,* 54–63.

Maron, R., Palanivel, V., Weiner, H.L., and Harn, D.A. (1998). Oral administration of schistosome egg antigens and insulin B-chain generates and enhances Th2-type responses in NOD mice. *Clin. Immunol. Immunopathol. 87,* 85–92.

Maron, R., Guerau-de-Arellano, M., Zhang, X., and Weiner, H.L. (2001). Oral administration of insulin to neonates suppresses spontaneous and cyclophosphamide induced diabetes in the NOD mouse. *J. Autoimmun. 16,* 21–28.

Martinez, N.R., Augstein, P., Moustakas, A.K., Papadopoulos, G.K., Gregori, S., Adorini, L., Jackson, D.C., and Harrison, L.C. (2003). Disabling an integral CTL epitope allows suppression of autoimmune diabetes by intranasal proinsulin peptide. *J. Clin. Invest. 111,* 1365–1371.

Marttila, J., Juhela, S., Vaarala, O., Hyoty, H., Roivainen, M., Hinkkanen, A., Vilja, P., Simell, O., and Ilonen, J. (2001). Responses of coxsackievirus B4-specific T-cell lines to 2C protein-characterization of epitopes with special reference to the GAD65 homology region. *Virology 284,* 131–141.

Mathieu, C., Casteels, K., Bouillon, R., and Waer, M. (1997). Protection against autoimmune diabetes in mixed bone marrow chimeras: mechanisms involved. *J. Immunol. 158,* 1453–1457.

McKown, K.M., Carbone, L.D., Kaplan, S.B., Aelion, J.A., Lohr, K.M., Cremer, M.A., Bustillo, J., Gonzalez, M., Kaeley, G., Steere, E.L., Somes, G.W., Myers, L.K., Seyer, J.M., Kang, A.H., and Postlethwaite, A.E. (1999). Lack of efficacy of oral bovine type II collagen added to existing therapy in rheumatoid arthritis. *Arthritis Rheum. 42,* 1204–1208.

Mendola, G., Casamitjana, R., and Gomis, R. (1989). Effect of nicotinamide therapy upon B-cell function in newly diagnosed type 1 (insulin-dependent) diabetic patients. *Diabetologia 32,* 160–162.

Menser, M.A., Forrest, J.M., Honeyman, M.C., and Burgess, J.A. (1974). Letter: Diabetes, HL-A antigens, and congenital rubella. *Lancet 2,* 1508–1509.

Menser, M.A., Forrest, J.M., and Bransby, R.D. (1978). Rubella infection and diabetes mellitus. *Lancet 1,* 57–60.

Metzler, B. and Wraith, D.C. (1993). Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: influence of MHC binding affinity. *Int. Immunol. 5,* 1159–1165.

Moncada, E., Subira, M.L., Oleaga, A., Goni, F., Sanchez-Ibarrola, A., Monreal, M., Sevilla, M., Goni, M.J., Yoldi, A., and Teran, D. (1990). Insulin requirements and residual beta-cell function 12 months after concluding immunotherapy in type I diabetic patients treated with combined azathioprine and thymostimulin administration for one year. *J. Autoimmun. 3,* 625–638.

Narendran, P., Mannering, S.I., and Harrison, L.C. (2003). Proinsulin—a pathogenic autoantigen in type 1 diabetes. *Autoimmun. Rev. 2,* 204–210.

Narendran, P., Neale, A., Jensen, K., Steptoe, R.J., and Harrison, L.C. (2004). *Ectopic expression of proinsulin by human peripheral blood cells*. IDS-7, University of Bristol, Cambridge, UK.

Nishimoto, H., Kikutani, H., Yamamura, K., and Kishimoto, T. (1987). Prevention of autoimmune insulitis by expression of I-E molecules in NOD mice. *Nature 328,* 432–434.

Norris, J.M. and Scott, F.W. (1996). A meta analysis of infant diet and insulin-dependent diabetes mellitus: do biases play a role. *Epidemiology 7,* 87–92.

Norris, J.M., Beaty, B., Klingensmith, G., Yu, L., Hoffman, M., Chase, H.P., Erlich, H.A., Hamman, R.F., Eisenbarth, G.S., and Rewers, M. (1996). Lack of association between early exposure to cow's milk protein and B cell autoimmunity: Diabetes Autoimmunity Study in the Young (DAISY). *JAMA 276,* 609–614.

Ou, D., Mitchell, L.A., Metzger, D.L., Gillam, S., and Tingle, A.J. (2000). Cross-reactive rubella virus and glutamic acid decarboxylase (65 and 67) protein determinants recognised by T cells of patients with type I diabetes mellitus. *Diabetologia 43,* 750–762.

Panto, F., Giordano, C., Amato, M.P., Pugliese, A., Donatelli, M., D'Acquisto, G., and Galluzzo, A. (1990). The influence of high dose intravenous immunoglobulins on immunological and metabolic pattern in newly diagnosed type I diabetic patients. *J. Autoimmun. 3,* 587–592.

Parish, C.R. (1996). Immune deviation: a historical perspective. *Immunol. Cell. Biol.* 74, 449–456.

Philips, J.C., Scheen, A.J., and Le Registre Belge du Diabete (2002). Info-congress. Study of the prevention of type 1 diabetes with nicotinamide: positive lessons of a negative clinical trial ENDIT) [Article in French]. *Rev. Med. Liege. 57,* 672–675.

Ploix, C., Bergerot, I., Fabien, N., Perche, S., Moulin, V., and Thivolet, C. (1998). Protection against autoimmune diabetes with oral insulin is associated with the presence of IL-4 type 2 T-cells in the pancreas and pancreatic lymph nodes. *Diabetes 47,* 39–44.

Ploix, C., Bergerot, I., Durand, A., Czerkinsky, C., Holmgren, J., and Thivolet, C. (1999). Oral administration of cholera toxin B-insulin conjugates protects NOD mice from autoimmune diabetes by inducing CD4+ regulatory T-cells. *Diabetes 48,* 2150–2156.

Pozzilli, P., Signore, A., Williams, A.J., and Beales, P.E. (1993). NOD mouse colonies around the world—recent facts and figures. *Immunol. Today 14,* 193–196.

Pozzilli, P., Visalli, B., Boccuni, M.L., Baroni, M.G., Buzzetti, R., Fioriti, E., Signore, A., Cavallo, M.G., Andreani, D., and Lucentini, L. (1994). Randomized trial comparing nicotinamide and nicotinamide plus cyclosporin in recent onset insulin-dependent diabetes (IMDIAB 1). The IMDIAB Study Group. *Diabet. Med. 11,* 98–104.

Pozzilli, P., Visalli, N., Signore, A., Baroni, M.G., Buzzetti, R., Cavallo, M.G., Boccuni, M.L., Fava, D., Gragnoli, C., Andreani, D., Lucentini, L., Matteoli, M.C., Crino, A., Cicconetti, C.A., Teodonio, C., Paci, F., Amoretti, R., Pisano, L., Pennafina, M.G., Santopadre, G., Marozzi, G., Multari, G., Suppa, M.A., Campea, L., De Mattia, G.C., Cassone Faldetta, M., Marietti, G., Perrone, F., Greco, A.V., and Ghirlanda, G. (1995). Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia 38,* 848–852.

Pozzilli, P., Visalli, N., Cavallo, M.G., Signore, A., Baroni, M.G., Buzzetti, R., Fioriti, E., Mesturino, C., Fiori, R., Romiti, A., Giovannini, C., Lucentini, L., Matteoli, M.C., Crino, A., Teodonio, C., Paci, F., Amoretti, R., Pisano, L., Suraci, C., Multari, G., Suppa, M., Sulli, N.,

De Mattia, G., Faldetta, M.R., and Suraci, M.T. (1997). Vitamin E and nicotinamide have similar effects in maintaining residual beta cell function in recent onset insulin-dependent diabetes (the IMDIAB IV study). *Eur. J. Endocrinol. 137,* 234–239.

Pozzilli, P., Pitocco, D., Visalli, N., Cavallo, M.G., Buzzetti, R., Crino, A., Spera, S., Suraci, C., Multari, G., Cervoni, M., Manca Bitti, M.L., Matteoli, M.C., Marietti, G., Ferrazzoli, F., Cassone Faldetta, M.R., Giordano, C., Sbriglia, M., Sarugeri, E., and Ghirlanda, G. (2000). No effect of oral insulin on residual beta-cell function in recent-onset type 1 diabetes (the IMDIAB VII). IMDIAB Group. *Diabetologia 43,* 1000–1004.

Pugliese, A., Zeller, M., Fernandez Jr, A., Zalcberg, L.J., Bartlett, R.J., Ricordi, C., Pietropaolo, M., Eisenbarth, G.S., Bennett, S.T., and Patel, D.D. (1997). The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat. Genet. 15,* 293–297.

Ratanatharathorn, V., Ayash, L., Lazarus, H.M., Fu, J., and Uberti, J.P. (2001). Chronic graft-versus-host disease: clinical manifestation and therapy. *Bone Marrow Transplant. 28,* 121–129.

Raz, I., Elias, D., Avron, A., Tamir, M., Metzger, M., and Cohen, I.R. (2001). Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet 358,* 1749–1753.

Redondo, M.J., Yu, L., Hawa, M., Mackenzie, T., Pyke, D.A., Eisenbarth, G.S., and Leslie, R.D. (2001). Heterogeneity of type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia 44,* 354–362.

Roep, B.O., Hiemstra, H.S., Schloot, N.C., De Vries, R.R., Chaudhuri, A., Behan, P.O., and Drijfhout, J.W. (2002). Molecular mimicry in type 1 diabetes: immune cross-reactivity between islet autoantigen and human cytomegalovirus but not Coxsackie virus. *Ann. N. Y. Acad. Sci. 958,* 163–5.

Rudy, G., Stone, N., Harrison, L.C., Colman, P.G., McNair, P., Brusic, V., French, M.B., Honeyman, M.C., Tait, B., and Lew, A.M. (1995). Similar peptides from two beta-cell autoantigens, proinsulin and glutamic acid decarboxylase, stimulate T cells of individuals at risk for insulin-dependent diabetes. *Mol. Med. 1,* 625–633.

Schmidt, D., Verdaguer, J., Averill, N., and Santamaria, P. (1997). A mechanism for the major histocompatibility complex-linked resistance to autoimmunity. *J. Exp. Med. 186,* 1059–1075.

Schnell, O., Eisfelder, B., Standl, E., and Ziegler, A.G. (1997). High-dose intravenous insulin infusion versus intensive insulin treatment in newly diagnosed IDDM. *Diabetes 46,* 1607–1611.

Secchi, A., Pastore, M.R., Sergi, A., Pontiroli, A.E., and Pozza, G. (1990). Prednisone administration in recent onset type I diabetes. *J. Autoimmun. 3,* 593–600.

Selam, J.L., Woertz, L., Lozano, J., Robinson, M., Chan, E., and Charles, M.A. (1993). The use of glipizide combined with intensive insulin treatment for the induction of remissions in new onset adult type I diabetes. *Autoimmunity 16,* 281–288.

Serreze, D.V., Leiter, E.H., Worthen, S.M., and Shultz, L.D. (1988). NOD marrow stem cells adoptively transfer diabetes to resistant (NOD × NON)F1 mice. *Diabetes 37,* 252–255.

Shah, S.C., Malone, J.I., and Simpson, N.E. (1989). A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N. Engl. J. Med. 320,* 550–554.

Shehadeh, N., Gelertner, L., Blazer, S., Perlman, R., and Etzioni, A. (2001). The importance of insulin content in infant diet: suggestion for a new infant formula period. *Acta Pediatr. 90,* 93–95.

Silverstein, J., Maclaren, N., Riley, W., Spillar, R., Radjenovic, D., and Johnson, S. (1988). Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N. Engl. J. Med. 319,* 599–604.

Singer, S.M., Tisch, R., Yang, X.D., and McDevitt, H.O. (1993). An Abd transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. *Proc. Natl. Acad. Sci. U.S.A. 90,* 9566–9570.

Singer, S.M., Tisch, R., Yang, X.D., Sytwu, H.K., Liblau, R., and McDevitt, H.O. (1998). Prevention of diabetes in NOD mice by a mutated I-Ab transgene. *Diabetes 47,* 1570–1577.

Skyler, J.S. and Rabinovitch, A. (1992). Cyclosporine in recent onset type I diabetes mellitus. Effects on islet beta cell function. Miami Cyclosporine Diabetes Study Group. *J. Diabetes Complications 6,* 77–88.

Skyler, J.S., Lorenz, T.J., Schwartz, S., Eisenbarth, G.S., Einhorn, D., Palmer, J.P., Marks, J.B., Greenbaum, C., Saria, E.A., and Byers, V. (1993). Effects of an anti-CD5 immunoconjugate (CD5-plus) in recent onset type I diabetes mellitus: a preliminary investigation. The CD5 Diabetes Project Team. *J. Diabet. Complications 7,* 224–232.

Slavin, A.J., Maron, R., and Weiner, H.L. (2001). Mucosal administration of IL-10 enhances oral tolerance in autoimmune encephalomyelitis and diabetes. *Int. Immunol. 13,* 825–833.

Stene, L.C., Ulriksen, J., Magnus, P., and Joner, G. (2000). Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. *Diabetologia 43,* 1093–1098.

Steptoe, R.J., Ritchie, J.M., and Harrison, L.C. (2003). Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes. *J. Clin. Invest. 111,* 1357–1363.

Steptoe, R.J., Stankovic, S., Lopaticki, S., Jones, L.K., Harrison, L.C., and Morahan, G. (2004). Persistence of recipient lymphocytes in NOD mice after irradiation and bone marrow transplantation. *J. Autoimmun. 22,* 131–138.

Strachan, D.P. (1989). Hay fever, hygiene, and household size. *BMJ 299,* 1259–1260.

Suzuki, T. (1987). Diabetogenic effects of lymphocyte transfusion on the NOD or NOD nude mouse. In J. Rygaard, N. Brunner, N. Graem, and M. Spang-Thomson (eds), *Immune deficient animals in biomedical research*, (pp. 112–116). Basel: Karger.

Tian, J., Atkinson, M.A., Clare-Salzler, M., Herschenfeld, A., Forsthuber, T., Lehmann, P.V., and Kaufman, D.L. (1996). Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J. Exp. Med. 183,* 1561–1567.

Trentham, D.A., Dynesius-Trentham, R.A., and Orav, E.J. (1993). Effects of oral administration of type II collagen on rheumatoid arthritis. *Science 261,* 1727–1730.

Tuomi, T., Groop, L.C., Zimmet, P.Z., Rowley, M.J., Knowles, W., and Mackay, I.R. (1993). Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. *Diabetes 42,* 359–362.

Turner, R., Stratton, I., Horton, V., Manley, S., Zimmet, P., Mackay, I.R., Shattock, M., Bottazzo, G.F., and Holman, R. (1997). UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. UK Prospective Diabetes Study Group. *Lancet 350,* 1288–1293.

Vague, P., Picq, R., Bernal, M., Lassmann-Vague, V., and Vialettes, B. (1989). Effect of nicotinamide treatment on the residual insulin secretion in type 1 (insulin-dependent) diabetic patients. *Diabetologia 32,* 316–321.

Ventura, A., Magazzu, G., and Greco, L. (1999). Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease (SIGEP study group for autoimmune disorders in celiac disease). *Gastroenterology 117,* 297–303.

Verge, C.F., Gianani, R., Kawasaki, E., Yu, L., Pietropaolo, M., Jackson, R.A., Chase, H.P., and Eisenbarth, G.S. (1996). Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes 45,* 926–933.

Vidal, J., Fernandez-Balsells, M., Sesmilo, G., Aguilera, E., Casamitjana, R., Gomis, R., and Conget, I. (2000). Effects of nicotinamide and intravenous insulin therapy in newly diagnosed type 1 diabetes. *Diabetes Care 23,* 360–364.

Viskari, H.R., Roivainen, M., Reunanen, A., Pitkaniemi, J., Sadeharju, K., Koskela, P., Hovi, T., Leinikki, P., Vilja, P., Tuomilehto, J., and Hyoty, H. (2002). Maternal first-trimester enterovirus infection and future risk of type 1 diabetes in the exposed fetus. *Diabetes 51,* 2568–2571.

von Herrath, M.G. and Harrison, L.C. (2003). Antigen-induced regulatory T cells in autoimmunity. *Nat. Rev. Immunol. 3,* 223–232.

Waldo, F.B., van den Wall Bake, A.W., Mestecky, J., and Husby, S. (1994). Suppression of the immune response by nasal immunization. *Clin. Immunol. Immunopathol. 72,* 30–34.

Weiner, H.L., Mackin, G.A., and Matsui, M. (1993). Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science 259,* 1321–1324.

Wicker, L.S., Miller, B.J., Chai, A., Terada, M., and Mullen, Y. (1988). Expression of genetically determined diabetes and insulitis in the nonobese diabetic (NOD) mouse at the level of bone marrow-derived cells. Transfer of diabetes and insulitis to nondiabetic (NOD X B10) F1 mice with bone marrow cells from NOD mice. *J. Exp. Med. 167,* 1801–1810.

Willis, J.A., Scott, R.S., Darlow, B.A., Lewy, H., Ashkenazi, I., and Laron, Z. (2002). Seasonality of birth and onset of clinical disease in children and adolescents (0–19 years) with type 1 diabetes mellitus in Canterbury, New Zealand. *J. Pediatr. Endocrinol. Metab. 15,* 645–647.

Yin, H., Berg, A.K., Tuvemo, T., and Frisk, G. (2002). Enterovirus RNA is found in peripheral blood mononuclear cells in a majority of type 1 diabetic children at onset. *Diabetes 51,* 1964–1971.

Zhang, Z.H., Davidson, L., Eisenbarth, G., and Weiner, H.L. (1991). Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc. Natl. Acad. Sci. U.S.A. 88,* 10252–10256.

CHAPTER

76

Emerging Therapies for Autoimmune Diseases

LUCIENNE CHATENOUD

Université René Descartes Paris 5, INSERM U580 Paris, France

Autoimmune diseases represent a major therapeutic challenge. In many cases, the disease is severe enough to significantly reduce longevity. In other cases, the disease causes major handicaps and discomfort that justify the usage of aggressive treatments generating their own hazards. Present treatments are palliative (substitutive), anti-inflammatory, or immunosuppressive without any specificity for the pathogenic mechanisms of the disease. With few exceptions [anti-tumor necrosis factor (TNF) antibodies in rheumatoid arthritis [RA0]] the treatment of autoimmune diseases has not significantly evolved from the mid-1980s. Therefore, much attention is drawn to modern technologies that have made new agents available. These approaches have been the matter of extensive experimental studies in the numerous and diversified animal models of autoimmune diseases. Some of them have already been tested in the clinic and even approved by regulatory authorities. The aim of this chapter is to review these strategies with particular emphasis on agents or methods that have entered the clinical arena. In addition, it is interesting to discuss how lessons drawn from the development of novel therapeutic strategies in one particular autoimmune disease may be translated into beneficial therapies for other autoimmune conditions.

LIMITATIONS OF CURRENT TREATMENTS

In some autoimmune diseases there is benefit from a substitutive treatment. This is typically the case in type 1 diabetes (use of insulin), thyroiditis (use of thyroid hormones), or pernicious anemia secondary to autoimmune gastritis (use of vitamin B12). In some cases, the approach is satisfactory; the treatment is fully efficient with minor constraints and side effects, as for thyroid hormones or vitamin B12. In other cases, such as type 1 diabetes, chronic insulin therapy is not an optimal treatment since it is associated with major constraints (multiple daily parenteral administration, need for self-monitoring of glycemia, serious risks linked to hypoglycemic episodes) and lack of effectiveness in preventing severe degenerative complications.

The treatment may aim at combating inflammation. Although many autoimmune diseases have a strong inflammatory component, it is fair to recognize that, with a few exceptions (RA), conventional anti-inflammatory drugs usually show limited efficacy and their chronic usage is associated with significant toxicity, notably renal and digestive.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

In diseases with a severe outcome, patients are given immunosuppressive agents such as cyclophosphamide, cyclosporine, or FK506, and more recently mycophenolate mofetil. These drugs are often (but not always) efficacious. Their usage, however, is curtailed by major difficulties. First, the drugs have a direct toxicity for humans, such as cyclosporine-induced nephrotoxicity or cyclophosphamide-induced bone marrow depression and carcinogenesis. Second, they must generally be used chronically at relatively high dosages, since disease relapse occurs when the treatment is stopped, thus exposing patients to over-immunosuppression leading to opportunistic infections and tumors. The risk can be minimized by adequate selection of drug dosage depending on a careful monitoring of blood levels and immune functions. Whatever the precautions taken, particularly when patients are not monitored by specialized centers, the long-term risks may become hardly acceptable in autoimmune patients.

Corticosteroids, which associate anti-inflammatory and immunosuppressive properties, are widely used in autoimmune diseases. They are frequently very effective but not satisfactory, however, due to their severe side effects, particularly when they are administered long-term at high dosages.

NEW GOALS FOR IMMUNOTHERAPY OF AUTOIMMUNE DISEASES

The first and most ambitious goal is that of inducing or, in the case of established autoimmune diseases, of restoring immune tolerance to target autoantigens. This may be defined operationally as the possibility to harness the pathologic immune response following a short-term treatment while keeping intact the capacity of the host to respond normally to exogenous antigens. Autoimmune diseases are the result of the rupture of tolerance to self-antigens. In most cases, notably in organ-specific autoimmune diseases, the breakdown of tolerance essentially involves the autoimmune response to the autoantigens of the target organ, which may represent a limited number of specificities. One must then attempt to restore tolerance to the corresponding autoantigen(s). In other cases, particularly in non-organ-specific autoimmune diseases such as systemic lupus erythematosus (SLE), the rupture of tolerance is more diffuse and may involve autoantigens that are present in many cell types, notably antigens expressed in apoptotic cells. Restoration of self-tolerance has the major advantage of avoiding the side effects linked to chronic immunosuppression. Since most autoimmune diseases are a chronic and/or relapsing/remitting process, for the effect to be long lasting one will probably need to repeat the tolerogenic treatment.

