Mucosal Immunology

Volume 1

Fourth Edition

Edited by

Jiri Mestecky
Departments of Microbiology and Medicine, University of Alabama, Birmingham, AL, USA;
Institute of Microbiology, Czech Academy of Sciences and Department of Immunology and Microbiology,
School of Medicine, Charles University, Prague, Czech Republic

Warren Strober
Chief, Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and
Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Michael W. Russell
Departments of Microbiology/Immunology and Oral Biology, University at Buffalo, Buffalo, NY, USA

Brian L. Kelsall
Mucosal Immunobiology Section, Laboratory of Molecular Immunology, National Institute of Allergy
and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Hilde Cheroutre
Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Bart N. Lambrecht
VIB Inflammation Research Center, Ghent University, and Department of Respiratory Medicine,
University Hospital Ghent, Ghent, Belgium

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD • PARIS
SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Mucosal Immunology

Volume 2

Fourth Edition

Edited by

Jiri Mestecky
Departments of Microbiology and Medicine, University of Alabama, Birmingham, AL, USA;
Institute of Microbiology, Czech Academy of Sciences and Department of Immunology and Microbiology,
School of Medicine, Charles University, Prague, Czech Republic

Warren Strober
Chief, Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and
Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Michael W. Russell
Departments of Microbiology/Immunology and Oral Biology, University at Buffalo, Buffalo, NY, USA

Brian L. Kelsall
Mucosal Immunobiology Section, Laboratory of Molecular Immunology, National Institute of Allergy
and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Hilde Cheroutre
Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Bart N. Lambrecht
VIB Inflammation Research Center, Ghent University, and Department of Respiratory Medicine,
University Hospital Ghent, Ghent, Belgium

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD • PARIS
SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB
225 Wyman Street, Waltham, MA 02451

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Catalog Number
A catalog record for this book is available from the Library of Congress

Set ISBN: 978-0-12-415847-4
Volume 1 ISBN: 978-0-12397203-3
Volume 2 ISBN: 978-0-12397202-6

Typeset by TNQ Books and Journals
www.tnq.co.in

Printed and bound in the United States of America

In Memoriam

Malcolm Artenstein
Thomas Brown
John Cebra
Merrill W. Chase
Arlette Darfeuille-Michaud
Anne Ferguson
Robert Good
Joseph Heremans
Graham Jackson
Martin Kagnoff
Otakar Koldovsky
Hilary Koprowski
Frederick Kraus
Henry Kunkel
Leo LeFrançois
Lloyd Mayer
Goro Mogi
Eva Orlans
Richard Rothberg
Roberta Shahin
Jaroslav Sterzl
Masaharu Tsuchiya
Robert Waldman
Martin Zeitz

For their lasting contributions
to the field of Mucosal Immunology

Arlette Darfeuille-Michaud, who completed Chapter 48 for this edition before her passing, discovered the adherent-invasive biotype of *Escherichia coli* and its role in inflammatory bowel disease. Leo LeFrançois, who co-authored Chapter 35, was a leader in the field of mucosal T cells including intestinal intraepithelial γδ- and αβ- T cells and he was one of the first to introduce the concept of tissue-resident memory T cells. Lloyd Mayer, an editor of the third edition, was a noted clinical immunologist who first demonstrated that intestinal epithelial cells had a critical role in gut immune responses and could present antigen to T cells. Hilary Koprowski pioneered the first oral vaccine against polio and later became director of the Wistar Institute where he contributed to an improved rabies vaccine. John Cebra's seminal finding that intestinal IgA antibody-secreting cells had their origins in Peyer's patches was instrumental in developing the concept of the common mucosal immune system. Jaroslav Sterzl more than 50 years ago initiated studies on the fundamental role of intestinal microbiota in the development and function of the immune system in gnotobiotic animals. Thomas Brown contributed importantly to studies on IgA function and the induction of IgA subclass antibody responses. Martin Zeitz's major contribution was to define the gastrointestinal abnormalities caused by HIV infection; he was the first to show that HIV infection caused an enteropathy resulting in malabsorption and entry of bacterial products into the internal milieu. He contributed Chapter 77 to this edition before his passing.

Contributors

Valérie Abadie (Ch 80) Sainte-Justine Hospital Research Centre, University of Montreal, Montreal, QC, Canada

Clara Abraham (Ch 30) Yale University, CT, USA

David H. Adams (Ch 90) University of Turku, Turku, Finland; NIHR Biomedical Research Unit in Liver Disease, Centre for Liver Research, University of Birmingham, Edgbaston, Birmingham, UK

William W. Agace (Ch 40) Lund University, Lund, Sweden; Danish Technical University, Copenhagen, Denmark

Jennifer Alexander-Brett (Ch 53) Washington University School of Medicine, Saint Louis, MO, USA

Omar Alkhairy (Ch 73) Karolinska University Hospital, Huddinge, Stockholm, Sweden; King Abdulaziz Medical City, Riyadh, Saudi Arabia

