Autoimmunity

METHODS IN MOLECULAR MEDICINE™

John M. Walker, SERIES EDITOR

- 112 **Molecular Cardiology: Methods and Protocols**, edited by Zhongjie Sun, 2005
- 111 **Chemosensitivity: Volume 2, In Vivo Models, Imaging, and Molecular Regulators**, edited by Rosalyn D. Blumethal, 2005
- 110 **Chemosensitivity: Volume 1, In Vitro Assays**, edited by Rosalyn D. Blumethal, 2005
- 109 **Adoptive Immunotherapy, Methods and Protocols**, edited by Burkhard Ludewig and Matthias W. Hoffman, 2005
- 108 **Hypertension, Methods and Protocols**, edited by Jérôme P. Fennell and Andrew H. Baker, 2005
- 107 **Human Cell Culture Protocols, Second Edition**, edited by Joanna Picot, 2005
- 106 **Antisense Therapeutics, Second Edition**, edited by M. Ian Phillips, 2005
- 105 **Developmental Hematopoiesis: Methods and Protocols**, edited by Margaret H. Baron, 2005
- 104 **Stroke Genomics: Methods and Reviews**, edited by Simon J. Read and David Virley, 2004
- 103 **Pancreatic Cancer: Methods and Protocols**, edited by Gloria H. Su, 2004
- 102 **Autoimmunity: Methods and Protocols**, edited by Andras Perl, 2004
- 101 **Cartilage and Osteoarthritis: Volume 2, Structure and In Vivo Analysis**, edited by Frédéric De Ceuninck, Massimo Sabatini, and Philippe Pastoureau, 2004
- 100 **Cartilage and Osteoarthritis: Volume 1, Cellular and Molecular Tools**, edited by Massimo Sabatini, Philippe Pastoureau, and Frédéric De Ceuninck, 2004
- 99 **Pain Research: Methods and Protocols**, edited by David Z. Luo, 2004
- 98 **Tumor Necrosis Factor: Methods and Protocols**, edited by Angelo Corti and Pietro Ghezzi, 2004
- 97 **Molecular Diagnosis of Cancer: Methods and Protocols, Second Edition**, edited by Joseph E. Roulston and John M. S. Bartlett, 2004
- 96 **Hepatitis B and D Protocols: Volume 2, Immunology, Model Systems, and Clinical Studies**, edited by Robert K. Hamatake and Johnson Y. N. Lau, 2004
- 95 **Hepatitis B and D Protocols: Volume 1, Detection, Genotypes, and Characterization**, edited by Robert K. Hamatake and Johnson Y. N. Lau, 2004
- 94 **Molecular Diagnosis of Infectious Diseases, Second Edition**, edited by Jochen Decker and Udo Reischl, 2004
- 93 **Anticoagulants, Antiplatelets, and Thrombolytics**, edited by Shaker A. Mousa, 2004
- 92 **Molecular Diagnosis of Genetic Diseases, Second Edition**, edited by Rob Elles and Roger Mountford, 2004
- 91 **Pediatric Hematology: Methods and Protocols**, edited by Nicholas J. Goulden and Colin G. Steward, 2003
- 90 **Suicide Gene Therapy: Methods and Reviews**, edited by Caroline J. Springer, 2004
- 89 **The Blood–Brain Barrier: Biology and Research Protocols**, edited by Sukriti Nag, 2003
- 88 **Cancer Cell Culture: Methods and Protocols**, edited by Simon P. Langdon, 2003
- 87 **Vaccine Protocols, Second Edition**, edited by Andrew Robinson, Michael J. Hudson, and Martin P. Cranage, 2003
- 86 **Renal Disease: Techniques and Protocols**, edited by Michael S. Goligorsky, 2003
- 85 **Novel Anticancer Drug Protocols**, edited by John K. Buolamwini and Alex A. Adjei, 2003
- 84 **Opioid Research: Methods and Protocols**, edited by Zhizhong Z. Pan, 2003
- 83 **Diabetes Mellitus: Methods and Protocols**, edited by Sabire Özcan, 2003
- 82 **Hemoglobin Disorders: Molecular Methods and Protocols**, edited by Ronald L. Nagel, 2003
- 81 **Prostate Cancer Methods and Protocols**, edited by Pamela J. Russell, Paul Jackson, and Elizabeth A. Kingsley, 2003
- 80 **Bone Research Protocols**, edited by Miep H. Helfrich and Stuart H. Ralston, 2003
- 79 **Drugs of Abuse: Neurological Reviews and Protocols**, edited by John Q. Wang, 2003
- 78 **Wound Healing: Methods and Protocols**, edited by Luisa A. DiPietro and Aime L. Burns, 2003