The second aim is the limitation of side effects of the tolerogenic treatment, either due to drug toxicity or over-immunosuppression. Biotechnology-derived products show a range of side effects, which are, however, often intrinsically different from those of conventional immunosuppressants. These side effects must be carefully evaluated, which is often difficult in preclinical studies because of the strict specificity of the products to humans (see below). One can study the mouse-specific equivalent of these products, but the conclusion drawn from these experiments is indirect since the drug tested is not the very same drug that will be used in the clinic. In some rare cases, the drugs may be tested in animal models when the human target molecule is transgenically expressed in the mouse.

The third aim is to achieve a medical benefit, which requires that the patients are still fully sensitive to the treatment, i.e., still showing a good function of the target organ at the initiation of therapy.

A last issue is that of the sensitization to the therapeutic protein, be it a humanized monoclonal antibody, a hormone, a cytokine, or an antigen. If neutralizing antibodies are produced, the therapeutic effect is lost. Additionally, if the hormone or the cytokine has an important biologic function, the sensitization may have serious consequences.

THE THERAPEUTIC ARMAMENTARIUM DERIVED FROM BIOTECHNOLOGY

Monoclonal Antibodies

Murine monoclonal antibodies (MAbs) produced by mouse or rat hybridomas (Kohler and Milstein, 1975) specific for immune cell receptors were introduced in clinical practice more than 25 years ago and their use initially developed in the field of solid organ transplantation. Two major side effects—namely, the sensitization against the xenogeneic molecule and the cytokine-releasing potential observed with some particular specificities—explain why the use of rodent MAbs remained initially mostly confined to transplantation with only very few attempts in autoimmunity. The advent of humanized MAbs that are less immunogenic and better tolerated has completely changed the picture and allowed a more widespread use of these interesting therapeutic tools. In fact, at variance with conventional immunosuppressants, MAbs specific for relevant lymphocyte receptors are unique in their capacity to induce, under adequate circumstances, immune tolerance to soluble proteins, foreign tissue alloantigens and autoantigens.

Conventional chemicals mostly act through removal and/or the functional inhibition of their targets. In contrast,

MAbs display a wide spectrum of pharmacologic and biologic activities highly relevant to their capacity to "reprogram" the immune system. Thus, depending on their fine specificity, MAbs will remove target cells, inhibit or block the functional capacity of the target without depleting it, neutralize major cytokines, and/or serve as receptor agonists triggering activation signals for specialized T-cell subsets, e.g., regulatory T cells.

The Problem of Sensitization can be Partly Overcome with Humanized and Human Monoclonal Antibodies

The repeated administration of murine MAbs invariably triggered a humoral immune response, of which the major clinical consequence was the neutralization of the antibody's therapeutic activity. Interestingly, this was not a global anti-mouse or anti-rat response; it was very restricted in its specificity with essentially anti-isotypic and anti-idiotypic antibodies being produced (Benjamin et al., 1986; Chatenoud, 1986; Chatenoud et al., 1986a). Anti-idiotypic antibodies that compete with the therapeutic antibody for antigen binding represent the neutralizing component of the response while anti-isotypic antibodies are mostly non-neutralizing (Baudrihaye et al., 1984). Another peculiarity of this humoral response is its oligoclonality (Chatenoud et al., 1986b), which explains why, at variance with what was observed in patients immunized to polyclonal antilymphocyte globulins, serum sickness was a rare consequence of sensitization to MAbs since the amount of immune complexes formed would be insufficient to elicit a generalized reaction. Antimonoclonal IgE responses associated with symptoms of anaphylaxis were reported but remained a very uncommon observation (Abramowicz et al., 1992; Abramowicz et al., 1996).

Until humanized MAbs or, more recently, fully human antibodies became available, the only way to cope with the problem of sensitization was to associate adequate doses of chemical immunosuppressants (Hricik et al., 1990). Two types of humanized antibodies have been derived from molecular engineering. Chimeric MAbs express intact rodent variable regions linked to human immunoglobulin constant domains (Elliott et al., 1994a). In fully reshaped or complementarity determining region (CDR)-grafted antibodies, the rodent hypervariable regions interacting with the antigen (i.e., CDRs) are included within human heavy and light chain immunoglobulin frameworks (Riechmann et al., 1988).

Humanized MAbs have significantly reduced, though not totally avoided, the risk of deleterious anti-idiotypic responses. The clinical data available indicate that both chimeric and reshaped humanized MAbs may be immunogenic when administered alone, without associated immunosuppressants, and after more than 2–3 repeated antibody courses (Elliott et al., 1994b). It has been the general experience that combining low doses of chemical immunosuppressants was an efficient way to overcome such sensitization (Nashan et al., 1997; Vincenti et al., 1998; Feldmann, 2002).

Fully human MAbs that are already used in clinical practice have been produced by different means. First, mice have been invalidated for the expression of endogenous (mouse) immunoglobulin genes and concurrently made transgenic for sufficient human constant and variable immunoglobulin-encoding sequences to provide for antibody diversity; B cells from these immunized mice will produce human antibodies that can be used in conventional fusions to obtain hybridomas yielding high-affinity MAbs derived from *in vivo* antigen-driven selection.

Second, there is a fully *in vitro* approach using cDNA libraries expressed on filamentous phages to derive high affinity antibodies to a wide variety of antigens, including those for which the conventional hybridoma technology fails due to poor immunogenicity (Marks et al., 1991). Third, human-mouse chimeras can be established using normal mice irradiated and reconstituted with bone marrow cells from SCID mice; after human lymphocytes from presensitized donors are inoculated, these mice are boosted with the antigen of interest, and sensitized human B cells are recovered and used for conventional fusions (Lubin et al., 1994).

Further Opportunities are Offered by Genetic Engineering

Engineering Fc Regions of Monoclonal Antibodies to Avoid Side Effects and Prolong Half-Life

Antibody engineering allows the design of tailor-made antibodies to fit at best therapeutic indications. Antibodies expressing human Fc portions have a significantly prolonged half-life. The choice of the human Fc portion will influence the antibody effector capacities, i.e., its activity in terms of complement fixation, opsonization and antibody-dependent cell cytotoxicity (ADCC). In addition, in the case of anti-CD3 antibodies, humanization can circumvent problems due to their intrinsic mitogenic and cytokine-releasing capacity that leads *in vivo* to a "flu-like" syndrome. This syndrome was regularly observed with murine anti-CD3 MAbs such as OKT3, and, although transient, it represented a major and troublesome side effect that totally precluded the use of CD3 MAbs for indications other than organ transplantation (Abramowicz et al., 1989; Chatenoud et al., 1990; Chatenoud, 2003). This mitogenic capacity is linked to the ability of the Fc portion of CD3 MAbs to interact with monocyte Fc receptors (Chatenoud, 2003). Thus "non-mitogenic" CD3 antibodies were obtained by inserting

adequate mutations into the Fc domains to hamper Fc receptor binding (Bolt et al., 1993; Alegre et al., 1994; Chatenoud, 2003). Phase I trials, using the two Fc-mutated CD3 MAbs presently available, OKT3γ1Ala-Ala and ChAglyCD3, in renal allograft recipients who presented with acute rejection episodes, confirmed that their use was free of major side effects (Friend et al., 1999; Woodle et al., 1999).

Engineering Variable Regions of Monoclonal Antibodies to Increase Affinity

X-ray crystallography has illuminated the nature of antigen–antibody interactions. The six CDRs do not contribute equally to the interactions for antigen-binding. Usually, a minimum of four CDRs are used, and the orientation of the CDR loops is critical for antibody specificity and affinity. Mutations within a CDR may create structural changes that, due to their close proximity, may influence other CDRs and greatly impact antigen binding. In addition, inadequate CDR/CDR interactions at the VL/VH interface may prevent domain assembly and antibody expression. This explains why antibody humanization requires detailed molecular modeling of antibody structures to achieve accurate structural predictions. It relies on computer-assisted three-dimensional models to predict the effects of point mutations or of perturbations in the position of the CDR loops during humanization. In this way it has been possible to obtain humanized CDR-grafted antibodies that show minimal reduction in their antigen-binding affinity compared to the parental rodent antibody.

In case the affinity of a given MAb is too low for *in vivo* use, phage display technology is an interesting approach to generate a better and improved fully human reagent with no requirement for prior immunization or use of hybridoma technology. The use of large phage libraries increases the possibility of isolating high-affinity antibodies. In case of failure, phage display can also be used to mimic artificially the processes used *in vivo* by the immune system to generate high-affinity antibodies. This has been achieved by shuffling the heavy or light chains by random or directed mutagenesis of CDRs (Barbas et al., 1994), as done by error-prone polymerase chain reaction (PCR) (Gram et al., 1992). Using such artificial affinity maturation of phage antibody repertoires, affinities of MAbs in the nanomolar to picomolar range have been generated that are perfectly suitable for therapeutic use (Barbas, 1995; Foote and Eisen, 1995).

PEGylation

The use is described of covalent attachment of polyethylene glycol (PEG) to various proteins including antibodies, antibody fragments, and cytokines essentially to prolong their half life (Choy et al., 2002; Yang et al., 2003; Torriani et al., 2004).

Main Targets

T-cell Antigens

CD3 Antibodies

The story of CD3 MAbs is paradoxical and remarkable. They were the first therapeutic antibody introduced in clinical practice in 1981, about 4 years before the molecular complexities and the key functional role of the CD3 molecule were discovered (Clevers et al., 1988; Davis and Chien, 1999). The OKT3 MAb, a mouse IgG2a (Kung et al., 1979), was initially used to treat and prevent renal allograft rejection (Cosimi et al., 1981a; Vigeral et al., 1986; Debure et al., 1988). This occurred without much *in vivo* preclinical data available due to the tight species-specificity of anti-T-cell MAbs in general and CD3 antibodies in particular. Chimpanzees are the only nonhuman primates harboring T lymphocytes that cross-react with MAbs to human CD3. In addition, antibodies to mouse CD3 were difficult to produce, the first one being characterized by Leo et al. in 1987 (Leo et al., 1987). Through the 1980s, controlled studies clearly demonstrated that MAb OKT3 was a potent immunosuppressant very efficient at reversing early acute renal allograft rejection episodes (Cosimi et al., 1981b; Ortho, 1985), an indication for which this MAb was rapidly licensed, both in the USA and Europe. Through the study of OKT3-treated patients, an enormous amount of knowledge was gained on the mode of action of murine anti-T-cell MAbs and their side effects, well-illustrated by over 3000 manuscripts published on the topic. These studies have been invaluable for the design of more refined approaches especially using humanized MAbs.

Over the last 10 years, as other immunosuppressants developed, the use of OKT3 was almost completely abandoned, essentially because of its cytokine-releasing potential (Chatenoud et al., 1986a; 1989; 1990; Cosimi, 1987; Abramowicz et al., 1989; Eason and Cosimi, 1999; Chatenoud, 2003).

CD3 MAbs used *in vitro* in functional studies and *in vivo*, both in the experimental and the clinical setting, are specific for the ε-chain of the CD3 complex. The experimental work conducted in different rat and mouse models suggested that, more than simply depressing all immune responses, CD3 MAbs could also induce immune tolerance to both alloantigens and autoantigens (Hayward and Shreiber, 1989; Nicolls et al., 1993; Plain et al., 1999) and, perhaps more impressively, could restore self-tolerance in established autoimmunity (Chatenoud et al., 1994; 1997; Belghith et al., 2003; Chatenoud, 2003). Based on these data, CD3 MAbs have again entered the clinical arena but now as well-tolerated humanized nonmitogenic MAbs (Bolt et al., 1993; Alegre et al., 1994) used not only in transplantation, but also in autoimmunity in protocols aimed at antigen-specific long-term effects rather than just immunosuppression.

CD3 MAbs and Autoimmune Diabetes

Trials are presently being conducted using anti-CD3 to treat patients with recent-onset type 1 diabetes based on our earlier data on diabetes-prone non-obese diabetic (NOD) mice. Short-term (5 days) treatment of overtly diabetic NOD mice with low-doses (5–20 μg/day) of CD3 MAbs, in either their mitogenic (whole 145 2C11) or nonmitogenic version [F(ab′)2 fragments of 145 2C11], induces disease remission by restoring self-tolerance (Chatenoud, et al., 1994; 1997; Belghith et al., 2003; Chatenoud, 2003). The effect is long-lasting and specific to β cell autoantigens (Chatenoud et al., 1994; 1997). Immune mechanisms mediating this tolerogenic capacity evolve in two distinct consecutive phases (Chatenoud, 2003). The first induction phase coincides with antibody administration and results in clearing of insulitis, explaining the rapid return to normoglycemia, with a transient Th2 polarization that is irrelevant to the long-term effect since there is prolonged remission of disease after anti-CD3 treatment of IL-4 deficient NOD mice (NOD IL-4$^{-/-}$) (Belghith et al., 2003; Chatenoud, 2003). The second maintenance phase results in upregulation and/or appearance of specialized subsets of CD4$^+$CD25$^+$ and CD4$^+$CD62L$^+$ regulatory T cells that mediate transferable active tolerance, and that effectively control pathogenic effector cells as shown by co-transfer experiments in immunodeficient NOD SCID mice (Chatenoud et al., 1994; 2001; Belghith et al., 2003). The proportions of regulatory CD4$^+$CD25$^+$ T cells increase in pancreatic and mesenteric lymph nodes of anti-CD3-treated tolerant mice (Belghith et al., 2003). Interestingly, CD4$^+$CD25$^+$ regulatory T cells induced by anti-CD3 may not be derived exclusively from "conventional" natural suppressor CD4$^+$CD25$^+$ T cells but also, and perhaps even essentially so, from peripheral CD4$^+$CD25$^-$ precursors (Belghith et al., 2003; see Chapters 9 and 10). In fact, CD3-specific MAb treatment induces diabetes remission also in NOD mice that are deficient for the co-stimulation molecule CD28 (NOD CD28$^{-/-}$), and are devoid of the thymic natural suppressor CD4$^+$CD25$^+$ population (Belghith et al., 2003). Also important, the immunoregulatory cytokine-transforming growth factor-β (TGF-β) appears to be a key player in this T-cell-mediated regulation, although its precise role, whether as a mediator of regulation or a growth and/or differentiation factor for regulatory T cells, is not yet determined. Thus, CD4$^+$ T cells from mice tolerant after anti-CD3 consistently produce high levels of TGF-β, and *in vivo* neutralization of TGF-β after injection of specific MAbs fully prevents anti-CD3-specific-induced remission (Belghith et al., 2003).

Clinical trials are underway to ascertain adequate modalities that will reproduce this remarkable effect. Results from an open trial using the OKT3γ1 Ala-Ala MAb in patients who present with recent-onset type 1 diabetes were very encouraging (Herold et al., 2002). Thus, at 1 year after a short-term treatment, a significant preservation of the β cell mass was observed in treated patients compared with controls (Herold et al., 2002). The results of a European multicenter randomized placebo-controlled trial using the ChAglyCD3 MAb, also in autoimmune type 1 diabetes, which we are conducting in collaboration with diabetology centers in Belgium (Belgium Diabetes Registry; Clinical coordinator Prof. B. Keymeulen) and Germany (Prof. A. Ziegler, Munich), fully confirmed the expectations. In fact, results obtained at 18 months of follow-up show not only a significant preservation of the β cell mass in ChAglyCD3-treated versus placebo-treated patients but also an impressive decrease in the insulin needs (Keymeulen et al., 2005). Interesting data have also been reported using the OKT3γ1Ala-Ala MAb in psoriatic arthritis (Utset et al., 2002) and in recipients of islet allografts (Hering et al., 2004).

CD4 Antibodies

The tolerogenic capacity of anti-CD4 MAbs in mice was highlighted from the outset, since, at variance with other xenogeneic MAbs, anti-CD4 did not elicit the usual antiglobulin response. In addition, humoral responses to foreign soluble protein antigens were specifically inhibited if delivery was under the cover of brief treatment with anti-CD4. The effect was obtained with both depleting and non-depleting CD4 antibodies, and it could be maintained long term merely by repeating antigen administration at regular intervals in absence of any further anti-CD4 treatment. Importantly, identical results were recently obtained in non-human primates using a humanized MAb specific for human CD4 and cross-reacting with monkey cells (Winsor-Hines et al., 2004). In this system CD4$^+$ T cells, but not B cells, are rendered tolerant, and effectively transfer such tolerance to naïve hosts. The single caveat is that only high CD4 MAb dosages are tolerogenic. In the mouse, anti-CD4 affords tolerance also to alloantigens (anti-CD4 MAbs are effective alone in the case of cardiac or islet allografts but must be combined to anti-CD8 MAbs in the case of skin allografts) and also often in the case of autoantigens (Wofsy and Seaman, 1987; Shizuru et al., 1988; Qin et al., 1993; Bushell et al., 1995; 2003; Waldmann and Cobbold, 2001; Cobbold et al., 2003). Effective treatment of an ongoing autoimmune disease was demonstrated for murine lupus in NZB/NZW F1 mice (Wofsy and Seaman, 1987), and in established diabetes in NOD mice (Maki et al., 1992), but only when using depleting CD4 antibodies.

The first pilot trials that used mouse MAbs to human CD4 were applied to patients presenting long-standing RA, psoriasis, inflammatory bowel disease, and uveitis (Goldberg et al., 1990; 1991; Emmrich et al., 1991; Horneff et al., 1991; Reiter et al., 1991; Wendling et al., 1991; Morel et al., 1992; Bachelez et al., 1998). In these trials, partial and short-term disappearance of circulating CD4$^+$ cells was observed,

together with coating of $CD4^+$ T-cells and dose-dependent saturation of CD4-binding sites. Results in terms of therapeutic effectiveness were encouraging but short-lasting, and were obscured by the antiglobulin response that rapidly developed and prompted trials of humanized CD4 MAbs.

The chimeric cM-T412 MAb (human IgG1), a depleting antihuman CD4 MAb, was used in RA with encouraging results in open studies, especially with high cumulative doses, ranging from 350–700 mg (Moreland et al., 1993; Van Der Lubbe et al., 1993; 1994); these, however, were not confirmed in a large randomized double-blind placebo-controlled study (Van Der Lubbe et al., 1995). Mild sensitization was reported in a majority of the patients (Moreland et al., 1993; Van Der Lubbe et al., 1993; 1994). Of major concern was the persisting $CD4^+$ cell depletion observed with MAb cM-T412, up to 60% from baseline for over 18–30 months (Moreland et al., 1994), contrary to what had initially been reported with the parental mouse M-T151 anti-CD4 MAb (Reiter et al., 1991). Apoptosis could mediate, at least in part, this massive depletion (Choy et al., 1993). The disappointing clinical results in ongoing RA are not necessarily surprising, since they are fully in keeping with preclinical data. In fact, in collagen-induced arthritis, a murine model for human RA, anti-CD4 in contrast to MAbs to TNF (discussed below) were effective for prevention but not for treatment of ongoing disease (Marinova-Mutafchieva et al., 2000). The cM-T412 MAb has also been used in patients with multiple sclerosis, but here again without any evident clinical benefit (van Oosten et al., 1997).

The nondepleting humanized MAb to CD4, OKT4cdr4A (the CDR-grafted version of OKT4A), was used in patients with severe psoriasis; one open pilot study and another placebo-controlled one were conducted, with promising data (Bachelez et al., 1998; Gottlieb et al., 2000).

Results from open-pilot studies suggested that CD4 MAb therapy, especially in combination with antibodies to CD52, could be effective in treating severe forms of vasculitis (Mathieson et al., 1990; Lockwood et al., 1993).

It is fair to conclude this section on anti-CD4 MAbs by pointing out that their development was seriously hampered by non-scientific considerations linked to patenting and marketing, explaining the incredible gap between the wealth of experimental data accrued and the paucity of controlled clinical trials conducted. It is hoped that CD4 antibodies will soon be made available for trials especially designed on the lessons drawn form preclinical studies.

CD25 Antibodies

CD25, the α-chain of the interleukin 2 (IL-2) receptor, is expressed on activated T cells (Waldmann, 1989), which explains the interest in it as a therapeutic target. Experimental data suggested an immunosuppressive capacity of anti-CD25 that significantly delayed rejection of heart allografts in the mouse (Kirkman et al., 1985) and of renal allografts in nonhuman primates (Reed et al., 1989). In autoimmunity anti-CD25 MAb prevented onset of collagen-induced arthritis (Banerjee et al., 1988), insulitis, and diabetes in NOD mice (Kelley et al., 1988) and lupus nephritis in NZB/NZW F1 mice (Kelley et al., 1988). In diabetes-prone Bio Breeding (BB) rats there was reversal of established disease after treatment with anti-CD25 and low-dose cyclosporine (Hahn et al., 1987).

Murine MAbs to human CD25 were effective for prevention but not for reversal of renal allograft rejection (Soulillou et al., 1990; Kirkman et al., 1991; Kriaa et al., 1993). By the late 1990s, two humanized MAbs to CD25—one chimeric [basiliximab (Simulect)] and one CDR-grafted (daclizumab [Zenapax])—were shown to be well-tolerated and effective as part of induction regimens to prevent organ allograft rejection, and were approved for use as induction therapies in organ transplantation (Nashan et al., 1997; Vincenti et al., 1998; Waldmann and O'Shea, 1998; Bumgardner et al., 2001). Moreover humanized CD25 antibodies appear beneficial for treatment of established rejection (Adu et al., 2003) and of some severe autoimmune diseases, uveitis in particular (Guex-Crosier et al., 1997; Nussenblatt, 2002).