Ines Ambite (Ch 106) Institute of Laboratory Medicine, Lund University, Lund, Scania, Sweden

Deborah J. Anderson (Ch 109) Boston University School of Medicine, Boston, MA, USA

David Artis (Ch 54) Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Robert L. Atmar (Ch 57) Baylor College of Medicine, Houston, TX, USA

Laetitia Aymeric (Ch 50) Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, Paris, France; Collège de France, Paris, France

Claus Bachert (Ch 100) Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan, Ghent, Belgium

Jantine E. Bakema (Ch 20) VU University Medical Center, Amsterdam, The Netherlands

Kristi Baker (Ch 19) Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA

Kenneth W. Beagley (Ch 107) Queensland University of Technology, Kelvin Grove, QLD, Australia

A.D. Befus (Ch 43) University of Alberta, Edmonton, AB, Canada

Mats Bemark (Ch 33) Mucosal Immunobiology and Vaccine Center, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden; Sahlgrenska University Hospital, Gothenburg, Sweden

M. Cecilia Berin (Ch 84) Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Margot Berings (Ch 100) Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan, Ghent, Belgium

Jay A. Berzofsky (Ch 75) National Institutes of Health, Bethesda, MD, USA

Martin Bilej (Ch 9) Institute of Microbiology of the Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

Nabanita Biswas (Chs 108, 110) Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Richard S. Blumberg (Chs 19, 27) Division of Gastroenterology, Hepatology and Endoscopy, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA

John Bienenstock (Introduction) McMaster University, Hamilton, ON, Canada

Dimitrios Bogdanos (Ch 87) Institute of Liver Studies, Transplantation Immunology and Mucosal Biology, King's College London School of Medicine, King's College Hospital, London, UK

Monica Boirivant (Ch 79) Immune-mediated Diseases Section, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Roma, Italy

Kobporn Boonnak (Ch 59) National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD, USA

Ken R. Bracke (Ch 97) Laboratory for Translational Research in Obstructive Pulmonary Diseases, Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium

Per Brandtzaeg (Chs 31, 103) Laboratory for Immunohistochemistry and Immunopathology (LIIPAT), Center for Immune Regulation (CIR), University of Oslo, and Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Jonathan Braun (Ch 5) Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Marie-Agnès Bringer (Ch 48) UMR1071 Inserm/ Université d'Auvergne, INRA, France

Andrew J. Broadbent (Ch 59) National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD, USA

Richard Bronson (Ch 111) Stony Brook University School of Medicine, Stony Brook, NY, USA

Guy G. Brusselle (Ch 97) Laboratory for Translational Research in Obstructive Pulmonary Diseases, Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium

Judith N. Bulmer (Ch 114) Newcastle University, Newcastle upon Tyne, UK

J.E. Butler (Ch 116) Carver College of Medicine, The University of Iowa, Iowa City, IA, USA

Paul A. Cardenas (Ch 6) National Heart and Lung Institute, Imperial College London, London, UK; Universidad de las Americas, Center for Translational Research, Quito, Ecuador

John J. Cebra (Introduction) University of Pennsylvania, Philadelphia, PA, USA

Marina Cella (Ch 52) Washington University School of Medicine, St Louis, MO, USA

Andrea Cerutti (Ch 32) Icahn School of Medicine at Mount Sinai, New York, NY, USA

Stephen J. Challacombe (Ch 102) Department of Oral Medicine, King's College London and Guys & St Thomas Hospitals, London, UK, and University of Sheffield, Sheffield, UK

Kuldeep Chattha (Ch 68) Canadian Food Inspection Agency, Lethbridge, AB, Canada

Hilde Cheroutre (Chs 1, 35, 72) Head, Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Tsutomu Chiba (Ch 88) Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Alejo Chorny (Ch 32) Icahn School of Medicine at Mount Sinai, New York, NY, USA

John D. Clements (Ch 61) Tulane University, School of Medicine, New Orleans, LA, USA

Marco Colonna (Ch 52) Washington University School of Medicine, St Louis, MO, USA

William O.C. Cookson (Ch 6) National Heart and Lung Institute, Imperial College London, London, UK

Lynette B. Corbeil (Ch 68) UCSD Medical Center, University of California – San Diego, San Diego, CA, USA

Blaise Corthésy (Ch 21) R&D Laboratory, Division of Immunology and Allergy, University State Hospital (CHUV), Lausanne, Switzerland

Allan W. Cripps (Chs 11, 101) Griffith University, Gold Coast, QLD, Australia

Koen van Crombruggen (Ch 100) Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan, Ghent, Belgium

Andre Pires da Cunha (Ch 41) Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Susanna Cunningham-Rundles (Ch 74) Weill Cornell Medical College, New York, NY, USA

Roy Curtiss, 3rd (Ch 64) Arizona State University, Tempe, AZ, USA

Arlette Darfeuille-Michaud (Ch 48) UMR1071 Inserm/ Université d'Auvergne, INRA, France

Wouter J. de Jonge (Ch 46) Tytgat Institute for Gastrointestinal and Liver Research, Academic Medical Centre, Meibergdreef, Amsterdam, The Netherlands