METHODS IN MOLECULAR MEDICINE™

Autoimmunity

Methods and Protocols

Edited by

Andras Perl, MD, PhD

*Departments of Medicine, Microbiology, and Immunology,
State University of New York, Upstate Medical University,
College of Medicine, Syracuse, NY*

HUMANA PRESS TOTOWA, NEW JERSEY

© 2004 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com

All rights reserved.

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in Molecular Medicine™ is a trademark of The Humana Press Inc.

All papers, comments, opinions, conclusions, or recommendations are those of the author(s), and do not necessarily reflect the views of the publisher.

This publication is printed on acid-free paper. (∞)

ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

Production Editor: Angela Burkey

Cover design by Patricia F. Cleary.

Cover illustration: Histopathology of EAU in the B10.RIII mouse (Chapter 21, Fig. 3.; see page 411 for complete legend and discussion on page 410).

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapress.com; or visit our Website: www.humanapress.com

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$25.00 per copy is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [1-58829-231-2/04 \$25.00].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

eISBN: 1-59259-805-6

ISSN: 1543-1894

Library of Congress Cataloging-in-Publication Data

Autoimmunity : methods and protocols / edited by Andras Perl.

p. ; cm. -- (Methods in molecular medicine ; 102)

Includes bibliographical references and index.

ISBN 1-58829-231-2 (alk. paper)

1. Autoimmune diseases--Laboratory manuals. 2. Autoimmunity--Laboratory manuals.
[DNLM: 1. Autoimmunity--physiology--Laboratory Manuals. 2. Autoimmune Diseases--physiopathology--Laboratory Manuals. 3. Models, Immunological--Laboratory Manuals. 4. T-Lymphocytes--immunology--Laboratory Manuals. QW 25 A938 2004] I. Perl, Andras. II. Series.
RC600.A852 2004
616.97'8--dc22

2004000177

*To my parents,
Ibolya and Miklos,
and family,
Katalin, Annmarie, Marcel, and Daniel,
for their inspiration, support, and love
in my pursuit of medicine and science*

Preface

“Research is to see what everybody else has seen, and to think what nobody else has thought.” — *Albert Szentgyörgyi*

Autoimmunity: Methods and Protocols is intended to serve as a ready-to-use guide to establish and interrogate human and animal models of autoimmune diseases. The first chapter, “Pathogenesis and Spectrum of Autoimmunity,” discusses major hypotheses driving this most tantalizing area of research since Paul Ehrlich proposed the concept of autoimmunity in 1900. Considering the great diversity and ever-changing spectrum of autoimmunity, it has not been possible to include models and experimental protocols for each known disorder. Rather, several chapters have been devoted to the most prevalent and complex diseases, such as rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus, and multiple sclerosis. The chapters are contributed by laboratories actively using the models presented. Each chapter contains an introductory section that discusses the relevance of the model for a particular disease and for autoimmunity in general.

Part I contains methods and protocols to assess immunological and biochemical pathways relevant for disease pathogenesis. Chapters in this section focus on methods to identify susceptibility genes, intercellular signaling via cytokines, intracellular signaling through the T-cell receptor and signal processing via protein kinases, identification and enumeration of autoantigen-specific T cells and autoantibodies, and the dysregulation of apoptosis and its role in modification of self-antigens. Part II contains protocols to establish and assess inflammatory arthritis, systemic lupus erythematosus, myocarditis, thyroiditis, experimental autoimmune encephalomyelitis, insulin-dependent diabetes mellitus, scleroderma, uveitis, and vitiligo. The methods center on the assessment of genetic, immunological, and biochemical parameters underlying spontaneous or exogenous antigen-induced diseases. Although the individual protocols focus on a specific disease, they can be adapted to investigate additional signaling pathways or pathogenic autoantigens.