Last but not least, none of the studies support a tolerance-promoting activity of anti-CD25 perhaps because CD25 is also expressed on a subset of T-cells endowed with regulatory/suppressor capacities that are critical in maintaining immune tolerance (Belghith et al., 2003; Sakaguchi, 2004). Thus, elimination or inhibition of the functional capacity of this subset using anti-CD25 MAbs might be counterproductive if the aim is tolerance induction.

Targeting Co-stimulatory Pathways

The CD28/CD80, 86 (B7.1, B7.2) Pathway

Delivery of activation signals through the T-cell receptor/CD3 pathway is insufficient to activate naïve T cells. Co-stimulation signals are required that are transduced through the specialized receptor CD28, which interacts with its specific ligands CD80 (B7.1) and CD86 (B7.2) at the surface of the antigen presenting cell (APC). Fusion proteins have been produced using the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4, CD152) molecule that is homologous to CD28 and expressed on T cells following activation (Linsley et al., 1991). CTLA-4 is also constitutively expressed on the specialized regulatory/suppressor T-cell subset $CD4^+CD25^+$ (Read et al., 2000; Takahashi et al., 2000). CTLA-4Ig is a fusion protein including the extracellular domain of CTLA-4 combined to the Fc domain of a

human IgG1 (Linsley et al., 1992), and shows higher avidity for CD80 than CD86. LEA29Y is a CTLA4-4Ig derived molecule with two amino-acid mutations that confer higher avidity for both CD80 and CD86. This explains why LEA29Y is more potent *in vitro* than CTLA-4Ig.

Treatment *in vivo* with CTLA-4Ig effectively blocked immune responses to alloantigens (Lin et al., 1993; Lakkis et al., 1997; Onodera et al., 1997), xenoantigens (Lenschow et al., 1992), and autoantigens. In NZB/NZW mice, treatment with CTLA-4Ig blocked autoantibody production and prolonged mouse half-life (Finck et al., 1994). Mostly CTLA4-Ig was more effective for preventing than reversing established immune responses and generally a robust and long-standing tolerance was not the general rule.

In the clinic, in a phase I open-label study on 43 patients with psoriasis, CTLA-4Ig resulted in >50% sustained (>6 months) improvement in disease activity in 46% of patients (Abrams et al., 1999). CTLA-4Ig also appeared promising in RA, according to a pilot, escalating dose, double-blind placebo-controlled study on 214 patients, given CTLA-4Ig, LEA29Y, or placebo I.V., four infusions in all. Patients were assessed at 2.5 months after the first injection. Both agents had an excellent safety profile, and clinical efficacy was superior to placebo (Moreland et al., 2002). These good results were confirmed in a second controlled study using CTLA-4Ig or placebo in combination with methotrexate, again with significant improvement for the CTL4-4Ig-treated group (Kremer et al., 2003).

The CD40/CD154 (CD40) Ligand Pathway

CD40 is a member of the TNF receptor superfamily and is constitutively expressed by various cell types including B lymphocytes and APCs. CD40 interacts with CD154 (CD40L), a member of the TNF superfamily exclusively expressed at the surface of activated T lymphocytes. The CD40/CD154 co-stimulation pathway is essential for both T–B and T–T cooperation. CD40/CD154 interactions lead to germinal-center formation and immunoglobulin class switching. Severe immunodeficiency is caused by disruption of this pathway in mice due to gene knockout, or spontaneous mutations in humans. Concerning T–T cooperation, studies in the mouse and in nonhuman primates using *in vivo* administration of antibodies to CD154, either alone or in combination with CTLA-4Ig, showed great promise in prolongation of allograft survival and prevention of autoimmune disease (Larsen et al., 1996; Larsen and Pearson, 1997; Niimi et al., 1998; Kirk et al., 1999), albeit rapidly tempered when the first clinical trials in renal allograft recipients and in patients with SLE showed severe thromboembolic events (Boumpas et al., 2003) with one of the MAbs used. While such events were not observed with the IDEC-131 MAb, this proved ineffective in patients with SLE (Davis et al., 2001; Kalunian et al., 2002; Kuwana et al., 2004).

Adhesion Molecules

The $\alpha 4\beta 1$ Integrin VLA-4

Integrins are adhesion molecules of fundamental importance to the recruitment of leukocytes in inflammation. The $\alpha 4\beta 1$ integrin VLA-4 is a leukocyte ligand for endothelial vascular cell adhesion molecule-1 (VCAM-1), fibronectin, and osteopontin. The interaction between VLA-4 at the surface of activated lymphocytes and monocytes with its ligand VCAM-1 is essential for cell migration into inflamed parenchyma. Promising data in experimental models of blockade of VLA-4 prompted use of a specific humanized monoclonal antibody (natalizumab) in randomized placebo-controlled trials, first in multiple sclerosis (MS) (Miller et al., 2003). In 213 patients with relapsing-remitting or relapsing secondary progressive MS given natalizumab or placebo every 28 days for 6 months, there was a marked reduction in the number of new brain lesions [gadolinium-enhanced magnetic resonance imaging (MRI)] in treated patients (Miller et al., 2003). The same antibody was applied in Crohn's disease (Ghosh et al., 2003). Similarly in 248 patients with moderate to severe Crohn's disease, two infusions of MAb given 4 weeks apart induced higher remission rates than did placebo and significant improvement in the Crohn's disease activity index, together with improved quality of life (Ghosh et al., 2003). Unfortunately, recent reports indicate that, upon chronic administration, this antibody exposes the risk of opportunistic brain infection caused by the JC virus. The first two cases were observed in MS patients receiving natalizumab associated with interferon-β (IFN-β). The last reported case affected a patient with Crohn's disease treated with natalizumab alone.

Leukocyte Function-Associated Antigen 1

A humanized MAb, efalizumab, specific for the CD11a subunit of leukocyte function-associated antigen 1 (LFA-1), has been tested in psoriasis. When administered subcutaneously once a week, improvement was observed within 2–4 weeks, and lasted for up to 2 years (Leonardi, 2004). Results from three randomized, placebo-controlled trials in patients exhibiting moderate-to-severe plaque psoriasis likewise showed promising results (Menter et al., 2004); here the follow-up lasted for 12 weeks as a double-blind trial, and there was an additional 12 weeks of extended treatment phase (Menter et al., 2004).

B-Cell Antigens (CD20)

Rituximab (Rituxan) is a human–mouse chimeric MAb specific to CD20, a B-cell antigen, which causes rapid depletion of B lymphocytes. Rituximab was approved in the

United States of America in 1997 and in Europe in 1998 (MabThera) to treat severe refractory CD20-positive non-Hodgkin B-cell lymphoma. The use of anti-CD20 has now been extended to first-line therapy and maintenance therapy in lymphoma, stem-cell transplantation procedures, and, recently, for autoimmune disorders, including RA, immune thrombocytopenic purpura, autoimmune hemolytic anemia, SLE, vasculitis, dermatomyositis, and MS (De Vita et al., 2002; Leandro et al., 2002; Silverman and Weisman, 2003; Looney et al., 2004a; 2004b; 2004c; Rastetter et al., 2004). A phase II randomized, double-blind, placebo-controlled trial in RA showed that treatment with anti-CD20 was safe and led to major and sustained clinical responses (Edwards et al., 2004). At the more fundamental level, these results highlight the pathogenic role of B-lymphocytes, not only as antibody producing cells but also as APCs, which hitherto had long been considered of marginal importance in some of these autoimmune diseases.

Leukocyte Antigens (CD52)

Antibodies to CD52 target a small (12 amino acids) glycosylphosphatidylinositol (GPI)-anchored protein of undefined function expressed at the surface of human B and T cells and monocytes/macrophages. Anti-CD52 MAbs are highly depleting and have potent efficacy in long-term acceptance of organ allografts and maintaining remission in established and otherwise intractable autoimmune diseases, notably MS, vasculitis, and RA (Mathieson et al., 1990; Lockwood et al., 1993; Lockwood et al., 1996; Calne et al., 1999).

The first rat MAb to CD52, Campath-1M, was characterized in 1983. A fully reshaped humanized version, Campath-1H (human IgG1) was derived by genetic engineering (Riechmann et al., 1988) and is marketed as alemtuzumab. Its depleting capacity has led to its extensive use *in vivo* to treat $CD52^+$ hematologic malignancies, and *in vitro* to purge bone marrow transplants to prevent graft-versus-host disease (GvHD). Upon the first injection, Campath-1H triggers an acute self-limited cytokine release that causes a transient "flu-like" syndrome.

Campath-1H has been given to patients with RA, of whom, after 3 and 6 months, 50% showed improvement in the Paulus score (Isaacs et al., 1992). It also proved very effective in severe systemic small-vessel vasculitis in which the pathogenesis depends mainly on T-cell-mediated mechanisms (see Chapter 65) (Mathieson et al., 1990; Lockwood et al., 1993; 1996). The long-term remissions that were obtained when combining antibodies to CD52 and CD4 were particularly impressive (Mathieson et al., 1990; Lockwood et al., 1993; 1996).

Results with anti-CD52 in MS are also promising (Moreau et al., 1994; Coles et al., 1999a; 1999b). The initial trials included patients with long-standing relapsing/remitting MS that was unresponsive to conventional treatments. Long-term follow-up showed a marked decrease in the appearance of new lesions in the CNS, assessed by MRI, that correlated with the persisting and significant depletion of peripheral $CD4^+$ T lymphocytes (Moreau et al., 1994; Coles et al., 1999a; 1999b; 2004). An unexpected adverse effect was the appearance of Graves' disease in a significant proportion (33%) of successfully treated patients (Coles et al., 1999b). Interestingly, current multicenter trials that include patients with MS of more recent onset do not appear to expose to this adverse event (A. Coles, personal communication).

In general, Campath-1H seems unique in its capacity to promote long-lasting remission of life-threatening autoimmune diseases that are unresponsive to conventional treatments, but results from trials in progress are needed for more definite conclusions especially the risk/benefit ratio.

Cytokines

Blocking Tumor Necrosis Factor Pathways

Humanized MAbs to TNF proved a major breakthrough in the treatment of RA, consequent to the pioneering experimental and clinical work of the groups of Feldmann and Maini (Brennan et al., 1989; Elliott et al., 1994a; Feldmann, 2002). The seminal finding was that neutralizing antibodies to TNF significantly decreased the production of most of the pro-inflammatory cytokines (i.e., IL-1, IL-6, IL-8, GM-CSF) normally produced in *in vitro* cultures of cells that infiltrate synovial membranes in RA (Brennan et al., 1989; Brennan and Feldmann, 1992; Feldmann, 2002). The relevance of this finding to events *in vivo* was validated in mice that express a human TNF transgene and develop a form of chronic arthritis fully preventable by MAbs to TNF (Brennan and Feldmann, 1992; Feldmann, 2002). In addition, in CIA, neutralizing antibodies to murine TNF given at the onset of disease decreased the severity of objective and histopathologic features (swollen joints and bone erosions) (Piguet et al., 1992; Williams et al., 1992; 1994). An unexpected but potentially relevant observation was that, in established arthritis, combination of a suboptimal dose of anti-TNF (which had no significant effect per se) with anti-CD4 greatly improved joint inflammation and helped heal paw-swelling and bone erosions (Williams et al., 1994; Marinova-Mutafchieva et al., 2000). Thus neutralizing inflammation, as with anti-TNF, effectively "sensitizes" the immune system to T-cell-directed immunointervention: this could be relevant to various autoimmune diseases other than RA.

The first randomized placebo-controlled double-blind study showing effectiveness of the chimeric neutralizing antibody to TNF, cA2 (human IgG1, now termed infliximab [Remicade]) in long-standing RA was reported in 1994 (Elliott et al., 1994a); there was significant therapeutic benefit lasting for several weeks after the end of treatment. Some patients with relapse of RA underwent two or more

courses of treatment; the mean duration of remissions progressively diminished due to sensitization to the MAb (Elliott et al., 1994b). In further studies, anti-TNF was combined with methotrexate, thus avoiding sensitization and obtaining longer-lasting remissions (Maini et al., 1998; Feldmann and Maini, 2001; Feldmann, 2002). Data from a phase III trial showed that anti-TNF arrested joint damage in more than 50% of patients; the effect was noted by 6 months after beginning of treatment and lasted for the 2 years of the study (Maini et al., 1999; Lipsky et al., 2000). Infliximab was, therefore, approved for use, in combination with methotrexate, both in the USA and Europe.

Given these results with infliximab, other biologic agents against TNF were developed. Another chimeric anti-TNF MAb named CDP571 also was clinically effective (Rankin et al., 1995), as were two fusion proteins linking the TNF receptor molecules p55 or p75 to a human IgG constant region [lenercept and etanercept (Enbrel), respectively] (Moreland et al., 1996; 1997; Furst et al., 2003). However, only etanercept was actively developed in the clinic and became approved. Other interesting candidates are on the way, one being the fully human D2E7 antibody (adalimumab) that has shown efficacy in phase II and III trials (Kempeni, 1999; den Broeder et al., 2002).

Also MAbs to TNF were used successfully in severe Crohn's disease and have been approved for this use (Van Dullemen et al., 1995; Present et al., 1999). Although the pathophysiology of Crohn's disease remains unclear (see Chapter 52) inflammatory cytokines are significantly involved (Van Deventer, 1997). The therapeutic benefit derived from anti-TNF treatment correlated with a decreased production of IFN-γ by mononuclear cells infiltrating the lamina propria of the colon (Plevy et al., 1997). Interestingly, at variance with what is observed in RA, TNF receptor fusion proteins were not effective in Crohn's disease.

Subsequently data from trials using anti-TNF in juvenile idiopathic arthritis (juvenile RA) (Lovell et al., 2000), ankylosing spondylitis (Brandt et al., 2000), psoriatic arthritis (Mease et al., 2000) and psoriasis have been published (Chaudhari et al., 2001).

One adverse effect reported, especially in patients undergoing repeated treatments with infliximab, was the increased incidence of tuberculosis (both pulmonary and extrapulmonary disease) (Keane et al., 2001; Gomez-Reino et al., 2003). This is one reason why combination therapy with drugs aimed at neutralizing TNF and IL-1 has been recently disallowed by the Food and Drug Administration in the USA. Other side effects that have been related to TNF blockers include the induction of autoantibodies and the occurrence of non-Hodgkin lymphoma (Brown et al., 2002; Wolfe and Michaud, 2004). Autoantibody formation is commonly seen in patients receiving prolonged treatment with infliximab (Louis et al., 2003; Caramaschi et al., 2004). These include antinuclear antibodies, anti-Sm, anti-RNP, and, in few cases, anti-double-stranded DNA autoantibodies; their presence is usually not associated with clinical signs of multisystem autoimmune disease. Concerning the occurrence of lymphoma Wolfe and Michaud recently reported an extensive study on 18,572 patients with RA who were enrolled in the National Data Bank for Rheumatic Diseases (Wolfe and Michaud, 2004). The overall standardized incidence ratio for lymphoma in RA patients not receiving methotrexate or biologics was 1.0 [95% confidence interval (CI) 0.4–2.5], and it was 2.9 (95% CI 1.7–4.9) in patients treated with TNF blockers, 2.6 (95% CI 1.4–4.5) in patients receiving infliximab (with or without etanercept), and 3.8 (95% CI 1.9–7.5) in patients receiving etanercept, with or without infliximab (Wolfe and Michaud, 2004). The authors concluded that current data are insufficient to firmly establish a causal relationship because lymphomas are increased in RA independently from the treatment. Although the risk appears higher when TNF blockers are administered, differences between therapies are slight, and confidence intervals for treatment groups overlap.

Antibodies to Interferon-Gamma

Treatment with antibodies to IFN-γ has been attempted in several experimental autoimmune diseases on the rationale that the central effects of IFN-γ in Th1-mediated immune responses include macrophage activation and upregulation of major histocompatibility complex molecules. MAbs to IFN-γ successfully prevented autoimmune diabetes in BB rats and NOD mice (Nicoletti et al., 1990; Debray-sachs et al., 1991). In (NZB × NZW) F1 mice, anti-IFN-γ treatment when started early (by 4 months of age) delayed the onset of glomerulonephritis and significantly prolonged survival; anti-IFN-γ appeared to act by inhibiting production of anti-DNA autoantibodies rather than affecting immune complex formation.

In patients with RA, a randomized double-blind trial compared the effectiveness of treatment with anti-IFN-γ versus anti-TNF or placebo (Sigidin et al., 2001). Promising data were obtained by 4 weeks post-treatment but longer term studies are needed.

Other Soluble Receptors and Protein Fusion Conjugates

The use of soluble receptors to TNF and CTLA-4Ig has already been discussed. Another human fusion protein was recently developed by coupling LFA-3 to human IgG1 to provide an agent (alefacept) that binds to CD2 on T lymphocytes; in controlled multicenter studies, this agent was used weekly in patients with chronic plaque psoriasis for one or two 12-week courses. The drug was well-tolerated, and a minimal 75% reduction of the disease score (PASI index) was observed 2 weeks from the last dose, lasting for 7 months (Krueger, 2003; Lebwohl et al., 2003).

Alefacept has been approved by regulatory authorities in the USA for this indication. There was a reduction of memory T cells ($CD45R0^+$ that include pathogenic cells) in treated patients that correlated with measurements of disease activity; lymphocytes expressing a naïve phenotype were spared (Krueger, 2003; Lebwohl et al., 2003).

A recombinant human IL-1 receptor antagonist (IL-1Ra), anakinra, has been tested in severe rheumatic diseases with encouraging results in patients with juvenile idiopathic arthritis resistant to other drugs (Verbsky and White, 2004). Data were also reported in refractory SLE (Moosig et al., 2004). In RA large placebo-controlled trials including international multicenter studies have demonstrated safety of the drug as well as a sustained clinical improvement (Nuki et al., 2002; Fleischmann et al., 2003). Importantly, data also suggested that IL-1Ra significantly reduced radiologic progression of bone erosions (Jiang et al., 2000).

A new strategy aimed at producing a massive though selective apoptosis of recently activated T cells has been developed by Strom and colleagues. They constructed cytolytic chimeric IL-2/Fc and IL-15/Fc fusion proteins that specifically bind with high affinity and kill cells bearing IL-2 and IL-15 receptors by complement-dependent and antibody-dependent cytotoxicity (Zheng et al., 1999; 2003a; 2003b; Ferrari-Lacraz et al., 2004). When administered alone, these IL-2/Fc and IL-15/Fc fusion proteins prevented autoimmune diabetes in NOD mice, and also CIA (Zheng et al., 1999; 2003a; 2003b; Ferrari-Lacraz et al., 2004). Moreover, combining these two agents together with rapamycin provided an extremely efficient means of restoring self-tolerance in NOD mice presenting with recent-onset disease, and inducing transplant tolerance in a particularly stringent model, i.e., implantation of allogeneic islets in overtly diabetic NOD mice (Zheng et al., 2003a; 2003b). Mechanistic studies showed that, while limiting the early expansion of activated T-cells and forcing their apoptosis, this regimen nevertheless preserved regulatory $CD4^+CD25^+$ T cells (Zheng et al., 2003a; 2003b).

Soluble Autoantigens

Immunologic tolerance to a wide spectrum of antigens (and autoantigens) can be induced by parenteral, nasal, or oral delivery of a soluble antigen. This approach has proven to be successful in several animal models of autoimmune diseases, either spontaneous or experimentally induced by immunization against autoantigens. Thus the onset of diabetes in NOD mice can be prevented by administration of insulin or GAD using various routes of administration subcutaneous, I.V., nasal, or oral. Similarly, experimental allergic encephalomyelitis (EAE) is preventable by administration of the soluble myelin antigen which is ultimately used to induce the disease.

The details of the underlying mechanisms are the subject of another chapter (see Chapter 75), but mention can be made that autoantigen-induced tolerance, whether using proteins, peptides, or altered peptide ligands, is beset by a number of difficulties. These include limitation of the treatment to early disease stages; loss of therapeutic effectiveness as disease progresses; a long lag time to achieve efficacy, which may represent a problem in the case of acute autoimmune responses; risk of disease acceleration by triggering rather than downregulating the autoimmune response; sensitization with potential risks of anaphylaxis and/or production of neutralizing antibodies leading to serious problems when the autoantigen molecule (e.g., insulin) is physiologically relevant.

Bone Marrow Transplantation

Autoimmune diseases include genetic components expressed in the lymphoid and macrophage lineages and thus qualify as stem-cell disorders (Ikehara et al., 1990). Hence, patients with serious autoimmune diseases can be considered for high-dose immunosuppression followed by hematopoietic stem-cell transplantation (HSCT) (Marmont et al., 1997; Tyndall et al., 1997; Ikehara, 1998). This strategy was initially based on clinical observations in patients with malignancies and concurrent autoimmune diseases (McAllister et al., 1997), as well as results of HSCT in experimental models (Karussis et al., 1992; 1993; van Bekkum, 1998). The latter showed that all types of HSCT, whether allogeneic or autologous, may induce high remission rates provided adequate conditioning regimens are administered. For example, excellent results were obtained in murine models of spontaneous autoimmunity such as autoimmune diabetes in the NOD mouse or BB rat, and lupus in (NZB × NZW) F1 mice (Ikehara et al., 1985). The mechanisms involve both central and peripheral chimerism. Although successful, the strategy is however hardly applicable in humans, due to the hazards associated with conditioning regimens and potential GvHD disease.

More attention has been given in serious autoimmune diseases to transplantation of autologous bone-marrow-derived stem cells, with the initial aim to induce very deep immunosuppression by reason of the protection afforded by bone marrow cell reconstitution, to reduce the disease activity, or even provide a cure. Other modes of action may be operative, such as the resetting of immunoregulatory circuits. Thus, regulatory T cells may recover before effector cells, or benefit may come from the initial elimination of the latter. There is also the possibly useful role of granulocyte-colony-stimulating factor (G-CSF), usually administered pretransplant to mobilize stem cells or post-transplant to accelerate reconstitution, which could be beneficial by downregulating effector cells as shown for NOD mice (Kared, 2004) and mice with EAE (Zavala et al., 2002).