Livija Deban (Ch 37) Cancer Research UK, London, UK; King's College London, London, UK

Timothy L. Denning (Ch 26) Georgia State University, Atlanta, GA, USA

James P. Di Santo (Ch 39) Innate Immunity Unit, Inserm U668, Institut Pasteur, Paris, France

Andreas Diefenbach (Ch 3) University of Mainz Medical Centre, Mainz, Germany

Victor J. DiRita (Ch 49) University of Michigan Medical School, Ann Arbor, MI, USA

Jordan Downey (Ch 93) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Ming-Qing Du (Ch 89) University of Cambridge, Cambridge, UK

Karen L. Edelblum (Ch 12) The University of Chicago, Chicago, IL, USA

Marjolein van Egmond (Ch 20) VU University Medical Center, Amsterdam, The Netherlands

H.-J. Epple (Ch 77) Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

Sidonia Fagarasan (Ch 23) Research Center for Integrative Medical Sciences, Riken Yokohama, Yokohama, Japan

John V. Fahey (Chs 108, 110) Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Michael J. Ferris (Ch 7) Louisiana State University Health Sciences Center, New Orleans, LA, USA

Stefan Fichtner-Feigl (Ch 91) University Medical Center Regensburg, Regensburg, Germany

Paul L. Fidel Jr. (Ch 112) Louisiana State University Health Science Center, New Orleans, LA, USA

Melanie Flach (Ch 3) University of Mainz Medical Centre, Mainz, Germany; Institute of Microbiology and Hygiene, University of Freiburg Medical Centre, Freiburg, Germany

Richard Flavell (Ch 38) School of Medicine, Yale University, New Haven, CT, USA; Howard Hughes Medical Institute, Chevy Chase, MD, USA

Howard B. Fleit (Ch 111) Stony Brook University School of Medicine, Stony Brook, NY, USA

Genoveffa Franchini (Ch 75) National Institutes of Health, Bethesda, MD, USA

Lucy C. Freytag (Ch 61) Tulane University, School of Medicine, New Orleans, LA, USA

Anja Fuchs (Ch 52) Washington University School of Medicine, St Louis, MO, USA

kohtaro Fujihashi (App2) University of Alabama at Birmingham, Birmingham, AL, USA

Ivan J. Fuss (Chs 81, 86) Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Nicola Gagliani (Ch 38) School of Medicine, Yale University, New Haven, CT, USA

Marta Rodriguez Garcia (Chs 108, 110) Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Wendy S. Garrett (Ch 42) Harvard School of Public Health, Boston, MA, USA; Harvard Medical School, Boston, MA, USA; Dana-Farber Cancer Institute, Boston, MA, USA; Broad Institute of Harvard and MIT, Cambridge, MA, USA

M. Eric Gershwin (Ch 87) University of California, Davis School of Medicine, Davis, CA, USA

Philippe Gevaert (Ch 100) Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan, Ghent, Belgium

Maree Gleeson (Ch 11) University of Newcastle, NSW, Australia

Gabriela Godaly (Ch 106) Institute of Laboratory Medicine, Lund University, Lund, Scania, Sweden

Randall M. Goldblum (Ch 115) Pediatric Child Health Research Center, University of Texas Medical Branch, Galveston, TX, USA

Naina Gour (Ch 93) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Mayda Gursel (Ch 62) Middle East Technical University, Department of Biological Sciences, Ankara, Turkey

George Hajishengallis (Ch 15) University of Pennsylvania School of Dental Medicine, Philadelphia, PA, USA

Hamida Hammad (Ch 29) Ghent University, Ghent, Belgium; VIB, Ghent, Belgium

Lennart Hammarström (Chs 71, 73) Department of Laboratory Medicine, Division of Clinical Immunology and Transfusion Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

Arno Hänninen (Ch 90) University of Turku, Turku, Finland; NIHR Biomedical Research Unit in Liver Disease, Centre for Liver Research, University of Birmingham, Edgbaston, Birmingham, UK

Lars Å. Hanson (Ch 117) Göteborg University, Göteborg, Sweden

Adrian Hayday (Ch 37) Cancer Research UK, London, UK; King's College London, London, UK

Ronit Herzog (Ch 74) Weill Cornell Medical College, New York, NY, USA

Douglas C. Hodgins (Ch 68) Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Stephen T. Holgate (Ch 96) School of Medicine, Southampton General Hospital, Southampton, UK

Jan Holmgren (Chs 51, 56) Department of Microbiology and Immunology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Michael J. Holtzman (Ch 53) Washington University School of Medicine, Saint Louis, MO, USA

Edward W. Hook III (Ch 112) University of Alabama at Birmingham, Birmingham, AL, USA

Samuel Huber (Ch 38) University Hospital Hamburg-Eppendorf, Hamburg, Germany

Julia L. Hurwitz (Ch 49) St Jude Children's Research Hospital, Memphis, TN, USA

Juraj Ivanyi (Ch 95) Guy's Campus of Kings College London, London, UK

Akiko Iwasaki (Ch 25) Howard Hughes Medical Institute, Yale University, New Haven, CT, USA