Autoimmunity: Methods and Protocols should supplement those laboratory manuals that contain recipes for standard cell and molecular biology and immunology techniques, such as cell culture, gene cloning, sequencing, and amplification by polymerase chain reaction, vector design for the generation of transgenic and knockout animals, flow cytometry, fluorescence microscopy, electrophoresis, and gene and protein microarray methods.

Although these general methods are not described in detail here, they are appropriately referenced in each section.

I am grateful to Professor John Walker for his invitation and help with organizing and formatting this book and to Dr. Paul Phillips for his continued encouragement and support. With both my colleagues in the field and newcomers in mind, step-by-step protocols and detailed troubleshooting guides supplement all chapters. I am grateful to the distinguished authors for the time, expertise, and devotion that made this book possible.

If the reader feels that a particularly relevant disease or model is missing, I should be held responsible. Refining and extracting new meaning from old models and developing new ones is a constantly ongoing process. Therefore, our readers are invited to approach the authors with questions and comments or offer new models and protocols for a future edition.

Andras Perl, MD, PhD

Contents

Preface	vii
Contributors	xi
1 Pathogenesis and Spectrum of Autoimmunity <i>Andras Perl</i>	1
PART I. MODELS OF PATHOGENESIS IN HUMAN AUTOIMMUNE DISORDERS	
2 Mapping the Systematic Lupus Erythematosus Susceptibility Genes <i>Swapan K. Nath, Jennifer A. Kelly, John B. Harley, and R. Hal Scofield</i>	11
3 T-Cell Signaling Abnormalities in Human Systemic Lupus Erythematosus <i>Madhusoodana P. Nambiar, Sandeep Krishnan, and George C. Tsokos</i>	31
4 TCR ζ -Chain Abnormalities in Human Systemic Lupus Erythematosus <i>Madhusoodana P. Nambiar, Sandeep Krishnan, Vishal G. Warke, and George C. Tsokos</i>	49
5 Protein Kinase A and Signal Transduction in T Lymphocytes: <i>Biochemical and Molecular Methods</i> <i>Islam U. Khan and Gary M. Kammer</i>	73
6 Apoptosis and Mitochondrial Dysfunction in Lymphocytes of Patients With Systemic Lupus Erythematosus <i>Andras Perl, Gyorgy Nagy, Peter Gergely, Ferenc Puskas, Yueming Qian, and Katalin Banki</i>	87
7 Methods for Inducing Apoptosis <i>Kathryn M. Roberts, Antony Rosen, and Livia A. Casciola-Rosen</i>	115
8 Measurement of Cytokines in Autoimmune Disease <i>Kyriakos A. Kirou, Christina Lee, and Mary K. Crow</i>	129
9 Evaluation of Autoimmunity to Transaldolase in Multiple Sclerosis <i>Brian Niland and Andras Perl</i>	155