The First International Symposium on Haemopoietic Stem Cell Therapy in autoimmune diseases took place in 1996 (Tyndall et al., 1997), and inaugurated collaborations

including publication by the European Group for Blood and Marrow Transplantation (EBMT), European League Against Rheumatism (EULAR), and the European Charcot Foundation of consensus reports of guidelines for autologous HSCT in autoimmune diseases, and for reports by participating centers on their results to the EBMT registry.

The source of the stem-cell transplant is mostly mobilized cells from peripheral blood, as these appear to afford a faster and more complete recovery compared with bone-marrow-derived stem cells. Mobilization of blood stem cells is performed using cyclophosphamide in combination with hematopoietic growth factors, G-CSF alone, or combined with GM-CSF. According to different studies, the grafts are either purged of mature T cells, which may contain autoreactive effectors, by selection of $CD34^+$ cells with or without additional MAb-dependent T-cell-depletion, or are not manipulated. Pretransplant conditioning regimens, for which the aim is to ablate as completely as possible the diseased (autoreactive) component of the immune system, include chemotherapy-based regimens, such as BEAM [BCNU (carmustine), etoposide, cytarabine, melphalan], cyclophosphamide with or without antilymphocyte globulins and with or without other drugs, or total body irradiation and busulfan.

Recent reports provide a conspectus of results on HSCT in rheumatic diseases and MS. Farge et al. reported on 57 patients with systemic sclerosis in European phase I/II studies from 1996 to 2002, with a response in two-thirds of these over a follow-up of 36 months, which was durable and with an "acceptable" morbidity/mortality risk (Farge et al., 2004). At 5 years, the progression probability was 48% and the projected survival was 72% (Farge et al., 2004) leading to randomized trials now in progress. In another report, Van Laar and Tyndall reviewed the results of recent phase I/II studies and data from the EBMT/EULAR registry on more than 400 patients with autoimmune diseases including RA, SLE, systemic sclerosis, and juvenile idiopathic arthritis (Van Laar and Tyndall, 2003). Treatment-related mortality was low in RA (1.4%) but relatively high (>10%) in patients with juvenile idiopathic arthritis, SLE, and systemic sclerosis, possibly related to visceral involvement. With the application of uniform and strict criteria, safety has improved. Long-term remissions of up to 4 years have been observed in systemic sclerosis and juvenile idiopathic arthritis, while relatively more relapses have occurred in patients with SLE and RA although sensitivity to antirheumatic drugs was restored in these patients thus resulting in improved disease control (Van Laar and Tyndall, 2003). Data from a multicenter retrospective study assessing autologous HSCT in the treatment of poor-risk patients with MS (Fassas et al., 2002) indicated possible benefit as judged by a confirmed progression-free survival probability of 74% at 3 years post-transplant; progression-free survival rates were higher in patients below 40 years of age (89%) and for those not suffering from permanent progressive MS (78%). Treatment-related mortality was high (6%), though not significantly different from that after autologous HSCT in lymphoma. It remains to be determined what level of risk is acceptable for patients with an autoimmune disease that is seldom life-threatening. In fact, autologous HSCT seems justified only if this will confer a clear-cut benefit compared with what can be achieved by existing treatments.

Cell Therapy and Gene Therapy

Cell Therapy

The culture *in vitro* of specialized subsets of immune cells that can be re-infused into a subject with an autoimmune disease is another emerging therapy that has benefited from experience with tumor immunology. Two cell types in particular have elicited interest—tolerogenic dendritic cells and regulatory T cells.

Dendritic cells are potent stimulators of immune responses but, when appropriately manipulated *in vitro*, express powerful tolerogenic properties shown by suppression *in vivo* of alloimune and autoimmune responses. Several factors influence this tolerogenic capacity of dendritic cells, including the precise subset of dendritic cell considered, and the degree of differentiation/maturation: immature or "semi-mature" dendritic cells are tolerogenic whereas mature dendritic cells are immunogenic. Several *in vitro* procedures have been described to derive tolerogenic dendritic cells, including treatment with CTLA-4Ig (Ben-yedidia et al., 1999), IL-10 (Takayama et al., 1998), vitamin D3 (Adorini, 2004), or TGF-β (Alard et al., 2004). The cellular and molecular mechanisms that drive the modulatory capacity of such tolerizing dendritic cells vary, depending on the model, and although only partly understood, these mechanisms mostly rely on a capacity to trigger states of peripheral tolerance, either anergy, immune deviation, or the induction of regulatory T cells.

The culture of regulatory T cells is another option. According to recent data, *in vitro* expanded $CD25^+$ regulatory T cells were highly effective in reversing established diabetes in NOD mice (Bluestone and Tang, 2004; Tang et al., 2004).

Gene Therapy

It is tempting to treat autoimmune diseases by administration of immunoregulatory cytokines. Although there have been some data from experimental models including use of IL-4 and IL-10 in NOD mice (Rapoport et al., 1993) and IL-10 in EAE (Rott et al., 1994), clinical application appears problematic with the possible exception of IL-10 in Crohn's disease (Tilg et al., 2002).

An alternative way of administering recombinant proteins or autoantigens is based on the principles of gene therapy with the advantage of a targeted delivery of high amounts of the selected therapeutic protein.

Gene therapy has successfully been used in several settings for the treatment of autoimmune diseases in animal models. Transduction with genes for regulatory cytokines, notably IL-4 (Yamamoto et al., 2001), IL-10 (Moritani et al., 1996), and TGF-β (Piccirillo et al., 1998) has been shown to protect mice from autoimmune diabetes (NOD), and also from EAE (Tarner et al., 2003). In some cases, immune cells were transduced, with the idea that these cells would deliver the cytokine *in situ* after they had homed to the target organ (Tarner et al., 2003; Yamamoto et al., 2001). It has also been possible to inhibit the progression of advanced diabetes in NOD mice by genetic induction of large amounts of an intracellular adhesion molecule (ICAM-1) fusion protein (Bertry-Coussot et al., 2002).

The difficulties associated with clinical application of gene therapy are considerable. These include the selection of an efficacious and safe vector, long-term maintenance of the expression of the transduced gene, difficulties in controlling the amount of protein produced with risks of uncontrollable hyperproduction, and uncertainty about the site of delivery of the expressed protein (see Bottino et al., 2003 for review). Ingenious procedures are being developed in genetic diseases, and in cancer, where the need for gene therapy is urgent. Perhaps only when the problems are solved in these settings will gene therapy become applicable to human autoimmune diseases. Meanwhile use should be encouraged in animal models of autoimmunity to provide original information on disease mechanisms and prepare for future clinical applications.

There are as yet early experimental studies aiming at promoting target cell reconstitution, applicable to islet cells in diabetes, by antecedent administration of precursor cells and subsequent genetic manipulation to commit them to the β-cell lineage. There is a double caveat: the intrinsic difficulty of the procedure, and the likelihood of relapse of the autoimmune process on newly generated cells (Bottino et al., 2003).

CONTRASTED APPLICATIONS OF EMERGENT IMMUNOTHERAPIES IN HUMAN AUTOIMMUNE DISEASES

It may seem surprising that the emergent therapies discussed above are not in fact being applied to various autoimmune diseases, but there are some good reasons for this. First, some autoimmune diseases are antibody-mediated (e.g., SLE or myasthenia gravis) whereas others are T-cell-mediated (e.g., type 1 diabetes and psoriasis). Of course B-cell-targeted therapies should be limited to the former and T-cell-targeted strategies more to the latter; this is not absolute, however, since antibody production requires T-cell help, and T-cell responses may depend on B-cell-mediated antigen presentation. Second, primary effector mechanisms vary considerably among autoimmune diseases since TNF appears to be of crucial importance in RA and Crohn's disease, whereas $CD4^+$ and $CD8^+$ T cells are main effectors in type 1 diabetes. Third, some autoimmune diseases will be more used to establish proof of principle because of their easy qualitative and report readout, as for psoriasis, which is often selected for phase I study even though not qualifying as a *bona fide* autoimmune disease and perhaps not truly representative of all autoimmune diseases. Rheumatoid arthritis is often primarily selected because of the large consumer market it represents even though it is a very difficult disease for early evaluation of drug efficacy, and also is not necessarily representative of other autoimmune diseases at the pathophysiologic level. On the other hand, trials in MS can only provide significant data after laborious long-term study, although the introduction of MRI has accelerated and improved the clinical evaluation.

All these issues point to differences among the various autoimmune diseases. It is necessary, though, to consider the following points: that IFN-β has essentially been developed for treatment of MS, but its immunomodulatory properties could probably benefit patients with other autoimmune diseases; that anti-TNF antibodies have so far been used in RA, spondyloarthropathies, Crohn's disease, and more recently psoriasis, yet this cytokine is probably involved in many other autoimmune diseases; and that anti-T-cell antibodies have been used in a rather limited fashion (e.g., anti-CD3 in type 1 diabetes, anti-CD52 in MS and RA, anti-CD4 in RA), although T cells are involved in the majority of autoimmune diseases.

Similar comments can be made for other agents: CTLA-4Ig has been limited to use in psoriasis and RA, LFA3-Ig to psoriasis, anti-CD25 to uveitis, and anti-VLA-4 to MS and Crohn's disease, even though there is no logical reason whatsoever for the preferential usage of these agents in any of these diseases.

One, therefore, hopes that, despite the commercial or ethical difficulties connected with the application of these biologic therapies in a multitude of diseases, the remarkable results achieved should ensure that these effective agents become accessible to all patients who could benefit from them.

WHAT ARE THE PERSPECTIVES FOR THE FUTURE?

In addition to policies based on disease severity (risk-to-benefit ratio), the overall policy will vary according to the phases of progression of a particular disease and the perception of urgency at the onset of the autoimmune response. When there is urgency, as pertains in the case of recently-diagnosed type 1 diabetes or MS, the hope is that the auto-

immune destruction can be halted before irreversible lesions develop. This is not done at present because patients with MS are usually given high-dose corticosteroid treatment at the time of diagnosis, which may not be optimal. Patients with type 1 diabetes are not given any immunotherapy because of the availability of a substitutive treatment, insulin. Here, anti-T-cell MAb, of which the most promising is anti-CD3, is an attractive approach: anti-CD3 acts rapidly (within 24 hours) and the effect is long-lasting after a short therapeutic course (Herold et al., 2002; Keymeulen et al., 2005).

When inflammation is more prominent, consideration should be given to new anti-inflammatory methods exemplified by MAbs to TNF or IL-1 RA. It should be realized, however, that these methods of countering inflammation have only short-term effects and should supplement rather than replace the many other approaches discussed above, notably in RA.

When the autoimmune disease exposes the patient to a lethal risk, or major morbidity, examples being MS, very severe SLE, vasculitis, or rapidly progressing juvenile RA, high-risk treatments must be considered such as high-dose nonspecific chemical immunosuppression associated with autologous bone marrow transplantation.

At the end of this discussion it may be wondered where precisely therapy with corticosteroids and chemical immunosuppressants fits in for the management of autoimmune disease. Currently, these drugs still represent the best-established method of managing an autoimmune disease, particularly so for corticosteroids, which are still impossible to replace in many autoimmune diseases. However, their lack of specificity for the underlying autoimmune responses, and the exposure of patients to the risks of over-immunosuppression and direct drug toxicity should lead to a progressive reduction in their usage. As a conclusion, it is hoped, if not anticipated, that emergent therapies based on the progress of biotechnology, including gene and cell therapy, will progressively complement and perhaps replace conventional treatments. The rapidity with which anti-TNF antibodies have become accessible to patients with RA and Crohn's disease is more than encouraging. Nevertheless, there are still numerous problems concerning the development and evaluation of the various drugs being studied. The multitude of these drugs and their potential clinical applications are remarkable. Major efforts should be made to identify the best applications and promote their development for the benefit of patients beyond commercial constraints.

References

Abramowicz, D., Schandene, L., Goldman, M., Crusiaux, A., Vereerstraeten, P., De Pauw, L., Wybran, J., Kinnaert, P., Dupont, E., and Toussaint, C. (1989). Release of tumor necrosis factor, interleukin-2, and gamma-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. *Transplantation 47*, 606–608.

Abramowicz, D., Crusiaux, A., and Goldman, M. (1992). Anaphylactic shock after retreatment with OKT3 monoclonal antibody. *N. Engl. J. Med. 327*, 736.

Abramowicz, D., Crusiaux, A., Niaudet, P., Kreis, H., Chatenoud, L., and Goldman, M. (1996). The IgE humoral response in OKT3-treated patients—Incidence and fine specificity. *Transplantation 61*, 577–581.

Abrams, J.R., Lebwohl, M.G., Guzzo, C.A., Jegasothy, B.V., Goldfarb, M.T., Goffe, B.S., Menter, A., Lowe, N.J., Krueger, G., Brown, M.J., Weiner, R.S., Birkhofer, M.J., Warner, G.L., Berry, K.K., Linsley, P.S., Krueger, J.G., Ochs, H.D., Kelley, S.L., and Kang, S. (1999). CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J. Clin. Invest. 103*, 1243–1252.

Adorini, L. (2004). Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting allograft rejection and autoimmune diseases. *Kidney Int. 65*, 1538.

Adu, D., Cockwell, P., Ives, N.J., Shaw, J., and Wheatley, K. (2003). Interleukin-2 receptor monoclonal antibodies in renal transplantation: meta-analysis of randomised trials. *BMJ 326*, 789.

Alard, P., Clark, S.L., and Kosiewicz, M.M. (2004). Mechanisms of tolerance induced by TGF beta-treated APC: CD4 regulatory T cells prevent the induction of the immune response possibly through a mechanism involving TGF beta. *Eur. J. Immunol. 34*, 1021–1030.

Alegre, M.L., Peterson, L.J., Xu, D., Sattar, H.A., Jeyarajah, D.R., Kowalkowski, K., Thistlethwaite, J.R., Zivin, R.A., Jolliffe, L., and Bluestone, J.A. (1994). A non-activating "humanized" anti-CD3 monoclonal antibody retains immunosuppressive properties *in vivo*. *Transplantation 57*, 1537–1543.

Bachelez, H., Flageul, B., Dubertret, L., Fraitag, S., Grossman, R., Brousse, N., Poisson, D., Knowles, R.W., Wacholtz, M.C., Haverty, T.P., Chatenoud, L., and Bach, J.F. (1998). Treatment of recalcitrant plaque psoriasis with a humanized non-depleting antibody to CD4. *J. Autoimmun. 11*, 53–62.

Banerjee, S., Wei, B.Y., Hillman, K., Luthra, H.S., and David, C.S. (1988). Immunosuppression of collagen-induced arthritis in mice with an anti-IL-2 receptor antibody. *J. Immunol. 141*, 1150–1154.

Barbas, C.F., 3rd (1995). Synthetic human antibodies. *Nat. Med. 1*, 837–839.

Barbas, C.F., 3rd, Hu, D., Dunlop, N., Sawyer, L., Cababa, D., Hendry, R.M., Nara, P.L., and Burton, D.R. (1994). *In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proc. Natl. Acad. Sci. U.S.A. 91*, 3809–3813.

Baudrihaye, M.F., Chatenoud, L., Kreis, H., Goldstein, G., and Bach, J.F. (1984). Unusually restricted anti-isotype human immune response to OKT3 monoclonal antibody. *Eur. J. Immunol. 14*, 686–691.

Belghith, M., Bluestone, J.A., Barriot, S., Megret, J., Bach, J.F., and Chatenoud, L. (2003). TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat. Med. 9,* 1202–1208.

Benjamin, R.J., Cobbold, S.P., Clark, M.R., and Waldmann, H. (1986). Tolerance to rat monoclonal antibodies. Implications for serotherapy. *J. Exp. Med. 163*, 1539–1552.

Ben-yedidia, T., Marcus, H., Reisner, Y., and Arnon, R. (1999). Intranasal administration of peptide vaccine protects human/mouse radiation chimera from influenza infection. *Int. Immunol. 11*, 1043–1051.

Bertry-Coussot, L., Lucas, B., Danel, C., Halbwachs-Mecarelli, L., Bach, J.F., Chatenoud, L., and Lemarchand, P. (2002). Long-term reversal of established autoimmunity upon transient blockade of the LFA–1/intercellular adhesion molecule-1 pathway. *J. Immunol. 168*, 3641–3648.

Bluestone, J.A. and Tang, Q. (2004). Therapeutic vaccination using CD4+CD25+ antigen-specific regulatory T cells. *Proc. Natl. Acad. Sci. U.S.A. 101 Suppl 2*, 14622–14626.

Bolt, S., Routledge, E., Lloyd, I., Chatenoud, L., Pope, H., Gorman, S.D., Clark, M., and Waldmann, H. (1993). The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains *in vitro* immunosuppressive properties. *Eur. J. Immunol. 23*, 403–411.

Bottino, R., Lemarchand, P., Trucco, M., and Giannoukakis, N. (2003). Gene- and cell-based therapeutics for type I diabetes mellitus. *Gene Ther. 10*, 875–889.

Boumpas, D.T., Furie, R., Manzi, S., Illei, G.G., Wallace, D.J., Balow, J.E., and Vaishnaw, A. (2003). A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum. 48*, 719–727.

Brandt, J., Haibel, H., Cornely, D., Golder, W., Gonzalez, J., Reddig, J., Thriene, W., Sieper, J., and Braun, J. (2000). Successful treatment of active ankylosing spondylitis with the anti-tumor necrosis factor alpha monoclonal antibody infliximab. *Arthritis Rheum. 43*, 1346–1352.

Brennan, F.M. and Feldmann, M. (1992). Cytokines in autoimmunity. *Curr. Opin. Immunol. 4*, 754–759.

Brennan, F., Chantry, D., Jackson, A., Maini, R., and Feldmann, M. (1989). Inhibitory affect of the TNF alpha on synovial all interleukin-1 production in rheumatoid arthritis. *Lancet. 2*, 244–247.

Brown, S.L., Greene, M.H., Gershon, S.K., Edwards, E.T., and Braun, M.M. (2002). Tumor necrosis factor antagonist therapy and lymphoma development: twenty-six cases reported to the Food and Drug Administration. *Arthritis Rheum. 46*, 3151–3158.

Bumgardner, G.L., Hardie, I., Johnson, R.W., Lin, A., Nashan, B., Pescovitz, M.D., Ramos, E., and Vincenti, F. (2001). Results of 3-year phase III clinical trials with daclizumab prophylaxis for prevention of acute rejection after renal transplantation. *Transplantation 72*, 839–845.

Bushell, A., Morris, P.J., and Wood, K.J. (1995). Transplantation tolerance induced by antigen pretreatment and depleting anti-CD4 antibody depends on CD4+ T cell regulation during the induction phase of the response. *Eur. J. Immunol. 25*, 2643–2649.

Bushell, A., Karim, M., Kingsley, C.I., and Wood, K.J. (2003). Pretransplant blood transfusion without additional immunotherapy generates CD25+CD4+ regulatory T cells: a potential explanation for the blood-transfusion effect. *Transplantation 76*, 449–455.

Calne, R., Moffatt, S.D., Friend, P.J., Jamieson, N.V., Bradley, J.A., Hale, G., Firth, J., Bradley, J., Smith, K.G., and Waldmann, H. (1999). Campath IH allows low-dose cyclosporine monotherapy in 31 cadaveric renal allograft recipients. *Transplantation 68*, 1613–1616.

Caramaschi, P., Biasi, D., Colombatti, M., Pieropan, S., Martinelli, N., Carletto, A., Volpe, A., Pacor, L.M., and Bambara, L.M. (2004). Anti-TNFalpha therapy in rheumatoid arthritis and autoimmunity. *Rheumatol. Int. 26*, 209–214.

Chatenoud, L. (1986). The immune response against therapeutic monoclonal antibodies. *Immunol. Today 7*, 367–368.

Chatenoud, L. (2003). CD3-specific antibody-induced active tolerance: from bench to bedside. *Nat. Rev. Immunol. 3*, 123–132.

Chatenoud, L., Baudrihaye, M.F., Chkoff, N., Kreis, H., Goldstein, G., and Bach, J.F. (1986a). Restriction of the human *in vivo* immune response against the mouse monoclonal antibody OKT3. *J. Immunol. 137*, 830–838.

Chatenoud, L., Jonker, M., Villemain, F., Goldstein, G., and Bach, J.F. (1986b). The human immune response to the OKT3 monoclonal antibody is oligoclonal. *Science 232*, 1406–1408.

Chatenoud, L., Ferran, C., Reuter, A., Legendre, C., Gevaert, Y., Kreis, H., Franchimont, P., and Bach, J.F. (1989). Systemic reaction to the anti-T-cell monoclonal antibody OKT3 in relation to serum levels of tumor necrosis factor and interferon-gamma. *N. Engl. J. Med. 320*, 1420–1421.

Chatenoud, L., Ferran, C., Legendre, C., Thouard, I., Merite, S., Reuter, A., Gevaert, Y., Kreis, H., Franchimont, P., and Bach, J.F. (1990). *In vivo* cell activation following OKT3 administration. Systemic cytokine release and modulation by corticosteroids. *Transplantation 49*, 697–702.

Chatenoud, L., Thervet, E., Primo, J., and Bach, J.F. (1994). Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc. Natl. Acad. Sci. U.S.A. 91*, 123–127.

Chatenoud, L., Primo, J., and Bach, J.F. (1997). CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J. Immunol. 158*, 2947–2954.