Bana Jabri (Ch 80) University of Chicago, Chicago, IL, USA

Susan Jackson (App1) University of Alabama at Birmingham, Birmingham, AL, USA

Jonathan Jacobs (Ch 5) Division of Digestive Diseases, Department of Medicine, Los Angeles, CA, USA

Sirpa Jalkanen (Ch 90) University of Turku, Turku, Finland; NIHR Biomedical Research Unit in Liver Disease, Centre for Liver Research, University of Birmingham, Edgbaston, Birmingham, UK

Edward N. Janoff (Ch 58) Mucosal and Vaccine Research Program Colorado (MAVRC); University of Colorado Denver, Aurora, CO, USA; Veterans Affairs Medical Center, Denver, CO, USA

Ann E. Jerse (Ch 107) Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Mangalakumari Jeyanathan (Ch 66) McMaster University, Hamilton, ON, Canada

Bruce A. Julian (Ch 105) School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Imre Kacskovics (Ch 116) Eötvös Loránd University, Budapest, Hungary

Charlotte S. Kaetzel (Chs 18, 19) Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, Lexington, KY, USA

Charu Kaushic (Ch 107) McMaster University, Hamilton, ON, Canada

Brian L. Kelsall (Chs 1, 24, 25, 78) Mucosal Immunobiology Section, Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Sarah Kessans (Ch 65) Arizona State University, Tempe, AZ, USA

Rebecca Kesselring (Ch 91) University Medical Center Regensburg, Regensburg, Germany

Mogens Kilian (Chs 21, 22) Department of Biomedicine (Medical Microbiology and Immunology), Aarhus University, Aarhus, Denmark

Hiroshi Kiyono (Ch 65) University of Tokyo, Minato-ku, Tokyo, Japan

Dennis M. Klinman (Ch 62) Cancer and Infammation Program, National Cancer Institute, Frederick, MD, USA

Marina Korotkova (Ch 117) Göteborg University, Göteborg, Sweden

Mitchell Kronenberg (Ch 36) Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Olga Krysko (Ch 100) Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan, Ghent, Belgium

Yuichi Kurono (Ch 101) Kagoshima University, Kagoshima, Japan

Miloslav Kverka (Ch 8) Laboratory of Cellular and Molecular Immunology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Bart N. Lambrecht (Chs 1, 25, 92, 94, 98) VIB Inflammation Research Center, Ghent University, and Department of Respiratory Medicine, University Hospital Ghent, Ghent, Belgium

Michael E. Lamm (Introduction) Case Western Reserve University, Cleveland, OH, USA

Olivier Lantz (Ch 36) Institut Curie, Paris, France

Gendie E. Lash (Ch 114) Newcastle University, Newcastle upon Tyne, UK

E.C. Lavelle (Ch 63) School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

Leo Lefrancois (Ch 35) Division of Immunology, University of Connecticut Health Center, Farmington, CT, USA

Patrick S.C. Leung (Ch 87) University of California, Davis School of Medicine, Davis, CA, USA

Myron M. Levine (Ch 56) University of Maryland School of Medicine, Baltimore, MD, USA

David J. Lim (Ch 101) University of California, Los Angeles, CA, USA

John Lippolis (Ch 116) Ruminant Disease of Cattle Research Unit, Ames, IA, USA

Nancy A. Louis (Ch 45) Emory University School of Medicine, Atlanta, GA, USA

Andrew D. Luster (Ch 40) Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Nataliya Lutay (Ch 106) Institute of Laboratory Medicine, Lund University, Lund, Scania, Sweden

Nils Lycke (Ch 33) Mucosal Immunobiology and Vaccine Center, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

Andrew J. Macpherson (Ch 23) University Hospital of Bern, Bern, Switzerland

Nicholas J. Mantis (Ch 21) Division of Infectious Disease, Wadsworth Center, New York State Department of Health, Albany, NY, USA

Harold Marcotte (Ch 71) Department of Laboratory Medicine, Division of Clinical Immunology and Transfusion Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden

David H. Martin (Ch 7) Louisiana State University Health Sciences Center, New Orleans, LA, USA

Hugh S. Mason (Ch 65) Arizona State University Tempe, AZ, USA

Helen M. Massa (Ch 101) Griffith University, Gold Coast, QLD, Australia

Nobuyuki Matoba (Ch 65) University of Louisville School of Medicine, Louisville, KY, USA

Lloyd Mayer (Ch 27) Immunology Center, Mount Sinai Medical Center, New York, NY, USA

Craig L. Maynard (Ch 34) University of Alabama at Birmingham, Birmingham, AL, USA

M. Juliana McElrath (Ch 60) Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

C. McEntee (Ch 63) School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

Jerry R. McGhee (Introduction) University of Alabama at Birmingham, Birmingham, AL, USA

Michael A. McGuckin (Ch 14) Mater Research Institute–The University of Queensland, Translational Research Institute, Woolloongabba, QLD, Australia