PART II. ANIMAL MODELS OF AUTOIMMUNITY

10	Animal Models for Autoimmune Myocarditis and Autoimmune Thyroiditis <i>Daniela Čiháková, Rajni B. Sharma, DeLisa Fairweather, Marina Afanasyeva, and Noel R. Rose</i>	175
11	Animal Models of Insulin-Dependent Diabetes <i>Edwin Liu, Liping Yu, Hiroaki Moriyama, and George S. Eisenbarth</i>	195
12	Generation, Maintenance, and Adoptive Transfer of Diabetogenic T-Cell Lines/Clones From the Nonobese Diabetic Mouse <i>Martha J. Milton, Michelle Poulin, Clayton Mathews, and Jon D. Piganelli</i>	213
13	Experimental Use of Murine Lupus Models <i>Stanford L. Peng</i>	227
14	The Anti-DNA Knock-In Model of Systemic Autoimmunity Induced by the Chronic Graft-vs-Host Reaction <i>Robert Eisenberg and Arpita Choudhury</i>	273
15	Murine Models of Lupus Induced by Hypomethylated T Cells <i>Bruce Richardson, Donna Ray, and Raymond Yung</i>	285
16	The Mouse Model of Collagen-Induced Arthritis <i>David D. Brand, Andrew H. Kang, and Edward F. Rosloniec</i>	295
17	Proteoglycan Aggrecan-Induced Arthritis: A Murine Autoimmune Model of Rheumatoid Arthritis <i>Tibor T. Glant and Katalin Mikecz</i>	313
18	Mouse Models of Multiple Sclerosis: <i>Experimental Autoimmune Encephalomyelitis and Theiler's Virus-Induced Demyelinating Disease</i> <i>Kevin G. Fuller, Julie K. Olson, Laurence M. Howard, J. Ludovic Croxford, and Stephen D. Miller</i>	339
19	Experimental Autoimmune Encephalomyelitis <i>Praveen Rao and Benjamin M. Segal</i>	363
20	Animal Models of Scleroderma <i>Gabriella Lakos, Shinsuke Takagawa, and John Varga</i>	377
21	Rodent Models of Experimental Autoimmune Uveitis <i>Rajeev K. Agarwal and Rachel R. Caspi</i>	395
22	Autoimmune Depigmentation Following Sensitization to Melanoma Antigens <i>Arthur A. Hurwitz and Qingyong Ji</i>	421
	Index	429

Contributors

MARINA AFANASYEVA • *Department of Medicine, University of Calgary, Calgary, Alberta, Canada*

RAJEEV K. AGARWAL • *Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD*

KATALIN BANKI • *Department of Pathology, State University of New York, Upstate Medical University College of Medicine, Syracuse, NY*

DAVID D. BRAND • *Department of Medicine, University of Tennessee Health Sciences Center; Veterans Affairs Medical Center, Memphis, TN*

LIVIA A. CASCIOLA-ROSEN • *Departments of Medicine and Dermatology, The Johns Hopkins University School of Medicine, Baltimore, MD*

RACHEL R. CASPI • *Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD*

ARPITA CHOUDHURY • *Division of Rheumatology, Department of Medicine, University of Pennsylvania, Philadelphia, PA*

DANIELA ČIHÁKOVÁ • *Division of Immunology, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD*

MARY K. CROW • *Mary Kirkland Center for Lupus Research, Hospital for Special Surgery, New York, NY*

J. LUDOVIC CROXFORD • *Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL*

GEORGE S. EISENBARTH • *Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO*

ROBERT EISENBERG • *Division of Rheumatology, Department of Medicine, University of Pennsylvania, Philadelphia, PA*

DELIISA FAIRWEATHER • *Division of Immunology, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD*

KEVIN G. FULLER • *Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL*

PETER GERGELY • *Division of Rheumatology, Department of Medicine, State University of New York, Upstate Medical University College of Medicine, Syracuse, NY*

TIBOR T. GLANT • *Section of Biochemistry and Molecular Biology, Department of Orthopedic Surgery and Department of Biochemistry, Rush University School of Medicine, Chicago, IL*

- JOHN B. HARLEY • *Arthritis and Immunology Program, Oklahoma Medical Research Foundation; Departments of Medicine and Pathology, University of Oklahoma Health Sciences Center, and the Department of Veterans Affairs Medical Center, Oklahoma City, OK*
- LAURENCE M. HOWARD • *Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL*
- ARTHUR A. HURWITZ • *Department of Microbiology and Immunology, State University of New York Upstate Medical University, Syracuse, NY; Laboratory of Molecular Immunoregulation, National Cancer Institute, Frederick, MD*
- QINGYONG JI • *Department of Microbiology and Immunology, State University of New York Upstate Medical University, Syracuse, NY*
- GARY M. KAMMER • *Section on Rheumatology and Clinical Immunology, Department of Internal Medicine; Department of Microbiology and Immunology, Wake Forest University Health Sciences, Winston-Salem, NC*
- ANDREW H. KANG • *Department of Medicine, University of Tennessee Health Sciences Center; Veterans Affairs Medical Center, Memphis, TN*
- JENNIFER A. KELLY • *Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK*
- ISLAM U. KHAN • *Section on Rheumatology and Clinical Immunology, Department of Internal Medicine, Wake Forest University Health Sciences, Winston-Salem, NC*
- KYRIAKOS A. KIROU • *Mary Kirkland Center for Lupus Research, Hospital for Special Surgery, New York, NY*
- SANDEEP KRISHNAN • *Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring; Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD*
- GABRIELLA LAKOS • *Section of Rheumatology, University of Illinois at Chicago College of Medicine, Chicago, IL*
- CHRISTINA LEE • *Mary Kirkland Center for Lupus Research, Hospital for Special Surgery, New York, NY*
- EDWIN LIU • *Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO*
- CLAYTON MATHEWS • *Diabetes Institute Division of Immunogenetics, Rangos Research Center, Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA*
- KATALIN MIKECZ • *Section of Biochemistry and Molecular Biology, Department of Orthopedic Surgery, Department of Biochemistry, Rush University Medical Center, Chicago, IL*