Chatenoud, L., Salomon, B., and Bluestone, J.A. (2001). Suppressor T cells—they're back and critical for regulation of autoimmunity! *Immunol. Rev. 182*, 149–163.

Chaudhari, U., Romano, P., Mulcahy, L.D., Dooley, L.T., Baker, D.G., and Gottlieb, A.B. (2001). Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet 357*, 1842–1847.

Choy, E.H., Adjaye, J., Forrest, L., Kingsley, G.H., and Panayi, G.S. (1993). Chimaeric anti-CD4 monoclonal antibody cross-linked by monocyte Fc gamma receptor mediates apoptosis of human CD4 lymphocytes. *Eur. J. Immunol. 23*, 2676–2681.

Choy, E.H., Hazleman, B., Smith, M., Moss, K., Lisi, L., Scott, D.G., Patel, J., Sopwith, M., and Isenberg, D.A. (2002). Efficacy of a novel PEGylated humanized anti-TNF fragment (CDP870) in patients with rheumatoid arthritis: a phase II double-blinded, randomized, dose-escalating trial. *Rheumatology (Oxford) 41*, 1133–1137.

Clevers, H., Alarcon, B., Wileman, T., and Terhorst, C. (1988). The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol. 6* 629–662.

Cobbold, S.P., Adams, E., Graca, L., and Waldmann, H. (2003). Serial analysis of gene expression provides new insights into regulatory T cells. *Semin. Immunol. 15*, 209–214.

Coles, A.J., Wing, M.G., Molyneux, P., Paolillo, A., Davie, C.M., Hale, G., Miller, D., Waldmann, H., and Compston, A. (1999a). Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Ann. Neurol. 46*, 296–304.

Coles, A.J., Wing, M.G., Smith, S., Corradu, F., Greer, S., Taylor, C., Weetman, A., Hale, G., Chatterjee, V.K., Waldmann, H., and Compston, A. (1999b). Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. *Lancet 354*, 1691–1695.

Coles, A., Deans, J., and Compston, A. (2004). Campath-1H treatment of multiple sclerosis: lessons from the bedside for the bench. *Clin. Neurol. Neurosurg. 106*, 270–274.

Cosimi, A.B. (1987). Clinical development of Orthoclone OKT3. *Transplant. Proc. 19*, 7–16.

Cosimi, A.B., Burton, R.C., Colvin, R.B., Goldstein, G., Delmonico, F.L., Laquaglia, M.P., Tolkoff-rubin, N., Rubin, R.H., Herrin, J.T., and Russell, P.S. (1981a). Treatment of acute renal allograft rejection with OKT3 monoclonal antibody. *Transplantation 32*, 535–539.

Cosimi, A.B., Colvin, R.B., Burton, R.C., Rubin, R.H., Goldstein, G., Kung, P.C., Hansen, W.P., Delmonico, F.L., and Russell, P.S. (1981b). Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N. Engl. J. Med. 305*, 308–314.

Davis, J.C., Jr., 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.

Davis, M.M. and Chien, Y.H. (1999). T cell antigen receptors. In W. Paul (ed.), *Fundamental Immunology,* (pp. 341–366). New York: Raven Press.

De Vita, S., Zaja, F., Sacco, S., De Candia, A., Fanin, R., and Ferraccioli, G. (2002). Efficacy of selective B cell blockade in the treatment of

rheumatoid arthritis: evidence for a pathogenetic role of B cells. *Arthritis Rheum. 46*, 2029–2033.

Debray-Sachs, M., Carnaud, C., Boitard, C., Cohen, H., Gresser, I., Bedossa, P., and Bach, J.F. (1991). Prevention of diabetes in NOD mice treated with antibody to murine IFN gamma. *J. Autoimmun. 4*, 237–248.

Debure, A., Chkoff, N., Chatenoud, L., Lacombe, M., Campos, H., Noel, L.H., Goldstein, G., Bach, J.F., and Kreis, H. (1988). One-month prophylactic use of OKT3 in cadaver kidney transplant recipients. *Transplantation 45*, 546–553.

den Broeder, A., van de Putte, L., Rau, R., Schattenkirchner, M., Van Riel, P., Sander, O., Binder, C., Fenner, H., Bankmann, Y., Velagapudi, R., et al. (2002). A single dose, placebo controlled study of the fully human anti-tumor necrosis factor-alpha antibody adalimumab (D2E7) in patients with rheumatoid arthritis. *J. Rheumatol. 29*, 2288–2298.

Eason, J.D. and Cosimi, A.B. (1999). Biologic immunosuppressive agents. In L. Ginns, A. Cosimi, and P. Morris (eds), *Transplantation,* (pp. 196–224). Malden, USA: Blackwell Science.

Edwards, J.C., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Emery, P., Close, D.R., Stevens, R.M., and Shaw, T. (2004). Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N. Engl. J. Med. 350*, 2572–2581.

Elliott, M.J., Maini, R.N., Feldmann, M., Kalden, J.R., Antoni, C., Smolen, J.S., Leeb, B., Breedveld, F.C., Macfarlane, J.D., Bijl, H., et al. (1994a). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet 344*, 1105–1110.

Elliott, M.J., Maini, R.N., Feldmann, M., Long-Fox, A., Charles, P., Bijl, H., and Woody, J.N. (1994b). Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. *Lancet 344*, 1125–1127.

Emmrich, J., Seyfarth, M., Fleig, W.E., and Emmrich, F. (1991). Treatment of inflammatory bowel disease with anti-CD4 monoclonal antibody. *Lancet 338*, 570–571.

Farge, D., Passweg, J., van Laar, J.M., Marjanovic, Z., Besenthal, C., Finke, J., Peter, H.H., Breedveld, F.C., Fibbe, W.E., Black, C., et al. (2004). Autologous stem cell transplantation in the treatment of systemic sclerosis: report from the EBMT/EULAR Registry. *Ann. Rheum. Dis. 63*, 974–981.

Fassas, A., Passweg, J.R., Anagnostopoulos, A., Kazis, A., Kozak, T., Havrdova, E., Carreras, E., Gravs, F., Kashyap, A., and Opeushaw, H. (2002). Hematopoietic cell transplantation for multiple sclerosis. A retrospective multicenter study. *J. Neurol. 249*, 1088–1097.

Feldmann, M. (2002). Development of anti-TNF therapy for rheumatoid arthritis. *Nat. Rev. Immunol. 2*, 364–371.

Feldmann, M., and Maini, T. (2001). Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu. Rev. Immunol. 19*, 163–196.

Ferrari-Lacraz, S., Zanelli, E., Neuberg, M., Donskoy, E., Kim, Y.S., Zheng, X.X., Hancock, W.W., Maslinski, W., Li, X.C., Strom, T.B., and Moll, T. (2004). Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. *J. Immunol. 173*, 5818–5826.

Finck, B.K., Linsley, P.S., and Wofsy, D. (1994). Treatment of murine lupus with CTLA4Ig. *Science 265*, 1225–1227.

Fleischmann, R.M., Schechtman, J., Bennett, R., Handel, M.L., Burmester, G.R., Tesser, J., Modafferi, D., Poulakos, J., and Sun, G. (2003). Anakinra, a recombinant human interleukin-1 receptor antagonist (r-metHuIL-1ra), in patients with rheumatoid arthritis: A large, international, multicenter, placebo-controlled trial. *Arthritis Rheum. 48*, 927–934.

Foote, J. and Eisen, H.N. (1995). Kinetic and affinity limits on antibodies produced during immune responses. *Proc. Natl. Acad. Sci. U.S.A. 92*, 1254–1256.

Friend, P.J., Hale, G., Chatenoud, L., Rebello, P., Bradley, J., Thiru, S., Phillips, J.M., and Waldmann, H. (1999). Phase I study of an engineered aglycosylated humanized CD3 antibody in renal transplant rejection. *Transplantation 68*, 1632–1637.

Furst, D.E., Weisman, M., Paulus, H.E., Bulpitt, K., Weinblatt, M., Polisson, R., Zaug, M., Kneer, J., Van der Auwera, P., and Stevens, R.M. (2003). Intravenous human recombinant tumor necrosis factor receptor p55-Fc IgG1 fusion protein, Ro 45–2081 (lenercept): results of a dose-finding study in rheumatoid arthritis. *J. Rheumatol. 30*, 2123–2126.

Ghosh, S., Goldin, E., Gordon, F.H., Malchow, H.A., Rask-Madsen, J., Rutgeerts, P., Vyhnalek, P., Zadorova, Z., Palmer, T., and Donoghue, S. (2003). Natalizumab for active Crohn's disease. *N. Engl. J. Med. 348*, 24–32.

Goldberg, D., Chatenoud, L., Morel, P., Boitard, C., Revillard, J.P., Bertoye, P., Bach, J.F., and Menkes, C.J. (1990). Preliminary trial of an anti-CD4 monoclonal antibody (MoAb) in rheumatoid arthritis (RA). *Arthritis Rheum. 33*, S153.

Goldberg, D., Morel, P., Chatenoud, L., Boitard, C., Menkes, C.J., Bertoye, P.H., Revillard, J.P., and Bach, J.F. (1991). Immunological effects of high dose administration of anti-CD4 antibody in rheumatoid arthritis patients. *J. Autoimmun. 4*, 617–630.

Gomez-Reino, J.J., Carmona, L., Valverde, V.R., Mola, E.M., and Montero, M.D. (2003). Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk: a multicenter active-surveillance report. *Arthritis Rheum. 48*, 2122–2127.

Gottlieb, A.B., Lebwohl, M., Shirin, S., Sherr, A., Gilleaudeau, P., Singer, G., Solodkina, G., Grossman, R., Gisoldi, E., Phillips, S., et al. (2000). Anti-CD4 monoclonal antibody treatment of moderate to severe psoriasis vulgaris: results of a pilot, multicenter, multiple-dose, placebo-controlled study. *J. Am. Acad. Dermatol. 43*, 595–604.

Gram, H., Marconi, L.A., Barbas, C.F., 3rd, Collet, T.A., Lerner, R.A., and Kang, A.S. (1992). *In vitro* selection and affinity maturation of antibodies from a naive combinatorial immunoglobulin library. *Proc. Natl. Acad. Sci. U. S. A. 89*, 3576–3580.

Guex-Crosier, Y., Raber, J., Chan, C.C., Kriete, M.S., Benichou, J., Pilson, R.S., Kerwin, J.A., Waldmann, T.A., Hakimi, J., and Roberge, F.G. (1997). Humanized antibodies against the alpha-chain of the IL-2 receptor and against the beta-chain shared by the IL-2 and IL-15 receptors in a monkey uveitis model of autoimmune diseases. *J. Immunol. 158*, 452–458.

Hahn, H.J., Lucke, S., Kloting, I., Volk, H.D., Baehr, R.V., and Diamantstein, T. (1987). Curing BB rats of freshly manifested diabetes by short-term treatment with a combination of a monoclonal anti-interleukin 2 receptor antibody and a subtherapeutic dose of cyclosporin A. *Eur. J. Immunol. 17*, 1075–1078.

Hayward, A.R. and Shreiber, M. (1989). Neonatal injection of CD3 antibody into nonobese diabetic mice reduces the incidence of insulitis and diabetes. *J. Immunol. 143*, 1555–1559.

Hering, B.J., Kandaswamy, R., Harmon, J.V., Ansite, J.D., Clemmings, S.M., Sakai, T., Paraskevas, S., Eckman, P.M., Sageshima, J., Nakano, M., et al. (2004). Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am. J. Transplant 4,* 390–401.

Herold, K.C., Hagopian, W., Auger, J.A., Poumian Ruiz, E., Taylor, L., Donaldson, D., Gitelman, S.E., Harlan, D.M., Xu, D., Zivin, R.A., and Bluestone, J.A. (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med. 346*, 1692–1698.

Horneff, G., Burmester, G.R., Emmrich, F., and Kalden, J.R. (1991). Treatment of rheumatoid arthritis with an anti-CD4 monoclonal antibody. *Arthritis Rheum. 34*, 129–140.

Hricik, D.E., Mayes, J.T., and Schulak, J.A. (1990). Inhibition of anti-OKT3 antibody generation by cyclosporine—results of a prospective randomized trial. *Transplantation 50*, 237–240.

Ikehara, S. (1998). Bone marrow transplantation for autoimmune diseases. *Acta Haematol. 99*, 116–132.

Ikehara, S., Ohtsuki, H., Good, R.A., Asamoto, H., Nakamura, T., Sekita, K., Muso, E., Tochino, Y., Ida, T., Kuzuya, H., Imura, H., and Hamashima, Y. (1985). Prevention of type I diabetes in nonobese diabetic mice by allogenic bone marrow transplantation. *Proc. Natl. Acad. Sci. U.S.A. 82*, 7743–7747.

Ikehara, S., Kawamura, M., Takao, F., Inaba, M., Yasumizu, R., Than, S., Hisha, H., Sugiura, K., Koide, Y., Yoshida, T.O., Ida, T., Imura, H., and Good, R.A. (1990). Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells. *Proc. Natl. Acad. Sci. U.S.A. 87*, 8341–8344.

Isaacs, J.D., Watts, R.A., Hazleman, B.L., Hale, G., Keogan, M.T., Cobbold, S.P., and Waldmann, H. (1992). Humanised monoclonal antibody therapy for rheumatoid arthritis. *Lancet 340*, 748–752.

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.

Kalunian, K.C., Davis, J.C., Jr., Merrill, J.T., Totoritis, M.C., and Wofsy, D. (2002). Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum. 46*, 3251–3258.

Kared, H., Adle-Biassette, A.M.H., Bach, J.-F., Chatenoud, L., and Zavala, F. (2004). G-CSF treatment prevents diabetes in NOD mice by recruiting plasmacytoid dendritic cells and functional CD4+CD25+ regulatory T cells. *Diabetes* in press.

Karussis, D.M., Slavin, S., Lehmann, D., Mizrachi-Koll, R., Abramsky, O., and Ben-Nun, A. (1992). Prevention of experimental autoimmune encephalomyelitis and induction of tolerance with acute immunosuppression followed by syngeneic bone marrow transplantation. *J. Immunol. 148*, 1693–1698.

Karussis, D.M., Vourka-Karussis, U., Lehmann, D., Ovadia, H., Mizrachi-Koll, R., Ben-Nun, A., Abramsky, O., and Slavin, S. (1993). Prevention and reversal of adoptively transferred, chronic relapsing experimental autoimmune encephalomyelitis with a single high dose cytoreductive treatment followed by syngeneic bone marrow transplantation. *J. Clin. Invest. 92*, 765–772.

Keane, J., Gershon, S., Wise, R.P., Mirabile-Levens, E., Kasznica, J., Schwieterman, W.D., Siegel, J.N., and Braun, M.M. (2001). Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N. Engl. J. Med. 345*, 1098–1104.

Kelley, V.E., Gaulton, G.N., Hattori, M., Ikegami, H., Eisenbarth, G., and Strom, T.B. (1988). Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nephritis. *J. Immunol. 140*, 59–61.

Kempeni, J. (1999). Preliminary results of early clinical trials with the fully human anti-TNFalpha monoclonal antibody D2E7. *Ann. Rheum. Dis. 58*(Suppl 1), I70–72.

Keymeulen, B., Vandemeulebroucke, E., Ziegler, A.G., Mathieu, C., Kaufman, L., Hale, G., Gorus, F., Goldman, M., Walter, M., Candon, S., et al. (2005). CD3 antibody treatment and insulin needs in new-onset type 1 diabetes. *New. Engl. J. Med. 352*, 2598–2608.

Kirk, A.D., Burkly, L.C., Batty, D.S., Baumgartner, R.E., Berning, J.D., Buchanan, K., Fechner Jh, J.R., Germond, R.L., Kampen, R.L., Patterson, N.B., Swanson, S.J., Tadaki, D.K., TenHoor, C.N., White, L., Knechtle, S.J., and Harlan, D.M. (1999). Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates [see comments. *Nat. Med. 5*, 686–693.

Kirkman, R.L., Barrett, L.V., Gaulton, G.N., Kelley, V.E., Ythier, A., and Strom, T.B. (1985). Administration of an anti-interleukin 2 receptor monoclonal antibody prolongs cardiac allograft survival in mice. *J. Exp. Med. 162*, 358–362.

Kirkman, R.L., Shapiro, M.E., Carpenter, C.B., Mckay, D.B., Milford, E.L., Ramos, E.L., Tilney, N.L., Waldmann, T.A., Zimmerman, C.E., and Strom, T.B. (1991). A randomized prospective trial of anti-Tac monoclonal antibody in human renal transplantation. *Transplantation 51*, 107–113.

Kohler, G. and Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature 256*, 495–497.

Kremer, J.M., Westhovens, R., Leon, M., Di Giorgio, E., Alten, R., Steinfeld, S., Russell, A., Dougados, M., Emery, P., Nuamah, I.F., et al. (2003). Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med *349*, 1907–1915.

Kriaa, F., Hiesse, C., Alard, P., Lantz, O., Noury, J., Charpentier, B., and Bazin, H. (1993). Prophylactic use of the anti-IL-2 receptor monoclonal antibody LO-Tact-1 in cadaveric renal transplantation: results of a randomized study. *Transplant Proc. 25*, 817–819.

Krueger, G.G. (2003). Clinical response to alefacept: results of a phase 3 study of intravenous administration of alefacept in patients with chronic plaque psoriasis. *J. Eur. Acad. Dermatol. Venereol. 17*(Suppl 2), 17–24.

Kung, P., Goldstein, G., Reinherz, E.L., and Schlossman, S.F. (1979). Monoclonal antibodies defining distinctive human T cell surface antigens. *Science 206*, 347–349.

Kuwana, M., Nomura, S., Fujimura, K., Nagasawa, T., Muto, Y., Kurata, Y., Tanaka, S., and Ikeda, Y. (2004). Effect of a single injection of humanized anti-CD154 monoclonal antibody on the platelet-specific autoimmune response in patients with immune thrombocytopenic purpura. *Blood 103*, 1229–1236.

Lakkis, F.G., Konieczny, B.T., Saleem, S., Baddoura, F.K., Linsley, P.S., Alexander, D.Z., Lowry, R.P., Pearson, T.C., and Larsen, C.P. (1997). Blocking the CD28-B7 T cell costimulation pathway induces long term cardiac allograft acceptance in the absence of IL-4. *J. Immunol. 158*, 2443–2448.

Larsen, C.P. and Pearson, T.C. (1997). The CD40 pathway in allograft rejection, acceptance, and tolerance. *Curr. Opin. Immunol. 9*, 641–647.

Larsen, C.P., Elwood, E.T., Alexander, D.Z., Ritchie, S.C., Hendrix, R., Tuckerburden, C., Cho, H.R., Aruffo, A., Hollenbaugh, D., Linsley, P.S., Winn, K.J., and Pearsen, T.C. (1996). Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature 381*, 434–438.

Leandro, M.J., Edwards, J.C., and Cambridge, G. (2002). Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann. Rheum. Dis. 61*, 883–888.

Lebwohl, M., Christophers, E., Langley, R., Ortonne, J.P., Roberts, J., and Griffiths, C.E. (2003). An international, randomized, double-blind, placebo-controlled phase 3 trial of intramuscular alefacept in patients with chronic plaque psoriasis. *Arch. Dermatol. 139*, 719–727.

Lenschow, D.J., Zeng, Y., Thistlethwaite, J.R., Montag, A., Brady, W., Gibson, M.G., Linsley, P.S., and Bluestone, J.A. (1992). Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4lg. *Science 257*, 789–792.

Leo, O., Foo, M., Sachs, D.H., Samelson, L.E., and Bluestone, J.A. (1987). Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. U.S.A. 84*, 1374–1378.

Leonardi, C.L. (2004). Efalizumab in the treatment of psoriasis. *Dermatol. Ther. 17*, 393–400.

Lin, H., Bolling, S.F., Linsley, P.S., Wei, R.Q., Gordon, D., Thompson, C.B., and Turka, L.A. (1993). Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J. Exp. Med. 178*, 1801–1806.

Linsley, P.S., Brady, W., Urnes, M., Grosmaire, L.S., Damle, N.K., and Ledbetter, J.A. (1991). CTLA–4 is a second receptor for the B cell activation antigen B7. *J. Exp. Med. 174*, 561–569.

Linsley, P.S., Wallace, P.M., Johnson, J., Gibson, M.G., Greene, J.L., Ledbetter, J.A., Singh, C., and Tepper, M.A. (1992). Immunosuppression *in vivo* by a soluble form of the CTLA-4 T cell activation molecule. *Science 257*, 792–795.

Lipsky, P.E., van der Heijde, D.M., St Clair, E.W., Furst, D.E., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Weisman, M., Emery, P., Feldmann, M., Harriman, G.R., Maini, R.N., Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. (2000). Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N. Engl. J. Med. 343*, 1594–1602.

Lockwood, C.M., Thiru, S., Isaacs, J.D., Hale, G., and Waldmann, H. (1993). Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. *Lancet 341*, 1620–1622.

Lockwood, C.M., Thiru, S., Stewart, S., Hale, G., Isaacs, J., Wraight, P., Elliott, J., and Waldmann, H. (1996). Treatment of refractory Wegener's granulomatosis with humanized monoclonal antibodies [see comments]. *Q. J. Med. 89*, 903–912.

Looney, R.J., Anolik, J., and Sanz, I. (2004a). B cells as therapeutic targets for rheumatic diseases. *Curr. Opin. Rheumatol. 16*, 180–185.

Looney, R.J., Anolik, J., and Sanz, I. (2004b). B lymphocytes in systemic lupus erythematosus: lessons from therapy targeting B cells. *Lupus 13*, 381–390.

Looney, R.J., Anolik, J.H., Campbell, D., Felgar, R.E., Young, F., Arend, L.J., Sloand, J.A., Rosenblatt, J., and Sanz, I. (2004c). B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum. 50*, 2580–2589.