Jiri Mestecky (Chs 1, 17, 55, 104, 105, 108, 112) Departments of Microbiology and Medicine, University of Alabama, Birmingham, AL, USA; Institute of Microbiology, Czech Academy of Sciences and Department of Immunology and Microbiology, School of Medicine, Charles University, Prague, Czech Republic

Zamaneh Mikhak (Ch 40) Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Robert D. Miller (Ch 10) University of New Mexico, Albuquerque, NM, USA

Zina Moldoveanu (App1, App2) University of Alabama at Birmingham, Birmingham, AL, USA

Paul C. Montgomery (Ch 99) Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI, USA

Tsafrir Mor (Ch 65) Arizona State University Tempe, AZ, USA

Markus F. Neurath (Chs 82, 91) University of Erlangen-Nuremberg, Erlangen, Germany

Katrijn Neyt (Ch 94) VIB Inflammation Research Center, Ghent, Belgium

Lindsay K. Nicholson (Ch 58) Mucosal and Vaccine Research Program Colorado (MAVRC); University of Colorado Denver, Aurora, CO, USA; Veterans Affairs Medical Center, Denver, CO, USA

Jan Novak (Ch 105) School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Stella Nowicki (Ch 115) Department of Microbiology and Immunology, Meharry Medical College, Nashville, TN, USA

D.T. O'Hagan (Ch 63) Novartis Vaccines and Diagnostics, Cambridge, MA, USA

Nancy L. O'Sullivan (Ch 99) Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, MI, USA

Pearay Ogra (Ch 117) University of Buffalo School of Medicine, Buffalo, NY, USA

Carlos Orihuela (Ch 49) University of Texas Health Science Center San Antonio, San Antonio, TX, USA

André J. Ouellette (Ch 16) Department of Pathology & Laboratory Medicine, Keck School of Medicine of the University of Southern California, Los Angeles, CA, USA

Robert L. Owen (Ch 13) University of California San Francisco, San Francisco, CA, USA; Veterans Affairs Medical Center, San Francisco, CA, USA

Oliver Pabst (Ch 41) Institute of Immunology, Hannover Medical School, Hannover, Germany

Charles A. Parkos (Ch 45) Emory University School of Medicine, Atlanta, GA, USA

Viviana Parreño (Ch 68) Instituto de Virología, Centro de Investigación en Ciencias Veterinarias y Agronómicas, Instituto Nacional de Tecnología Agropecuaria, Castelar, Buenos Aires, Argentina

Mickey V. Patel (Chs 108, 110) Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Claudina Perez-Novo (Ch 100) Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan, Ghent, Belgium

Darren J. Perkins (Ch 30) University of Maryland, Baltimore (UMB), MD, USA

Calman Prussin (Ch 83) Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Jeffrey Pudney (Ch 109) Boston University School of Medicine, Boston, MA, USA

Sukanya Raghavan (Ch 51) Department of Microbiology and Immunology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Pascal Rainard (Ch 116) INRA Centre Val de Loire et Universite' Francois Rabelais de Tours, UMR, Nouzilly, France

Sasirekha Ramani (Ch 57) Baylor College of Medicine, Houston, TX, USA

Troy D. Randall (Ch 4) University of Alabama at Birmingham, Birmingham, AL, USA

Milan Raska (Chs 67, 105) School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; Palacky University Olomouc, Olomouc, Czech Republic

Gourapura J. Renukaradhya (Ch 68) Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Department Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA

Maria Rescigno (Ch 28) European Institute of Oncology, Milano, Italy

Kenneth L. Rosenthal (Ch 66) McMaster University, Hamilton, ON, Canada

Marc E. Rothenberg (Ch 44) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Frank M. Ruemmele (Ch 85) Université Sorbonne Paris Cité, Université Paris Descartes, Hôpital Necker Enfants Malades, Service de Gastroentérologie Pédiatrique, Paris, France

Michael W. Russell (Chs 1, 15, 18, 21, 22, 55, 98, 104, 112) Departments of Microbiology/Immunology and Oral Biology, University at Buffalo, Buffalo, NY, USA

Linda J. Saif (Ch 68) Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Department Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA

Irene Salinas (Ch 10) University of New Mexico, Albuquerque, NM, USA

Marko Salmi (Ch 90) University of Turku, Turku, Finland; NIHR Biomedical Research Unit in Liver Disease, Centre for Liver Research, University of Birmingham, Edgbaston, Birmingham, UK

Henri Salmon (Ch 116) INRA Centre Val de Loire et Universite' Francois Rabelais de Tours, UMR, Nouzilly, France

Hugh A. Sampson (Ch 84) Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Philippe Sansonetti (Ch 50) Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, Paris, France; Collège de France, Paris, France

T. Schneider (Ch 77) Charité – Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany

Nicolas Serafini (Ch 39) Innate Immunity Unit, Inserm U668, Institut Pasteur, Paris, France