- STEPHEN D. MILLER • *Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL*
- MARTHA J. MILTON • *Diabetes Institute Division of Immunogenetics, Rangos Research Center, Department of Pediatrics, Children’s Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA*
- HIROAKI MORIYAMA • *Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO*
- GYORGY NAGY • *Division of Rheumatology, Department of Medicine, College of Medicine, Upstate Medical University, State University of New York, Syracuse, NY*
- MADHUSOODANA P. NAMBIAR • *Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring; Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD*
- SWAPAN K. NATH • *Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK*
- BRIAN NILAND • *Departments of Medicine, Microbiology, and Immunology, College of Medicine, Upstate Medical University, State University of New York, Syracuse, NY*
- JULIE K. OLSON • *Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Northwestern University Feinberg School of Medicine, Chicago, IL*
- STANFORD L. PENG • *Department of Internal Medicine/Rheumatology, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO*
- ANDRAS PERL • *Division of Rheumatology, Departments of Medicine and of Microbiology and Immunology, College of Medicine, Upstate Medical University, State University of New York, Syracuse, NY*
- JON D. PIGANELLI • *Diabetes Institute Division of Immunogenetics, Rangos Research Center, Department of Pediatrics, Children’s Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA*
- MICHELLE POULIN • *Diabetes Institute Division of Immunogenetics, Rangos Research Center, Department of Pediatrics, Children’s Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA*
- FERENC PUSKAS • *Division of Rheumatology, Department of Medicine, College of Medicine, Upstate Medical University, State University of New York, Syracuse, NY*
- YUEMING QIAN • *Division of Rheumatology, Department of Medicine, College of Medicine, Upstate Medical University, State University of New York, Syracuse, NY*

- PRAVEEN RAO • *Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY*
- DONNA RAY • *Department of Internal Medicine, University of Michigan, Ann Arbor, MI*
- BRUCE RICHARDSON • *Department of Internal Medicine, University of Michigan and the Ann Arbor Veteran's Affairs Hospital, Ann Arbor, MI*
- KATHRYN M. ROBERTS • *Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD*
- NOEL R. ROSE • *Division of Immunology, Department of Pathology, and Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Medical Institutions, Baltimore, MD*
- ANTONY ROSEN • *Department of Medicine, Cell Biology, and Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD*
- EDWARD F. ROSLONIEC • *Veterans Affairs Medical Center and the Departments of Medicine and Pathology, University of Tennessee Health Sciences Center, Memphis, TN*
- R. HAL SCOFIELD • *Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Departments of Medicine and Pathology, University of Oklahoma Health Sciences Center; Department of Veterans Affairs Medical Center, Oklahoma City, OK*
- BENJAMIN M. SEGAL • *Department of Neurology, University of Rochester School of Medicine and Dentistry, Rochester, NY*
- RAJNI B. SHARMA • *Division of Immunology, Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD*
- SHINSUKE TAKAGAWA • *Section of Rheumatology, University of Illinois at Chicago College of Medicine, Chicago, IL*
- GEORGE C. TSOKOS • *Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring; Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD*
- JOHN VARGA • *Section of Rheumatology, University of Illinois at Chicago College of Medicine, Chicago, IL*
- VISHAL G. WARKE • *Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring; Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD*
- LIPING YU • *Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO*
- RAYMOND YUNG • *Department of Internal Medicine, University of Michigan and the Ann Arbor Veteran's Affairs Hospital, Ann Arbor, MI*