Louis, M., Rauch, J., Armstrong, M., and Fitzcharles, M.A. (2003). Induction of autoantibodies during prolonged treatment with infliximab. *J. Rheumatol. 30*, 2557–2562.

Lovell, D.J., Giannini, E.H., Reiff, A., Cawkwell, G.D., Silverman, E.D., Nocton, J.J., Stein, L.D., Gedalia, A., Ilowite, N.T., Wallace, C.A., Whitmore, J., and Finck, B.K. (2000). Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N. Engl. J. Med. 342*, 763–769.

Lubin, I., Segall, H., Marcus, H., David, M., Kulova, L., Steinitz, M., Erlich, P., Gan, J., and Reisner, Y. (1994). Engraftment of human peripheral blood lymphocytes in normal strains of mice. *Blood 83*, 2368–2381.

Maini, R.N., Breedveld, F.C., Kalden, J.R., Smolen, J.S., Davis, D., Macfarlane, J.D., Antoni, C., Leeb, B., Elliott, M.J., Woody, J.N., Schaible, T.F., and Feldmann, M. (1998). Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis [see comments.] *Arthritis Rheum. 41*, 1552–1563.

Maini, R., St Clair, E.W., Breedveld, F., Furst, D., Kalden, J., Weisman, M., Smolen, J., Emery, P., Harriman, G., Feldmann, M., and Lipsky, P. (1999). Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet 354*, 1932–1939.

Maki, T., Ichikawa, T., Blanco, R., and Porter, J. (1992). Long-term abrogation of autoimmune diabetes in nonobese diabetic mice by immunotherapy with anti-lymphocyte serum. *Proc. Natl. Acad. Sci. U.S.A. 89*, 3434–3438.

Marinova-Mutafchieva, L., Williams, R.O., Mauri, C., Mason, L.J., Walmsley, M.J., Taylor, P.C., Feldmann, M., and Maini, R.N. (2000). A comparative study into the mechanisms of action of anti-tumor necrosis factor alpha, anti-CD4, and combined anti-tumor necrosis factor alpha/anti-CD4 treatment in early collagen-induced arthritis. *Arthritis Rheum. 43*, 638–644.

Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., Mccafferty, J., Griffiths, A.D., and Winter, G. (1991). By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J. Mol. Biol. 222*, 581–597.

Marmont, A.M., Van Lint, M.T., Gualandi, F., and Bacigalupo, A. (1997). Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. *Lupus 6*, 545–548.

Mathieson, P.W., Cobbold, S.P., Hale, G., Clark, M.R., Oliveira, D.B., Lockwood, C.M., and Waldmann, H. (1990). Monoclonal-antibody therapy in systemic vasculitis. *N. Engl. J. Med. 323*, 250–254.

McAllister, L.D., Beatty, P.G., and Rose, J. (1997). Allogeneic bone marrow transplant for chronic myelogenous leukemia in a patient with multiple sclerosis. *Bone Marrow Transplant. 19*, 395–397.

Mease, P.J., Goffe, B.S., Metz, J., VanderStoep, A., Finck, B., and Burge, D.J. (2000). Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet 356*, 385–390.

Menter, A., Kosinski, M., Bresnahan, B.W., Papp, K.A., and Ware, J.E., Jr. (2004). Impact of efalizumab on psoriasis-specific patient-reported outcomes. Results from three randomized, placebo-controlled clinical trials of moderate to severe plaque psoriasis. *J. Drugs Dermatol. 3*, 27–38.

Miller, D.H., Khan, O.A., Sheremata, W.A., Blumhardt, L.D., Rice, G.P., Libonati, M.A., Willmer-Hulme, A.J., Dalton, C.M., Miszkiel, K.A., and O'Connor, P.W. (2003). A controlled trial of natalizumab for relapsing multiple sclerosis. *N. Engl. J. Med. 348*, 15–23.

Moosig, F., Zeuner, R., Renk, C., and Schroder, J.O. (2004). IL-1-RA in refractory systemic lupus erythematosus. *Lupus 13*, 605–606.

Moreau, T., Thorpe, J., Miller, D., Moseley, I., Hale, G., Waldmann, H., Clayton, D., Wing, M., Scolding, N., and Compston, A. (1994). Preliminary evidence from magnetic resonance imaging for reduction in disease activity after lymphocyte depletion in multiple sclerosis [published erratum appears in *Lancet* 1994 *344*(8920), 486.] *Lancet 344*, 298–301.

Morel, P., Revillard, J.P., Nicolas, J.F., Wijdenes, J., Rizova, H., and Thivolet, J. (1992). Anti-CD4 monoclonal antibody therapy in severe psoriasis. *J. Autoimmun. 5*, 465–477.

Moreland, L.W., Bucy, R.P., Tilden, A., Pratt, P.W., Lobuglio, A.F., Khazaeli, M., Everson, M.P., Daddona, P., Ghrayeb, J., Kilgarriff, C., Sanders, E., and Koopman, W.J. (1993). Use of a chimeric monoclonal anti-CD4 antibody in patients with refractory rheumatoid arthritis. *Arthritis Rheum. 36*, 307–318.

Moreland, L.W., Pratt, P.W., Bucy, R.P., Jackson, B.S., Feldman, J.W., and Koopman, W.J. (1994). Treatment of refractory rheumatoid arthritis with a chimeric anti-CD4 monoclonal antibody. Long-term followup of CD4+ T cell counts. *Arthritis Rheum. 37*, 834–838.

Moreland, L.W., Margolies, G., Heck, L.W., Jr., Saway, A., Blosch, C., Hanna, R., and Koopman, W.J. (1996). Recombinant soluble tumor necrosis factor receptor (p80) fusion protein: toxicity and dose finding trial in refractory rheumatoid arthritis. *J. Rheumatol. 23*, 1849–1855.

Moreland, L.W., Baumgartner, S.W., Schiff, M.H., Tindall, E.A., Fleischmann, R.M., Weaver, A.L., Ettlinger, R.E., Cohen, S., Koopman, W.J., 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 [see comments]. *N. Engl. J. Med. 337*, 141–147.

Moreland, L.W., Alten, R., Van den Bosch, F., Appelboom, T., Leon, M., Emery, P., Cohen, S., Luggen, M., Shergy, W., Nuamah, I., and Becker, J.C. (2002). Costimulatory blockade in patients with rheumatoid arthritis: a pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. *Arthritis Rheum. 46*, 1470–1479.

Moritani, M., Yoshimoto, K., Ii, S., Kondo, M., Iwahana, H., Yamaoka, T., Sano, T., Nakano, N., Kikutani, H., and Itakura, M. (1996). Prevention of adoptively transferred diabetes in nonobese diabetic mice with IL-10-transduced islet-specific Th1 lymphocytes. A gene therapy model for autoimmune diabetes. *J. Clin. Invest. 98*, 1851–1859.

Nashan, B., Moore, R., Amlot, P., Schmidt, A.G., Abeywickrama, K., and Soulillou, J.P. (1997). Randomised trial of basiliximab versus placebo for control of acute cellular rejection in renal allograft recipients. CHIB 201 International Study Group [published erratum appears in *Lancet 350*(9089), 1484]. *Lancet 350*, 1193–1198.

Nicoletti, F., Meroni, P.L., Landolfo, S., Gariglio, M., Guzzardi, S., Barcellini, W., Lunetta, M., Mughini, L., and Zanussi, C. (1990). Prevention of diabetes in BB/Wor rats treated with monoclonal antibodies to interferon-gamma. *Lancet 336*, 319.

Nicolls, M.R., Aversa, G.G., Pearce, N.W., Spinelli, A., Berger, M.F., Gurley, K.E., and Hall, B.M. (1993). Induction of long-term specific tolerance to allografts in rats by therapy with an anti-CD3-like monoclonal antibody. *Transplantation 55*, 459–468.

Niimi, M., Pearson, T.C., Larsen, C.P., Alexander, D.Z., Hollenbaugh, D., Aruffo, A., Linsley, P.S., Thomas, E., Campbell, K., Fanslow, W.C., Geha, R.S., Morris, P.J., and Wood, K.J. (1998). The role of the CD40 pathway in alloantigen-induced hyporesponsiveness *in vivo*. *J. Immunol. 161*, 5331–5337.

Nuki, G., Bresnihan, B., Bear, M.B., and McCabe, D. (2002). Long-term safety and maintenance of clinical improvement following treatment with anakinra (recombinant human interleukin-1 receptor antagonist) in patients with rheumatoid arthritis: extension phase of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum. 46*, 2838–2846.

Nussenblatt, R.B. (2002). Bench to bedside: new approaches to the immunotherapy of uveitic disease. *Int. Rev. Immunol. 21*, 273–289.

Onodera, K., Chandraker, A., Schaub, M., Stadlbauer, T.H., Korom, S., Peach, R., Linsley, P.S., Sayegh, M.H., and Kupiec Weglinski, J.W. (1997). CD28-B7 T cell costimulatory blockade by CTLA4Ig in sensitized rat recipients: induction of transplantation tolerance in association with depressed cell-mediated and humoral immune responses. *J. Immunol. 159*, 1711–1717.

Ortho. (1985). A randomized clinical trial of OKT3 monoclonal antibody for acute rejection of cadaveric renal transplants. Ortho Multicenter Transplant Study Group. *N. Engl. J. Med. 313*, 337–342.

Piccirillo, C.A., Chang, Y., and Prud'homme, G.J. (1998). TGF-beta1 somatic gene therapy prevents autoimmune disease in nonobese diabetic mice. *J. Immunol. 161*, 3950–3956.

Piguet, P.F., Grau, G.E., Vesin, C., Loetscher, H., Gentz, R., and Lesslauer, W. (1992). Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a recombinant soluble TNF receptor. *Immunology 77*, 510–514.

Plain, K.M., Chen, J., Merten, S., He, X.Y., and Hall, B.M. (1999). Induction of specific tolerance to allografts in rats by therapy with non-mitogenic, non-depleting anti-CD3 monoclonal antibody: association with TH2 cytokines not anergy. *Transplantation 67*, 605–613.

Plevy, S.E., Landers, C.J., Prehn, J., Carramanzana, N.M., Deem, R.L., Shealy, D., and Targan, S.R. (1997). A role for TNF-alpha and mucosal T helper-1 cytokines in the pathogenesis of Crohn's disease. *J. Immunol. 159*, 6276–6282.

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.

Qin, S., Cobbold, S.P., Pope, H., Elliott, J., Kioussis, D., Davies, J., and Waldmann, H. (1993). "Infectious" transplantation tolerance. *Science 259*, 974–977.

Rankin, E.C., Choy, E.H., Kassimos, D., Kingsley, G.H., Sopwith, A.M., Isenberg, D.A., and Panayi, G.S. (1995). The therapeutic effects of an engineered human anti-tumour necrosis factor alpha antibody (CDP571) in rheumatoid arthritis. *Br. J. Rheumatol. 34*, 334–342.

Rapoport, M.J., Jaramillo, A., Zipris, D., Lazarus, A.H., Serreze, D.V., Leiter, E.H., Cyopick, P., Danska, J.S., and Delovitch, T.L. (1993). Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J. Exp. Med. 178*, 87–99.

Rastetter, W., Molina, A., and White, C.A. (2004). Rituximab: expanding role in therapy for lymphomas and autoimmune diseases. *Annu. Rev. Med. 55*, 477–503.

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.

Reed, M.H., Shapiro, M.E., Strom, T.B., Milford, E.L., Carpenter, C.B., Weinberg, D.S., Reimann, K.A., Letvin, N.L., Waldmann, T.A., and Kirkman, R.L. (1989). Prolongation of primate renal allograft survival by anti-Tac, an anti-human IL-2 receptor monoclonal antibody. *Transplantation 47*, 55–59.

Reiter, C., Kakavand, B., Rieber, E.P., Schattenkirchner, M., Riethmuller, G., and Kruger, K. (1991). Treatment of rheumatoid arthritis with monoclonal CD4 antibody M-T151. Clinical results and immunopharmacologic effects in an open study, including repeated administration. *Arthritis Rheum. 34*, 525–536.

Riechmann, L., Clark, M., Waldmann, H., and Winter, G. (1988). Reshaping human antibodies for therapy. *Nature 332*, 323–327.

Rott, O., Fleischer, B., and Cash, E. (1994). Interleukin-10 prevents experimental allergic encephalomyelitis in rats. *Eur. J. Immunol. 24*, 1434–1440.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol. 22*, 531–562.

Shizuru, J.A., Taylor-Edwards, C., Banks, B.A., Gregory, A.K., and Fathman, C.G. (1988). Immunotherapy of the nonobese diabetic mouse: treatment with an antibody to T-helper lymphocytes. *Science 240*, 659–662.

Sigidin, Y.A., Loukina, G.V., Skurkovich, B., and Skurkovich, S. (2001). Randomized, double-blind trial of anti-interferon-gamma antibodies in rheumatoid arthritis. *Scand. J. Rheumatol. 30*, 203–207.

Silverman, G.J. and Weisman, S. (2003). Rituximab therapy and autoimmune disorders: prospects for anti-B cell therapy. *Arthritis Rheum. 48*, 1484–1492.

Soulillou, J.P., Cantarovich, D., Le Mauff, B., Giral, M., Robillard, N., Hourmant, M., Hirn, M., and Jacques, Y. (1990). Randomized controlled trial of a monoclonal antibody against the interleukin-2 receptor (33B3.1) as compared with rabbit antithymocyte globulin for prophylaxis against rejection of renal allografts. *N. Engl. J. Med. 322*, 1175–1182.

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.

Takayama, T., Nishioka, Y., Lu, L., Lotze, M.T., Tahara, H., and Thomson, A.W. (1998). Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness. *Transplantation 66*, 1567–1574.

Tang, Q., Henriksen, K.J., Bi, M., Finger, E.B., Szot, G., Ye, J., Masteller, E.L., McDevitt, H., Bonyhadi, M., and Bluestone, J.A. (2004). *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med. 199*, 1455–1465.

Tarner, I.H., Slavin, A.J., McBride, J., Levicnik, A., Smith, R., Nolan, G.P., Contag, C.H., and Fathman, C.G. (2003). Treatment of autoimmune disease by adoptive cellular gene therapy. *Ann. N. Y. Acad. Sci. 998*, 512–519.

Tilg, H., van Montfrans, C., van den Ende, A., Kaser, A., van Deventer, S.J., Schreiber, S., Gregor, M., Ludwiczek, O., Rutgeerts, P., Gasche, C., Koningsberger, J.C., Abreu, L., Kuhn, I., Cohard, M., LeBeaut, A., Grint, P., and Weiss, G. (2002). Treatment of Crohn's disease with recombinant human interleukin 10 induces the proinflammatory cytokine interferon gamma. *Gut 50*, 191–195.

Torriani, F.J., Rodriguez-Torres, M., Rockstroh, J.K., Lissen, E., Gonzalez-Garcia, J., Lazzarin, A., Carosi, G., Sasadeusz, J., Katlama,

C., Montaner, J., Sette, H., Jr., Passe, S., De Pamphilis, J., Duff, F., Schreuk, U.M., Dieterich, D.T., APRICOT Study Group. (2004). Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N. Engl. J. Med. 351*, 438–450.

Tyndall, A., Black, C., Finke, J., Winkler, J., Mertlesmann, R., Peter, H.H., and Gratwohl, A. (1997). Treatment of systemic sclerosis with autologous haemopoietic stem cell transplantation [letter] [see comments]. *Lancet 349*, 254.

Utset, T.O., Auger, J.A., Peace, D., Zivin, R.A., Xu, D., Jolliffe, L., Alegre, M.L., Bluestone, J.A., and Clark, M.R. (2002). Modified anti-CD3 therapy in psoriatic arthritis: a phase I/II clinical trial. *J. Rheumatol. 29*, 1907–1913.

van Bekkum, D.W. (1998). New opportunities for the treatment of severe autoimmune diseases: bone marrow transplantation. *Clin. Immunol. Immunopathol. 89*, 1–10.

Van Der Lubbe, P.A., Reiter, C., Breedveld, F.C., Kruger, K., Schattenkirchner, M., Sanders, M.E., and Riethmuller, G. (1993). Chimeric CD4 monoclonal antibody cM-T412 as a therapeutic approach to rheumatoid arthritis. *Arthritis Rheum. 36*, 1375–1379.

Van Der Lubbe, P.A., Reiter, C., Miltenburg, A.M., Kruger, K., De Ruyter, A.N., Rieber, E.P., Bijl, J.A., Riethmuller, G., and Breedveld, F.C. (1994). Treatment of rheumatoid arthritis with a chimeric CD4 monoclonal antibody (cM-T412): immunopharmacological aspects and mechanisms of action. *Scand. J. Immunol. 39*, 286–294.

Van Der Lubbe, P.A., Dijkmans, B.A., Markusse, H.M., Nassander, U., and Breedveld, F.C. (1995). A randomized, double-blind, placebo-controlled study of CD4 monoclonal antibody therapy in early rheumatoid arthritis. *Arthritis Rheum. 38*, 1097–1106.

Van Deventer, S.J. (1997). Tumour necrosis factor and Crohn's disease. *Gut 40*, 443–448.

Van Dullemen, H.M., Van Deventer, S.J., Hommes, D.W., Bijl, H.A., Jansen, J., Tytgat, G.N., and Woody, J. (1995). Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology 109*, 129–135.

Van Laar, J.M. and Tyndall, A. (2003). Intense immunosuppression and stem-cell transplantation for patients with severe rheumatic autoimmune disease: a review. *Cancer Control 10*, 57–65.

van Oosten, B.W., Lai, M., Hodgkinson, S., Barkhof, F., Miller, D.H., Moseley, I.F., Thompson, A.J., Rudge, P., McDougall, A., McLeod, J.G., Ader, H.J., and Polman, C.H. (1997). Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology 49*, 351–357.

Verbsky, J.W. and White, A.J. (2004). Effective use of the recombinant interleukin 1 receptor antagonist anakinra in therapy resistant systemic onset juvenile rheumatoid arthritis. *J. Rheumatol. 31*, 2071–2075.

Vigeral, P., Chkoff, N., Chatenoud, L., Campos, H., Lacombe, M., Droz, D., Goldstein, G., Bach, J.F., and Kreis, H. (1986). Prophylactic use of OKT3 monoclonal antibody in cadaver kidney recipients. Utilization of OKT3 as the sole immunosuppressive agent. *Transplantation 41*, 730–733.

Vincenti, F., Kirkman, R., Light, S., Bumgardner, G., Pescovitz, M., Halloran, P., Neylan, J., Wilkinson, A., Ekberg, H., Gaston, R., et al. (1998). Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N. Engl. J. Med. 338*, 161–165.

Waldmann, H. and Cobbold, S. (2001). Regulating the immune response to transplants. a role for CD4+ regulatory cells? *Immunity 14*, 399–406.

Waldmann, T.A. (1989). The multi-subunit interleukin-2 receptor. *Annu. Rev. Biochem. 58*, 875–911.

Waldmann, T.A. and O'Shea, J. (1998). The use of antibodies against the IL-2 receptor in transplantation. *Curr. Opin. Immunol. 10*, 507–512.

Wendling, D., Wijdenes, J., Racadot, E., and Morel-Fourrier, B. (1991). Therapeutic use of monoclonal anti-CD4 antibody in rheumatoid arthritis. *J. Rheumatol. 18*, 325–327.

Williams, R.O., Feldmann, M., and Maini, R.N. (1992). Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A. 89*, 9784–9788.

Williams, R.O., Mason, L.J., Feldmann, M., and Maini, R.N. (1994). Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A. 91*, 2762–2766.

Winsor-Hines, D., Merrill, C., O'Mahony, M., Rao, P.E., Cobbold, S.P., Waldmann, H., Ringler, D.J., and Ponath, P.D. (2004). Induction of immunological tolerance/hyporesponsiveness in baboons with a non-depleting CD4 antibody. *J. Immunol. 173*, 4715–4723.

Wofsy, D. and Seaman, W.E. (1987). Reversal of advanced murine lupus in NZB/NZW F1 mice by treatment with monoclonal antibody to L3T4. *J. Immunol. 138*, 3247–3253.

Wolfe, F. and Michaud, K. (2004). Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. *Arthritis Rheum. 50*, 1740–1751.

Woodle, E.S., Xu, D., Zivin, R.A., Auger, J., Charette, J., O'laughlin, R., Peace, D., Jollife, L.K., Haverty, T., Bluestone, J.A., and Thistlethwaite Jr, J.R. (1999). Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3gamma1(Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation 68*, 608–616.

Yamamoto, A.M., Chernajovsky, Y., Lepault, F., Podhajcer, O., Feldmann, M., Bach, J.F., and Chatenoud, L. (2001). The activity of immunoregulatory T cells mediating active tolerance is potentiated in nonobese diabetic mice by an IL-4-based retroviral gene therapy. *J. Immunol. 166*, 4973–4980.

Yang, K., Basu, A., Wang, M., Chintala, R., Hsieh, M.C., Liu, S., Hua, J., Zhang, Z., Zhou, J., Li, M., Phyu, H., Petti, G., Mendez, M., Janjua, H., Peng, P., Longley, C., Borowski, V., Mehlig, M., and Filpula, D. (2003). Tailoring structure-function and pharmacokinetic properties of single-chain Fv proteins by site-specific PEGylation. *Protein Eng. 16*, 761–770.

Zavala, F., Abad, S., Ezine, S., Taupin, V., Masson, A., and Bach, J.F. (2002). G-CSF therapy of ongoing experimental allergic encephalomyelitis via chemokine- and cytokine-based immune deviation. *J. Immunol. 168*, 2011–2019.

Zheng, X.X., Steele, A.W., Hancock, W.W., Kawamoto, K., Li, X.C., Nickerson, P.W., Li, Y., Tian, Y., and Strom, T.B. (1999). IL-2 receptor-targeted cytolytic IL-2/Fc fusion protein treatment blocks diabetogenic autoimmunity in nonobese diabetic mice. *J. Immunol. 163*, 4041–4048.