Dolly Sharma (Ch 117) University of Buffalo School of Medicine, Buffalo, NY, USA

Zheng Shen (Ch 108) Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Hai Ning Shi (Ch 2) Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Penelope J. Shirlaw (Ch 102) Department of Oral Medicine, King's College London and Guys & St Thomas Hospitals, London, UK, and University of Sheffield, Sheffield, UK

Sourima B. Shivhare (Ch 114) Newcastle University, Newcastle upon Tyne, UK

Phillip D. Smith (Ch 26) University of Alabama at Birmingham, Birmingham, AL, USA; VA Medical Center, Birmingham, AL, USA

Patrick M. Smith (Ch 42) Harvard School of Public Health, Boston, MA, USA

Daniel J. Smith (Ch 69) The Forsyth Institute, Cambridge, MA, USA; Harvard School of Dental Medicine, Boston, MA, USA

Lesley E. Smythies (Ch 26) University of Alabama at Birmingham, Birmingham, AL, USA

Jo Spencer (Chs 33, 89) King's College London School of Medicine, Guy's King's College, Thomas Hospitals, London, UK

Warren Strober (Chs 1, 24, 47, 70, 72, 78, 81, 86, 88) Chief, Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Kanta Subbarao (Ch 59) National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD, USA

Catharina Svanborg (Ch 106) Institute of Laboratory Medicine, Lund University, Lund, Scania, Sweden

Ann-Mari Svennerholm (Ch 51) Department of Microbiology and Immunology, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Martin A. Taubman (Ch 69) The Forsyth Institute, Cambridge, MA, USA; Harvard School of Dental Medicine, Boston, MA, USA

Esbjörn Telemo (Ch 117) Göteborg University, Göteborg, Sweden

Martin H. Thornhill (Ch 102) Department of Oral Medicine, King's College London and Guys & St Thomas Hospitals, London, UK, and University of Sheffield, Sheffield, UK

David J. Thornton (Ch 14) Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester, UK

Eva Thuenemann (Ch 65) Institute of Food Research, Norwich Research Park, Norwich, UK

Helena Tlaskalova-Hogenova (Ch 8) Laboratory of Cellular and Molecular Immunology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Debra Tristram (Ch 113) Department of Pediatrics, Albany Medical Center, Albany, NY, USA

Palak Trivedi (Ch 90) University of Turku, Turku, Finland; NIHR Biomedical Research Unit in Liver Disease, Centre for Liver Research, University of Birmingham, Edgbaston, Birmingham, UK

Elaine Tuomanen (Ch 49) St Jude Children's Research Hospital, Memphis, TN, USA

Jaroslav Turanek (Ch 67) Veterinary Research Institute, Brno, Czech Republic

Jerrold R. Turner (Ch 12) The University of Chicago, Chicago, IL, USA

Brian J. Underdown (Ch 70) McMaster University, Hamilton, ON, Canada

Mary J. van Helden (Ch 94) VIB Inflammation Research Center, Ghent, Belgium

Ronald S. Veazey (Ch 76) Tulane National Primate Research Center, Tulane University School of Medicine, Covington, LA, USA

Elena F. Verdu (Ch 8) Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada

Anastasia Vlasova (Ch 68) Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Department Veterinary Preventive Medicine, The Ohio State University, Wooster, OH, USA

Harissios Vliagoftis (Ch 43) University of Alberta, Edmonton, AB, Canada

Stefanie N. Vogel (Ch 30) University of Maryland, Baltimore (UMB), MD, USA

W. Allan Walker (Ch 2) Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Xiaolei Wang (Ch 76) Tulane National Primate Research Center, Tulane University School of Medicine, Covington, LA, USA

Tomohiro Watanabe (Ch 88) Department of Gastroenterology and Hepatology, Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University Graduate School of Medicine, Kyoto, Japan; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Casey T. Weaver (Ch 34) University of Alabama at Birmingham, Birmingham, AL, USA

Howard L. Weiner (Ch 41) Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Jerry M. Wells (Ch 8) Host-Microbe Interactomics Group, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

Ting Wen (Ch 44) Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Judith Whittum-Hudson (Ch 112) Wayne State University, Detroit, MI, USA

Jeffrey A. Whitsett (Ch 14) Perinatal Institute, Cincinnati Children's Hospital, OH, USA; University of Cincinnati, OH, USA

Ifor R. Williams (Ch 13) Emory University School of Medicine, Atlanta, GA, USA

Marsha Wills-Karp (Ch 93) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Charles R. Wira (Chs 108, 110) Geisel School of Medicine at Dartmouth, Lebanon, NH, USA

Jenny M. Woof (Chs 17, 20) Division of Cancer Research, University of Dundee Medical School, Dundee, UK

Andrew C. Wotherspoon (Ch 89) The Royal Marsden NHS Trust, London, UK

Zhou Xing (Ch 66) McMaster University, Hamilton, ON, Canada

Huanbin Xu (Ch 76) Tulane National Primate Research Center, Tulane University School of Medicine, Covington, LA, USA

Colby Zaph (Ch 54) University of British Columbia, Vancouver, BC, Canada

Sebastian Zeissig (Ch 27) Department of Internal Medicine I, Kiel University, Kiel, Germany

M. Zeitz (Ch 77) University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Preface to the First Edition

Only 25 years ago, a multidisciplinary group of some three dozen individuals met for the first time in Vero Beach, Florida, under the auspices of the National Institute of Child Health and Human Development (NICHHD) to discuss a recently identified immunoglobulin, secretory IgA. Since that historic workshop, seven international congresses have been held to discuss secretory immunoglobulins and mucosal immunology, and there have been a number of scientific meetings on immunological mechanisms in such mucosal sites as respiratory tract, gut, genital tract, mammary glands, and periodontal tissues. The last International Congress of Mucosal Immunology, held in 1992 in Prague, Czechoslovakia, was attended by nearly 1000 participants.

The recognition that defenses are mediated via mucosal barriers dates back several 1000 years. Ingestion of Rhus leaves to modify the severity of reactions to poison ivy is a centuries old practice among native North Americans. The modern concepts of local immunity, however, were developed by Besredka in the early 1900s, followed by the discovery of IgA in 1953 and its isolation and characterization in 1959. Studies in the early 1960s demonstrated the presence of IgA in a unique form in milk and, shortly thereafter, in other external secretions. These studies were followed by the discovery of the secretory component and the identification of the J chain. These remarkable observations were soon complemented by the characterization of the bronchus-associated lymphoid tissue (BALT) and the gut-associated lymphoid tissue (GALT), the observation of circulation of antigen-sensitized or reactive IgA B cells from BALT and GALT to other mucosal surfaces such as the genital tract and the mammary glands, and the definition of mucosal T cells. Since 2004, our concept of the mucosal immune system has been expanded to include M cells and mechanisms of mucosal antigen processing, regulatory T lymphocytes and other effector cell mechanisms, neuropeptides, and the network of interleukins and other cytokines. Finally, the biological significance of the mucosal immune system increasingly is being realized in the context of human infections acquired via mucosal portals of entry, including conventional infections as well as new syndromes such as acquired immune deficiency associated with infection by HIV.

Despite the tremendous progress made in the acquisition of new knowledge concerning the common mucosal immune system, mucosal infections, and oral immunization, no single text covering the entire spectrum of mucosal immunity was available. Therefore, this handbook was organized to develop a perspective of the basic biology of the components that constitute the framework of the common mucosal immune system, as well as of the infectious and immunologically mediated disease processes of the mucosae. Virtually all chapters have been authored by original investigators responsible for key observations on which current concepts are based.

Part I, Cellular Basis of Mucosal Immunity, provides an introductory overview and a historical perspective of the mucosal immune system (Chapter 1), followed by 10 comprehensive chapters (Section A) on development and physiology of mucosal defense (Chapters 2–11). These chapters address structure and function of mucosal epithelium, cellular basis of antigen transport, mucosal barrier, innate humoral factors, bacterial adherence, development and function of mucosal immunoglobulin, and epithelial and hepatobiliary transport. Section B (Chapters 12–19) focuses on cells, regulation, and specificity in inductive and effector sites. The inductive site chapters discuss characteristics of mucosa-associated lymphoid tissue (MALT), Peyer's patches, regulation of IgA B cell development, diversity and function of mucosal antigen-presenting cells, oral tolerance, peptidergic circuits, role of B-1 cells, and lymphocyte homing. The chapters on effector sites (Section C) present information about cytokines, mucosal Ig-producing cells, regulatory T cells, intraepithelial cells, mucosal IgE, inflammation and mast cells, cytokines in liver, cytotoxic T cells in mucosal effector sites, and immunity to viruses (Chapters 20–29). Section D addresses mucosal immunization and the concepts of mucosal vaccines. These chapters discuss passive immunization, vaccine development for mucosal surfaces, antigen delivery systems, mucosal adjuvants, and approaches for generating specific secretory IgA antibodies (Chapters 30–34).

Part II, Mucosal Diseases, addresses the secretory immune system with special reference to mucosal diseases. Section E consists of chapters on the stomach, intestine, and liver, and includes diseases of GALT and intestinal tract, a chain and related lymphoproliferative disorders, gastritis and peptic ulcer, malabsorption syndrome, food allergy, intestinal infections, and diseases of the liver and biliary

tract (Chapters 35–42). Section F covers selected areas of lung and lower airway and includes chapters on BALT and pulmonary diseases, mucosal immunity in asthma, respiratory infections, and inhalant allergy (Chapters 43–46). Section G presents information on the oral cavity, upper airway, and mucosal regions in the head and neck (Chapters 47–50), as well as ocular immunity, tonsils and adenoids, and middle ear. Sections H and I are devoted to mammary glands and genitourinary tract, respectively. These sections consist of chapters on milk, immunological effects of breast feeding (Chapters 51 and 52), IgA nephropathy, immunology of female and male reproductive tracts, endocrine regulation of genital immunity, mucosal immunopathophysiology of HIV infection, and genital infections relative to maternal and infant disease (Chapters 53–58).