Zheng, X.X., Sanchez-Fueyo, A., Domenig, C., and Strom, T.B. (2003a). The balance of deletion and regulation in allograft tolerance. *Immunol. Rev. 196*, 75–84.

Zheng, X.X., Sanchez-Fueyo, A., Sho, M., Domenig, C., Sayegh, M.H., and Strom, T.B. (2003b). Favorably tipping the balance between cytopathic and regulatory T cells to create transplantation tolerance. *Immunity 19*, 503–514.

CHAPTER

77

Advocatus Diaboli: What is not known about Autoimmune Disease

JAMES W. GODING

Department of Immunology and Department of Physiology, Monash University, Clayton 3800, Victoria, Australia

WHY IS AUTOIMMUNITY STILL IN SUCH A MUDDLE?

It is a challenging privilege to conclude *The Autoimmune Diseases, 4th edition* by setting out what remains unknown. Perhaps a scientist who is not at the center of autoimmunity might be hesitant in listing gaps in knowledge that must be painfully obvious to those in the field. But, as befits the title, I will argue that we remain in a state of deep ignorance concerning the nature of autoimmunity and its induction, maintenance, and effector mechanisms. We are even uncertain of where the perimeters of autoimmune disease begin and end.

It is sometimes argued that scientists should mount a frontal attack on the so-called "big questions." In biology, this approach is rarely successful, and often delusional. I doubt whether many immunologists realized just how complex the question of tolerance would turn out to be. The "military concept" of a frontal attack seems eerily reminiscent of the ubiquitous "mission statement" that is currently so fashionable in the business world. It usually fails because essential but apparently unrelated information is missing, or simply because the necessary technology is not yet available. The time has to be ripe. Technological progress may make a question suddenly tractable, as witnessed by the occasional simultaneous appearance of a batch of papers on the same hitherto intractable subject. Sometimes major advances are unexpected, unplanned, and even initially unbelievable, coming as the result of asking a totally different question. Such was the case for the discovery of the relevant autoantigen of myasthenia gravis—the acetylcholine receptor (Patrick and Lindstrom, 1973; Lennon et al., 1975).

WHAT WE DO KNOW

Many of the central questions in immunology have been answered. The clonal selection theory continues to underpin our thinking. It has been intermittently under attack, but not fatally (Melchers, 2004). The revolutions that have flowed from the discovery of T and B cells, MHC restriction, and DNA rearrangements to create receptor genes seem comfortably embedded in the past. We now have a fairly clear idea of the mechanisms of antigen recognition by both B- and T cells, and of the genetic mechanisms by which clonal diversity is achieved. It might, therefore, be felt that immunology has reached maturity, and that the functioning of the immune system is reasonably well understood (see Chapter 2). Yet even this would be an overstatement. A glance backwards for just a few years will reveal that immunology is continuing to undergo quite revolutionary changes. Indeed, the rate at which discoveries are being made makes it clear that we should expect more radical discoveries to be just around the corner.

The Autoimmune Diseases, edited by Noel R. Rose and Ian R. Mackay.
ISBN-13: 978-0-12-595961-2 ISBN-10: 0-12-595961-3

Let me take some examples. First, suppressor T cells have reappeared, renamed "regulatory T cells." This is a most unfortunate term, because all T cells are regulatory. We still need to ask what regulates the regulators. If all that this population does is suppress, we should "bite the bullet" and call them suppressors, and hope that we are on more solid footing the second time around (Shevach, 2004; Schwartz, 2005; von Boehmer, 2005). But clearly, for the present, there are many unknowns regarding regulation and the T cells contributing to it (see Chapter 9).

Second, the innate immune system that until a few years ago was seen as "yesteryearish" and boring, has suddenly become the focus of renewed interest and excitement. It has been found to possess a recognition system of sophistication far greater hitherto imagined (Janeway and Medzhitov, 2002). Moreover, in hagfish, the "innate" system has a diversification system involving DNA rearrangements that previously appeared to be exclusive to the adaptive immune system in vertebrates (Pancer et al., 2004). Is this really an adaptive system rather than a "fixed" system, and has nature invented adaptive immunity twice? It is too early to say whether similar gene rearrangements occur in vertebrates.

Third, the renewed interest in the innate immune system has forced us to rethink some cherished concepts. The intractability of the problem of autoimmunity reflects our incomplete understanding of the normal functioning of the immune system, particularly how immune responses are initiated and how mechanisms of self-tolerance operate–opposite sides of the same coin. It is now apparent that mere recognition of antigen by lymphocyte surface receptors is insufficient to activate the adaptive immune system: it needs a "kick start" from the innate system (Medzhitov and Janeway, 1999). Given the well-documented existence of self-reactive albeit low avidity T and B cells in normally healthy individuals, this is just as well (Andre et al., 1996).

WILL THE "REAL" AUTOANTIGEN PLEASE STAND UP?

Autoimmune processes can be analyzed with much greater clarity if they are divided into initiation, maintenance, and effector mechanisms. But let us begin by considering autoantigens, which must be common to all three stages.

The topic of autoimmunity has no shortage of antigens. In most cases, it is not clear whether any individual autoantigen is involved in the pathogenesis of the associated disease, or is merely an innocent bystander. A "real" autoantigen would be one wherein there is definitive evidence that autoimmune attack against it causes disease in humans, either as an initiator or a final target or both.

This requirement is most readily met for diseases in which the damage is mediated by antibodies, such as those demonstrable in myasthenia gravis, Graves' disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, pemphigus vulgaris, and various more uncommon diseases, including those associated with autoantibodies to growth factors such as granulocyte/macrophage colony-stimulating factor (Trapnell et al., 2003) and erythropoietin (Guest and Levitt, 2003), and the folate receptor (Ramaekers et al., 2005). On the other hand, the "real" antigens that determine tissue damage in many common human autoimmune diseases including type I diabetes mellitus, systemic sclerosis, rheumatoid arthritis, and multiple sclerosis remain unidentified, and the relevance to human disease of autoantigens identified or studied in corresponding animal models remains uncertain.

The technology required to identify antigens that are recognized by autoantibodies is well established and relatively straightforward. In marked contrast, the identification of autoantigens recognized by T cells is very much more of a challenge, particularly for those that are restricted by MHC class I and recognized by $CD8^+$ T cells. In particular, the entire role and activities of $CD8^+$ T cells in autoimmune disease is near to a "closed book." Numerous T-cell antigens that are centrally involved in pathogenesis probably remain to be identified, and many mysteries of autoimmunity probably stem from this technological difficulty.

What should we make of the plethora of serologically detectable autoantigens, many of which are intracellular? These reactivities have considerable utility as diagnostic markers, but do they have anything to do with pathogenesis? It is possible to have high levels of many such antibodies and yet be perfectly healthy. Could they merely represent fortuitous cross-reactions with the "real" antigen? Claims that antibodies can penetrate cells and cause damage by binding to intracellular autoantigens remain perennially controversial. The main reason why T cells are so important for defense of the body is because every cell in the body displays on its surface in the MHC groove a more or less random sample of all the proteins that the cell is making. This allows T cells to "see inside" other cells, while B cells and antibodies cannot, and is only possible because of a very complex set of mechanisms involving intracellular proteolysis, transport of peptides across cell membranes, and machinery to load the MHC groove with endogenous or exogenous peptides.

It is interesting but poorly understood why so many intracellular autoantigens recognized by autoantibodies are multi-subunit proteins or protein/nucleic acid complexes. The conventional explanation, which seems convincing, is that the entire multimeric complex is dragged by one subunit into an antigen-presenting cell, perhaps via the membrane immunoglobulin receptors of B cells that are part of the inflammatory component of autoimmunity. Endocytosis is

followed by fragmentation and presentation of peptides from all subunits in the groove of class II MHC molecules on the B-cell surface, ultimately leading to activation of additional helper T cells and consequent "epitope spreading." In many such instances where there are autoantibodies to intracellular molecular complexes, the disease process is multiorgan and multisystem, and may reflect the relatively nonspecific inflammatory effects of circulating antigen/antibody complexes.

Sometimes an autoantigen is ubiquitous, yet the tissue damage is localized to discrete tissue sites. The most dramatic example is primary biliary cirrhosis (Ichiki et al., 2004; see Chapter 54). It is very difficult to see how the antimitochondrial antibodies could be involved in pathogenesis of highly localized tissue damage affecting bile ducts alone. Is the "real" antigen something else? Or are the tissues and cells that are damaged unusually susceptible to some form of effector phase?

INDUCTION OF AUTOIMMUNITY

The strong association of almost all, or perhaps even all, autoimmune diseases with particular HLA alleles is a very clear pointer towards a primary involvement of T-cells in the initiation of disease (see Chapter 20). It also strongly suggests that autoimmunity must be initiated by a single antigen, or at the most by a very small number of antigens that bind preferentially to the relevant HLA allelic product. However, even for diseases with the highest risk HLA alleles, penetrance of risk is incomplete, and the great majority of individuals with the high risk allele do not develop autoimmune disease. Why? It seems all too easy to invoke environmental factors. Incomplete penetrance could result from other mechanisms, such as low probability pathogenic somatic mutations. Somatic mutations are now extremely well documented as playing a causative role in cancer, and the time-dependent buildup of somatic mutations is widely accepted as explaining the dramatic increase in the incidence of cancer with aging. A similar process may well be a part explanation for the age-specific increase in incidence of certain autoimmune diseases. Autoimmune disease does occur in younger age groups, but so does cancer. Somatic mutations are a normal physiologic process in B cells during affinity maturation in secondary responses. They do not seem to be involved in normal T-cell development, but I can see no reason why pathogenic somatic mutations could not occur in lymphocytes in a similar way to those that cause cancer in any somatic cell type. We are just beginning to see evidence in support of a role for somatic mutations in autoimmunity, and it seems very likely that many more examples will be found in the next few years (Clementi et al., 2004; Holzelova et al., 2004; Puck and Straus, 2004).

Mutations in the germline are easy to detect because they are amplified by massive cell division. Cells are extremely small; there are extremely large numbers of them, and mutations are rare events that take place in single cells. Accordingly, the vast majority of somatic mutations would not be expected to have detectable biologic consequences. It has been reasonably argued that the only way that somatic mutations would reveal themselves would be if they caused cancer or autoimmunity. The "Clonal Selection Theory" introduced the concept of the "forbidden clone" as a general explanation for autoimmunity. This hypothesis was formulated long before the mechanisms of receptor diversification were understood or even imagined, and it now seems likely that the great majority of initially arising receptor specificities are forbidden (i.e., autoreactive). Such clones could persist due to failure of deletion, or could arise *de novo* by somatic mutation.

Somatic mutations in genes for lymphocyte receptors for antigen are obvious candidates to make them autoreactive. However, the appeal of this simple hypothesis is greatly diminished by the clear evidence that autoreactive T and B cells are widespread in the body, yet most of the time they do no damage. The term "forbidden clone" has disappeared from the lexicon, although it does aptly describe the regulation-resistant memory-type "autoaggressive" T cell characteristic of organ-specific autoimmunity.

Mutation of genes encoding receptors for antigen is not the only way in which somatic mutations could cause autoimmunity. Somatic mutations in genes encoding any of the pathways involved in immune responses, such as costimulatory molecules, regulation of apoptosis, or signaling pathways in lymphocytes or antigen-presenting cells, could lower the threshold of activation and thereby cause the development of a destructive effector phenotype.

There is considerable evidence, both in humans and in mice, that autoimmunity can be the result of an unfavorable genotype caused by inherited germline mutations that affect the threshold of lymphocyte activation (Bach, 2005; Lang et al., 2005; McGaha et al., 2005). Such germline mutations might be expected to be expressed in large numbers of cells and cause generalized disease such as systemic lupus erythematosus (SLE) by inducing polyclonal activation. I can see no reason why a similar process could not occur for somatic mutations that lower the activation threshold in individual T or B cells. Because the specificity of lymphocytes is clonally distributed, such somatic mutations would be expected to cause antigen-specific tissue damage.

If, as I strongly suspect, a rogue T-cell clone is the most common cause of autoimmunity, how could such a clone be identified? It will probably be necessary to enrich for the culprits, but this will not be easy. I envisage the initiating event to be a single adverse mutation in a single cell, although a multistep process similar to oncogenesis is also

possible. It is clear that the infiltrating population of attackers in naturally occurring autoimmunity is not monoclonal, perhaps due to recruitment of other T- and B-cell clones and resultant epitope spreading. Will "rounding up the usual suspects" be sufficient to apprehend the culprit? What seems to be required is a massive program of amplifying, cloning, and sequencing cDNA from enriched populations. Sequencing of genes for antigen receptors has generally been unrewarding. A more promising target may be the numerous genes encoding molecules involved in setting their threshold of activation, such as kinases, phosphatases, and many others. Such a brute force approach may seem inelegant, but at least it is now technically feasible. If the abundance of particular mRNAs in these pathways change in a selective way, microarrays might help focus the search, but the final answer will probably come from DNA sequencing.

What about environmental factors as an explanation of the incomplete penetrance of high-risk HLA alleles? In spite of massive searches for a "smoking gun," there are extremely few instances where an environmental villain has been unmasked at a molecular level. We have known for well over half a century that beta hemolytic streptococci cause rheumatic fever. Some progress has been made in identifying the relevant microbial antigen molecules (see Chapter 62), but we still have a very inadequate understanding of how these proteins trigger autoimmunity, and we have even less understanding of the mechanism of damage to the heart. It seems quite unlikely that it is due to antibodies. The case for an environmental trigger is also strong for spondylarthropathies including reactive arthritis, but the molecules and mechanisms by which autoimmunity is triggered are not understood at all (see Chapter 33).

A rare but fascinating example of what is almost certainly autoimmunity provoked by an external antigen is the case of pure red cell aplasia associated with autoantibodies to erythropoietin that appear to have been provoked by therapy with recombinant erythropoietin (Bennett et al., 2004). This seems to be a case in which autoimmunity is induced by an external agent that is very closely related to self, but which presumably has some critical differences that make it immunogenic. Possible candidates include aggregation, which is known to increase immunogenicity, and oxidation damage, which could render the protein "non-self." It may be significant that changes in the preparation and storage of recombinant erythropoietin to minimize damage by aggregation and oxidation seem to have reduced the incidence of such autoimmune reactions. It seems unlikely that the case of pure red cell aplasia points to any general principle regarding the provoking of autoimmunity by an external antigen that is closely related to "self" because it is possible to make anti-allotype antibodies that are specific for an allelic product of self that differs by only a single amino acid, and yet these antibodies do not react with the "self" allele (Warner et al., 1977; see below).

The case in which an environmental factor in a naturally occurring autoimmune disease seems best understood at the molecular level is celiac disease (McManus and Kelleher, 2003; see Chapter 51). Peptides from the wheat protein gliadin bind to the relevant HLA class II allelic products, triggering T-cell activation and thereby initiating local disease in the duodenum. The gliadin peptides are covalently modified by tissue transglutaminase, increasing their affinity of binding to the relevant HLA molecules. Transglutaminase is a serologically detectable autoantigen in the disease, although it is not clear whether it also represents an important target in the effector phase. The identification of an environmental trigger for celiac disease raises hopes that other autoimmune diseases may likewise have identifiable environmental triggers. In the vast majority of other cases, the initiating antigen for autoimmune disease remains unknown, and it is not even clear whether it is environmental, or intrinsic to the patient.

Returning to celiac disease, this is not without difficulties as a general model for induction of autoimmunity. Is it a true autoimmune disease or merely an example of cell-mediated hypersensitivity to foods? There is little evidence that such autoantibodies or T-cell reactivity to transglutaminase are involved in generating tissue damage.

A similar mechanism to that of celiac disease, also involving covalent post-translational modification of self proteins and peptides, has been proposed for rheumatoid arthritis, where some autoantigenic peptides have been found to have a covalent modification involving conversion of arginine to citrulline (Meyer, 2004). However, the case for involvement of this process in pathogenesis of the disease is less clear than for celiac disease, even though with refined assays the specificity of autoantibodies to cyclic citrullinated peptides is remarkably high (Rantapaa-Dahlqvist et al., 2003).

Does inflammation play a role in initiation of autoimmunity? The hypothesis is attractive because the activation of the innate immune system induces inflammation and maturation of dendritic cells, which are key events in the initiation of immune responses. This concept has been given many colorful names, particularly the "danger hypothesis" (Matzinger, 1994). The need for adjuvants to provoke strong immune responses is probably a reflection of the same requirement, which Janeway has termed "immunologists' dirty little secret" (Medzhitov and Janeway, 1999). To some extent these terms have been overtaken by a more precise understanding of the role of dendritic cells and Toll-like receptors in the initiation of T-cell responses (see Chapter 4).

Activation of lymphocytes must occur in stages. In some mouse models of type I diabetes mellitus, pancreatic islets become surrounded by a cuff of lymphocytes without any apparent damage. A distinct additional signal or event seems to be required for them to enter the islets and destroy

them. Is activation of the innate immune system and inflammation all that is required to change "sleeper" autoreactive T cells into active assassins (Poirot et al., 2004; Lang et al., 2005)?

Inflammation is clearly not a general provoker of autoimmunity. A popular hypothesis is that autoimmunity results from a combination of an inflammatory stimulus and a high-risk genotype. This is unlikely to be the complete answer, because all individuals with "high-risk" HLA alleles will encounter injuries and infection throughout life, yet few will develop autoimmunity. On the contrary, the "clean environment" of modern Western life is associated with rising incidence of autoimmune disease (Feillet and Bach, 2004). Similarly, nonobese diabetic (NOD) mice are highly prone to type 1 diabetes mellitus caused by autoimmune destruction of their pancreatic beta cells, but, contrary to the danger hypothesis as a cause for autoimmunity (see above), the cleaner the mice, the higher the incidence of diabetes (Zaccone et al., 2004). Moreover, the provocation of autoantibodies to erythropoietin by the recombinant hormone seems to have occurred in the absence of any adjuvant or obvious inflammatory stimulus (Bennett et al., 2004; see above).

In summary, we can say with confidence that HLA antigens and T cells must be of primary importance in the induction of autoimmunity. We must acknowledge our ignorance concerning the role of environmental triggers and inflammation. We can suspect that somatic mutations in T cells that influence specificity by setting the threshold of activation may provide an explanation for the incomplete penetrance of the known genetic risk factors.

MAINTENANCE OF AUTOIMMUNITY

Is autoimmunity antigen-driven? This would seem to be the case in general, and certainly for celiac disease where removal of gluten from the diet results in the intestinal mucosa returning to normal: but is this autoimmunity? The widely assumed premise that most cases of autoimmunity are driven by endogenous antigen is supported by fading levels of autoantibodies in organ-specific diseases when the relevant autoantigen is completely eliminated ("burn out") as exemplified by thyroiditis, gastritis, and type 1 diabetes, but in other examples definite proof of autoantigen requirement is lacking. For B cells at least, there is a further reason to believe that the process of autoimmunity is autoantigen-driven, because autoantibody-secreting B cells usually have mutated antibody genes that are typical of antigen-driven secondary responses. The situation for T cells is less clear, but lesions do depend on the presence of T cells of the "activated/memory" phenotype.

The ideal experimental model to test whether autoimmunity is driven by endogenous antigen would be a disease in which all the tissue bearing the relevant autoantigen could be removed without killing the animal. The "classical" experiment that is frequently cited in this regard is that of Triplett (1962), in which removal of the pituitary from embryonic frogs was claimed to prevent the induction of tolerance to this organ, as evidenced by rejection of autologous pituitary grafts after the frogs had matured. In view of its potential importance, it is remarkable that it took 20 years before attempts to repeat it were made, and it was found to be irreproducible (Rollins-Smith and Cohen, 1982).

In mice, some organs that are subject to autoimmune attack are not required for life, such as the ovary, thyroid, or even the stomach. They could be removed and life maintained. Two key questions could be asked. If a mouse had evidence of an autoimmune attack against an organ, and that organ were *completely* removed, would the autoimmune process fade away? Even if it did, would autoimmune memory cells persist? And if the relevant organ were removed sufficiently early in life, would this prevent the development of autoimmune attack against that organ? Suppose, for example, that NOD mice that have begun to produce autoimmune attack on the pancreatic islet cells were pancreatectomized and maintained on insulin, would the autoantibodies disappear? And would they return if the mice were reconstituted with syngeneic pancreas at a later stage?

But would removal of the organ that bears the brunt of immunologic attack be enough to remove all the sites of antigen expression? Probably not, because low-level "ectopic" antigen expression in other tissues is very common. Ectopic antigen expression may even help establish and maintain self-tolerance, particularly if it is in the thymus or the bone marrow, or possibly anywhere, since regulatory T cells can be induced peripherally (see Chapter 9). Many self antigens are expressed at low levels in the thymus, driven by the AIRE (autoimmune regulator) gene, and germline mutations of this gene cause autoimmunity (Chapters 37 and 38; Finnish-German APECED Consortium, 1997; Nagamine et al., 1997; Ramsdell and Ziegler, 2003).

EFFECTOR MECHANISMS IN AUTOIMMUNITY

The instances where the mechanisms of tissue damage are well characterized are almost entirely confined to antibody-mediated effects. These are generally diseases where the "real" autoantigen is clearly defined, such as myasthenia gravis, Graves' disease, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, acquired hemophilia, and pemphigus vulgaris. In a few cases, autoantibodies have been found to neutralize growth factors such as erythropoietin or granulocyte-macrophage colony-

stimulating factor, presumably by direct binding, which prevents their association with the cognate cellular receptors. Antibodies may mediate damage by direct binding, activation of complement, facilitation of phagocytosis, or a combination of these. We also have some, but still insufficient, understanding of autoimmune tissue damage due to circulating antigen–antibody complexes, such as lupus and various forms of vasculitis (see Chapters 27, 28, 65).

However, much less clear are the instances where demonstrable autoantibodies cannot be implicated as a cause of damage, such as type 1 diabetes mellitus, primary biliary cirrhosis, and autoimmune hepatitis, and in some diseases of suspected autoimmune etiology such as multiple sclerosis where the role of autoantibodies remains controversial (Berger et al., 2003; Hafler et al, 2005). There is considerable evidence that damage is mediated by the direct action of T-cells, either as cytotoxic $CD8^+$ T cells or by the secretion of cytokines from $CD4^+$ cells and probably $CD8^+$ cells as well. In nearly all these cases, the "real" antigen in humans has not been definitively identified, nor has the precise mechanism of tissue damage been established. We know even less about the mechanism of tissue damage in rheumatic heart disease, systemic sclerosis, rheumatoid arthritis, or polymyositis.

Not all damage comes from antibody or T cells. Recent evidence has pointed to a significant role for natural killer (NK) cells in the destructive infiltration of the pancreatic islets in NOD mice (Poirot et al., 2004). NKT cells also seem to play a major role in downmodulating the susceptibility to autoimmunity in some animal models, although evidence for a role of these cells in human disease is far less clear (Godfrey, 2000; Esteban et al., 2003).

WE STILL DON'T UNDERSTAND TOLERANCE

Sometimes it is argued that "tolerance is dead," and that the key to understanding self/non-self discrimination lies at the level of the dendritic cell and its state of activation. This seems no more justified by the facts than Ehrlich's often misinterpreted "*horror autotoxicus*" that came to be taken to mean that autoimmunity was impossible.

There is abundant and very strong evidence that the initial repertoire of specificity of T and B cells is random and, therefore, includes anti-self specificities, and that many of these are deleted in the thymus and bone marrow, respectively, a process known as "central tolerance" (see Chapters 8, and 13). Few immunologists would doubt the existence of this process. Equally well, most would acknowledge that central mechanisms cannot be the whole story because it seems unlikely that each and every self antigen is expressed in the primary lymphoid organs, and there is good evidence that some self-reactive cells slip out. There is also good evidence for B cells that somatic mutations that drive affinity maturation in secondary responses are likely to generate anti-self reactivities (see Chapters 8, and 13). Mechanisms of "peripheral tolerance" must exist to deal with these potential sources of autoimmunity.

There has been a tendency in recent times to downplay the importance of structural differences between self and non-self, and to emphasize other factors in making the choice for the immune system of whether to respond or not. I take the traditional view of non-self as being structurally different from self, and that this is the main driving force for immune responses.

At least in the case of B cells, the further the phylogenetic distance between self and non-self proteins the greater the sequence divergence and the stronger the immune response to them. The reason is obviously that the closer the sequence of the antigen is to that of the corresponding self protein, the greater the fraction of self-reactive cells that can be silenced via self tolerance. The fact that it is sometimes possible to generate autoantibodies to self proteins does not negate this basic fact.

If there is any doubt about this point, consider the case of IgM allotypes in the mouse. There are two allelic forms of μ-chain, which differ by only one amino acid in the constant region, out of a total of 434 amino acids (Schreier et al., 1986). Immunization of mice with IgM of the non-self allele in the absence of adjuvants generates antibodies that react with the non-self allele but not the self allele (Warner et al, 1977). This tells us several things. It demonstrates the ability of the immune system to discriminate between self and non-self is extremely precise. It also tells us that the lymphocyte repertoire does not respond to the self allele when challenged with the non-self allele. If we accept the notion that the activation signal delivered by helper T cells to B cells is not antigen-specific, it follows that B cells with specificity for the self allele must be either absent or silenced.

The situation for T cells is considerably more complex, because, when the antigen comprises living cells such as transplanted organs or the mixed lymphocyte reaction, allogeneic interactions are generally stronger than xenogeneic. However, these situations are highly artificial. Their relevance to naturally occurring immune reactions is still unclear, and the reasons for the strength of alloreactivity are still not well understood.

There have been many experimental models of tolerance, some with more rationale than others. One very popular model, the rationale of which I have never understood, has been the induction of "tolerance" by intravenous injection of large doses of de-aggregated human IgG in mice. If these experiments purport to reveal a universal truth, it would seem logical that administration of high doses of mouse IgG to humans would also induce unresponsiveness. This experiment has been done unintentionally in countless attempts to

use monoclonal antibodies as therapeutic agents. Administration of large quantities of pure, de-aggregated endotoxin-free mouse monoclonal antibodies to humans always induces an anti-mouse response, in the absence of any overt inflammatory co-stimulus. This is why therapeutic mouse monoclonal antibodies must be humanized for successful use as therapeutic agents. So much for the de-aggregated IgG tolerance model.

Experiments in which autoantibodies can be induced by immunization with self antigens almost always involves the use of complete Freund's adjuvant, and their relevance to naturally occurring tolerance and autoimmunity must be evaluated taking this into account. These experiments certainly prove that self tolerance can sometimes be broken under extreme conditions, and that self tolerance is not always complete (see below).

In recent years, the attention of many immunologists has shifted from central tolerance to the periphery. One of the most important recent insights has been the appreciation of the central role of dendritic cells in the initiation of T-cell responses. However, we should not forget that many other cell types, notably B cells and macrophages, also play an important role in presentation of antigen to T cells, albeit perhaps more importantly once a primary response has been established.

It is agreed that under some circumstances dendritic cells can induce activation of T cells, while under others they can induce tolerance. It has become orthodoxy to explain the decision on the basis that encounter of the T cell with antigen in the absence of costimulation from activated dendritic cells leads to tolerance, while activation of dendritic cells induces T-cell activation (Abbas and Sharpe, 2005). This model seems too simplistic. To give one example of the inadequacy of this model, recent work has shown that induction of T cell tolerance by resting dendritic cells requires engagement of the costimulatory molecules PD-1 and CTLA-4 on T cells (Abbas and Sharpe, 2005; Probst et al., 2005). Much remains to be learned about decision-making of activation, non-response, or tolerance at the synaptic interface between the dendritic cell and the T cell.

To summarize, the field of self/non-self discrimination and tolerance abounds with paradoxes. The mere presence of non-self is not necessarily sufficient for an immune response to occur. There is abundant evidence that, in addition to the presence of antigen, activation of dendritic cells is essential for the initiation of T-cell responses. And yet, the strong human anti-mouse antigen response to mouse IgG in the absence of adjuvants seems to argue that activation of inflammation is not essential for initiation of immune responses. This should not be taken to imply that activation of the innate immune system is not important for initiation of immune responses. There seems little doubt that inflammation potentiates the induction of immune responses. This is almost certainly a key factor in how adjuvants work. However, the role (if any) of inflammation in the induction of human autoimmune disease is still unclear. Obviously, we still don't understand all the rules of the game.

SELF TOLERANCE IS NOT ABSOLUTE, AND IS INCOMPLETE

The immune system evolved to produce a workable but imperfect defense system involving a series of compromises between the conflicting demands of destroying non-self but not self. Given the enormous diversity of biologic structures and the corresponding diversity of immune receptors, the distinction between self and non-self cannot be absolute. It must depend on choices based on thresholds of affinity of recognition and setting the sensitivity of signaling mechanisms to optimize survival for populations as a whole. In genetically diverse populations it seems inevitable that some individuals will have these thresholds set too far at one or other extreme. A system capable of defending the body against an almost infinite diversity of external threats would be expected to set the threshold for activation at a level where some individuals would succumb to autoimmunity.

There are many reasons why self-tolerance may be incomplete. Sequestration of a particular self antigen away from the immune system would be expected to result in immunologic ignorance. Presentation of soluble antigens in monomeric forms can induce anergy rather than deletion of B-cells with cognate receptors. There is also no reason to think that there would be evolutionary pressure to select for mechanisms of robust and unbreakable tolerance to self antigens that are unlikely to cause trouble in most individuals. Accordingly, the numerous examples of induction of autoantibodies by immunizing with autologous antigens in complete Freund's adjuvant do not invalidate the general concept of self tolerance any more than the concept of "*horror autotoxicus*" rules out the possibility of autoimmune disease.

THE HOLY GRAIL: INDUCTION OF ANTIGEN-SPECIFIC TOLERANCE

> ". . . evolution is selecting . . . to maintain the probability of debilitating autoimmunity acceptably low and of an effective ridding response acceptably high. What medicine is trying to do is alter these probabilities." (Cohn, 2002).

The triumph of vaccination as a means to prevent disease is a reflection of the ease of turning on specific immune responses. Much of this knowledge was obtained empirically, with little understanding of the underlying

mechanisms. Preventing or turning off immune responses has been more challenging. Apart from tissue matching involving ABO blood groups and HLA tissue antigens, there have been very few instances where immunologic knowledge has been successfully applied to implement antigen-specific non-responsiveness in clinical medicine. The only example that I can think of is in the prevention of Rh sensitization of mothers at childbirth by the administration of anti-Rh antibodies. This highly effective procedure has been in use for many decades, but it is still not clear how it works. It could be as simple as the rapid clearance of antigen from the body before there is time for an immune response to be activated, although more complex mechanisms have not been ruled out.

Given all these uncertainties, it is not surprising that treatment remains unsatisfactory. We still rely almost entirely on drugs that suppress immune responses non-specifically. The challenge facing treatment of autoimmunity is to be able to prevent immune responses from occurring in an antigen-specific way. This form of treatment would be expected to have far fewer side effects than non-specific immunosuppression. What are the prospects?

Antigen-specific intervention in autoimmune disease seems most promising at the level of prevention. It has been possible to prevent autoimmune disease in mice by expressing the relevant autoantigen in the thymus from early embryonic development (Alderuccio et al., 1993; see Chapter 39), but it is difficult to see how this successful strategy could be applied to autoimmune disease in humans. However, some attempts are currently being made to prevent the onset of autoimmune disease in high-risk individuals. For example, clinical trials are under way to try to prevent type 1 diabetes mellitus by intranasal administration of proinsulin. This intervention seems to lack a convincing rationale, and there is a risk that it could provoke the disease instead of preventing it. Nonetheless, preliminary results are encouraging (see Chapter 75).

Turning off an immune response that has already been initiated is an even greater challenge. In the vast majority of cases, once an immune response gets going, the only known way to stop it is by nonspecific immunosuppression. Some success in modifying ongoing immune responses has been claimed in the area of desensitization for allergy, but this traditional procedure still requires examination in the context of contemporary immunology. In any event, this is a special case and its relevance to treatment of autoimmunity is uncertain.

Wouldn't it be wonderful if we could indeed turn off autoimmunity by antigen-specific means? Success would seemingly demand the identification of the "real" antigens that are driving the process, finding a way to turn off the response in an antigen-specific manner. However, the diseases in which we most wish to achieve this, such as multiple sclerosis, the "real" autoantigen remains unknown. Moreover, we still do not understand the rules that govern whether encounter of a naïve or activated T-cell with cognate antigen will be ignored, or will induce activation or tolerance. Until we do, the induction of antigen-specific tolerance will remain a dream.

At the present time, the goal of cure or even amelioration of autoimmune disease by antigen-specific means seems a long way off, but it would be scarcely appropriate to conclude this book on a note of pessimism. The history of medicine is littered with pronouncements that a seemingly distant goal is "impossible," but with the pronouncement becoming refuted, sometimes not long after the pessimist has spoken. It is now half a century since the first successful renal transplant (Morris, 2004). An ability to induce antigen-specific tolerance would revolutionize both transplantation and autoimmunity. Given the current pace of immunologic research, who can say that such a goal is impossible?

References

Abbas, A.K. and Sharpe, A.H. (2005) Dendritic cells giveth and taketh away. *Nat. Immunol. 6,* 227–228.

Alderuccio, F., Toh, B.H., Tan, S.S., Gleeson, P.A., and van Driel, I.R. (1993). An autoimmune disease with multiple molecular targets abrogated by the transgenic expression of a single autoantigen in the thymus. *J. Exp. Med. 178,* 419–426.

Andre, I., Gonzalez, A., Wang, B., Katz, J., Benoist, C., and Mathis, D. (1996). Checkpoints in the progression of autoimmune disease, lessons from diabetes models. *Proc. Natl. Acad. Sci. U.S.A. 93*, 2260–2263.

Bach, J.-F. (2005) A toll-like trigger for autoimmune disease. *Nat. Med. 11,* 120–121.

Bennett, C.L., Luminari, S., Nissenson, A.R., Tallman, M.S., Klinge, S.A., McWilliams, N., McKoy, J.M., Kim, B., Lyons, E.A., Trifilio, S.M., Raisch, D.W., Evens, A.M., Kuzel, T.M., Schumock, G.T., Belknap, S.M., Locatelli, F., Rossert, J., and Casadevall, N. (2004). Pure red-cell aplasia and epoetin therapy. *N. Engl. J. Med. 351*, 1403–1408.

Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F., and Reindl, M. (2003). Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. *N. Engl. J. Med. 349*, 139–145.

Clementi, R., Dagna, L., Dianzani, U., Dupre, L., Dianzani, I., Ponzoni, M., Cometa, A., Chiocchetti, A., Sabbadini, M.G., Rugarli, C., Ciceri, F., Maccario, R., Locatelli, F., Danesino, C., Ferrarini, M., and Bregni, M. (2004). Inherited perforin and Fas mutations in a patient with autoimmune lymphoproliferative syndrome and lymphoma. *N. Engl. J. Med. 351*, 1419–1424.

Cohn, M. (2002). The immune system, a weapon of mass destruction invented by evolution to even the odds during the war of the DNAs. *Immunol. Rev. 185*, 24–38.

Esteban, L.M., Tsoutsman, T., Jordan, M.A., Roach, D., Poulton, L.D., Brooks, A., Naidenko, O.V., Sidobre, S., Godfrey, D.I., and Baxter, A.G. (2003). Genetic control of NKT cell numbers maps to major diabetes and lupus loci. *J. Immunol. 171*, 2873–2878.

Feillet, H. and Bach, J.F. (2004). On the mechanisms of the protective effect of infections on type 1 diabetes. *Clin. Dev. Immunol. 11*, 191–194.

Finnish-German APECED Consortium (1997). An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. The Finnish-German APECED Consortium.

Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy. *Nat. Genet. 17*, 399–403.

Godfrey, D.I., Hammond, K.J., Poulton, L.D., Smyth, M.J., and Baxter, A.G. (2000). NKT cells: facts, functions and fallacies. *Immunol. Today 21*, 573–583.

Guest, S.S. and Levitt, L. (2003). Pure red-cell aplasia secondary to antierythropoietin antibodies. *N. Engl. J. Med. 349*, 2572–2573.

Hafler, D.A., Slavik, J.M., Anderson, D.E., O'Connor, K.C., De Jager, P., and Baecher-Allan, C. (2005). Multiple sclerosis. *Immunol. Rev. 204*, 208–231.

Holzelova, E., Vonarbourg, C., Stolzenberg, M.C., Arkwright, P.D., Selz, F., Prieur, A.M., Blanche, S., Bartunkova, J., Vilmer, E., Fischer, A., Le Deist, F., and Rieux-Laucat, F. (2004). Autoimmune lymphoproliferative syndrome with somatic Fas mutations. *N. Engl. J. Med. 351*, 1409–1418.

Ichiki, Y., Shimoda, S., Ishibashi, H., and Gershwin, M.E. (2004). Is primary biliary cirrhosis a model autoimmune disease? *Autoimmun. Rev. 3*, 331–336.

Janeway, C.A., Jr. and Medzhitov, R. (2002). Innate immune recognition, *Annu. Rev. Immunol. 20*, 197–216.

Lang, K.S., Recher, M., Junt, T., Navarini, A.A., Harris, N.L., Freigang, S., Odermatt, B., Conrad, C., Ittner, L.M., Bauer, S., Luther, S.A., Uematsu, S., Akira, S., Hengartner, H., and Zinkernagel, R.M. (2005). Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat. Med. 11*, 138–145.

Lennon, V.A., Lindstrom, J.M., and Seybold, M.E. (1975). Experimental autoimmune myasthenia: A model of myasthenia gravis in rats and guinea pigs. *J. Exp. Med. 141*, 1365–1375.

Matzinger, P. (1994). Tolerance, danger, and the extended family. *Annu. Rev. Immunol. 12*, 991–1045.

McGaha, T.L, Sorrentino, B., and Ravetch, J.V. (2005) Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science 307,* 590–593.

McManus, R. and Kelleher, D. (2003). Celiac disease—the villain unmasked? *N. Engl. J. Med. 348*, 2573–2574.

Medzhitov, R. and Janeway, C.A., Jr. (1999). Innate immune induction of the adaptive immune response. *Cold Spring Harb. Symp. Quant. Biol. 64*, 429–435.

Melchers, F. (2004). The death of a dogma? *Nat. Immunol. 5*, 1199–1201.

Meyer, O. (2004) Is the celiac disease model relevant to rheumatoid arthritis? *Joint Bone Spine 71,* 4–6.

Morris, P.J. (2004). Transplantation—a medical miracle of the 20th century. *N. Engl. J. Med. 351*, 2678–2680.

Nagamine, K., Peterson, P., Scott, H.S., Kudoh, J., Minoshima, S., Heino, M., Krohn, K.J., Lalioti, M.D., Mullis, P.E., Antonarakis, S.E., Kawasaki, K., Asakawa, S., Ito, F., and Shimizu, N. (1997). Positional cloning of the APECED gene. *Nat. Genet. 17*, 393–398.

Pancer, Z., Amemiya, C.T., Ehrhardt, G.R., Ceitlin, J., Gartland, G.L., and Cooper, M.D. (2004). Somatic diversification of variable lymphocyte receptors in the agnathan sea lamprey. *Nature 430*, 174–180.

Patrick, J. and Lindstrom, J. (1973). Autoimmune response to acetylcholine receptor. *Science 180*, 871–872.

Poirot, L., Benoist, C., and Mathis, D. (2004). Natural killer cells distinguish innocuous and destructive forms of pancreatic islet autoimmunity. *Proc. Natl. Acad. Sci. U.S.A. 101*, 8102–8107.

Probst, H.C., McCoy, K., Okazaki, T., Honjo, T., and van den Broek, M. (2005). Resting dendritic cells induce peripheral CD8(+) T cell tolerance through PD-1 and CTLA-4. *Nat. Immunol. 6*, 280–286.

Puck, J.M. and Straus, S.E. (2004). Somatic mutations—not just for cancer anymore. *N. Engl. J. Med. 351*, 1388–1390.

Ramaekers, V.T., Rothenberg, S.P., Sequeira, J.M., Opladen, T., Blau, N., Quadros, E.V., and Selhub, J. (2005). Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *N. Engl. J. Med. 352*, 1985–1991.

Ramsdell, F. and Ziegler, S.F. (2003). Transcription factors in autoimmunity. *Curr. Opin. Immunol. 15*, 718–724.

Rantapaa-Dahlqvist, S., de Jong, B.A., Berglin, E., Hallmans, G., Wadell, G., Stenlund, H., Sundin, U., and van Venrooij, W.J. (2003). Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum. 48*, 2741–2749.

Rollins-Smith, L.A. and Cohen, N. (1982). Self-pituitary grafts are not rejected by frogs deprived of their pituitary anlagen as embryos. *Nature 299*, 820–821.

Schreier, P.H., Quester, S., and Bothwell, A. (1986). Allotypic differences in murine mu genes. *Nucleic Acids Res. 14*, 2381–2389.

Schwartz, R.H. (2005). Natural regulatory T cells and self-tolerance. *Nat. Immunol. 6*, 327–330.

Shevach, E.M. (2004). Regulatory/suppressor T cells in health and disease. *Arthritis Rheum. 50*, 2721–2724.

Trapnell, B.C., Whitsett, J.A., and Nakata, K. (2003). Pulmonary alveolar proteinosis. *N. Engl. J. Med. 349*, 2527–2539.

Triplett, E.L. (1962). On the mechanism of immunologic self recognition. *J. Immunol. 89*, 505–510.

von Boehmer, H. (2005). Mechanisms of suppression by suppressor T cells. *Nat. Immunol. 6*, 338–344.

Warner, N.L., Goding, J.W., Gutman, G.A., Warr, G.W., Herzenberg, L.A., Osborne, B.A., van der Loo, W., Black, S.J., and Loken, M.R. (1977). Allotypes of mouse IgM immunoglobulin, *Nature 265*, 447–449.

Zaccone, P., Raine, T., Sidobre, S., Kronenberg, M., Mastroeni, P., and Cooke, A. (2004). *Salmonella typhimurium* infection halts development of type 1 diabetes in NOD mice. *Eur. J. Immunol. 34*, 3246–3256.

Index

C

D

E

F

H

J

K

L

O

P

Q

R

S

T

U

V

Erratum

The Publisher regrets that Figures **2.2** and **2.4–2.7** in the color plate are missing explanatory labels. Black and white versions of the figures showing the correct labelling can be found on the following pages:

Figure 2.2 *(page 13)*; **Figure 2.4** *(page 16)*; **Figure 2.5** *(page 17)*; **Figure 2.6** *(page 19)*; **Figure 2.7** *(page 20)*

Additionally, the Publisher regrets that the following credit lines were omitted from the text:

Figures **2.1, 2.2, 2.4,** and **2.6**:

From Delves, P & Roitt, I. (2000). Advances in immunology: the immune system (first of two parts). N. Engl. J. Med. 343, 37–49. Copyright © 2000 Massachusetts Medical Society. All rights reserved.

Figures **2.5** and **2.7**:

From Delves, P & Roitt, I. (2000). Advances in immunology: the immune system: second of two parts. N. Engl. J. Med. 343, 108–117. Copyright © 2000 Massachusetts Medical Society. All rights reserved.

The Publisher apologizes for any inconvenience these omissions may have caused.