The information reviewed in the different chapters in this handbook will be of considerable interest to diverse groups of clinicians, basic and clinical immunologists, biologists, veterinarians, and public health workers interested in understanding the application of basic biology to virtually all immunological or infection-mediated disease processes of external mucosal surfaces. This handbook will be of particular importance to students of medicine and pediatrics, including individuals studying gastroenterology and pulmonology, ophthalmology, gynecology, infectious disease, otolaryngology, periodontal disease, sexually transmitted disease, and especially mucosal immunology.

Pearay L. Ogra
Jiri Mestecky
Michael E. Lamm
Warren Strober
Jerry R. McGhee
John Bienenstock

Preface to the Second Edition

Since the publication of the First Edition of Mucosal Immunology (then called Handbook of Mucosal Immunology) in 1994, an enormous amount of new information has become available concerning the structure and function of the mucosal immune system. The Editors therefore decided to update and expand the original text to encompass this new information and to maintain the volume as the primary reference work of the field. The broadened content of the second edition, and therefore its increased size, reflects the rapid expansion of new information and interest, and hence the impact our discipline is having on immunology and biology in general. It is becoming obvious that the phylogenetic development of the entire immune system is inseparable from that of its mucosal compartment. Indeed, one can provide many convincing arguments that stimulation with environmental antigens, which are encountered in everyday life primarily at mucosal surfaces, results in a strategic distribution of cells involved in the initiation of humoral and cellular immune responses at such sites. Notable advances have been made in our knowledge of the regulation of mucosal immune responses. This involves a better understanding of how immune responses are generated and thus how the mucosal immune system maintains host defense at mucosal surfaces. In addition, it involves deeper insights recently acquired into the nature of negative or tolerogenic responses (mucosal tolerance) in the mucosal immune system and how the mucosal immune system avoids untoward responses to ubiquitous antigens and the possible induction of self-reactive responses.

The implications of these advances have been applied in several clinical areas. These include the development of several new mucosal vaccine preparations consisting of either live attenuated organisms or nonreplicating antigens that provide protection for several mucosal viral infections such as rotavirus and influenza virus infection, and for mucosal bacterial infections such as infection with *Salmonella typhi* and *Vibrio cholerae*. These vaccines are the harbinger of others to come based on recent increases in the understanding of mucosal adjuvants. On the other side of the coin, the induction of oral tolerance is now being tested as an approach to the treatment of autoimmune diseases such as juvenile diabetes mellitus, rheumatoid arthritis, and multiple sclerosis. Finally, the balance between mucosal responsiveness and unresponsiveness is being explored within the context of a series of newly developed models of chronic mucosal inflammation. These models are providing a wealth of new information not only concerning immune response in general, but also concerning the pathogenesis of various types of mucosal inflammation such as inflammatory bowel disease and gluten-sensitive enteropathy. These advances in our understanding of mucosal immune system function, of course, are based on numerous new studies of the way individual components of the system operate. This edition of Mucosal Immunology addresses these issues with new discussions and analyses such as mucosal B cell function and the development of IgA-producing plasma cells; the function of epithelial cells as antigen-presenting cells and secretors of cytokines and chemokines; and the function of mucosal T cells both in the lamina propria and in the epithelial cell compartments.

The Second Edition is a considerably larger volume with nearly 100 chapters. Several important changes have been made in this edition. The sections on the development and physiology of mucosal defense, inductive and effector tissues and cells of the mucosal immune system, functional characteristics of mucosal cells and tissues, mucosal immunity and infections, antigen delivery systems, mucosal adjuvants, and the male genital tract have either been enlarged or newly added. As a result of these changes, as well as the dedicated work of our many contributors, we hope Mucosal Immunology will continue to be the starting place for the knowledge and study of the mucosal immune system.

Publication of this rather large volume would not have been possible without the dedication and collaboration of the authors of the individual chapters. We recognize with gratitude the contributions of a most helpful staff at Academic Press, especially Dr Kerry Willis

and Mr Aaron Johnson. We also recognize the contributions of Ms Diane Zimmerman (University of Texas Medical Branch), Ms Ruby Zuppert (Case Western Reserve University), Ms Wendy Abbott and Ms Sheila D. Turner (University of Alabama at Birmingham), Ms Sarah Kaul (National Institutes of Health), Ms Linda Builder (McMaster University), and Ms Maria Bethune (University of Alabama at Birmingham).

It is our pleasure to submit this volume to interested readers. We sincerely hope that this second edition will provide new stimuli for research not only in mucosal immunology but also in the related fields of theoretical and practical immunology.

Pearay L. Ogra
Jiri Mestecky
Michael E. Lamm
Warren Strober
John Bienenstock
Jerry R. McGhee

Preface to the Third Edition

Mucosal immunology has grown since 2004 from a discipline of perhaps peripheral interest to the mainstream immunologist into a major subspecialty with implications for the physiology of the entire immune system. An enormous and highly variable load of foreign substances, which includes indigenous mucosal microbiota as well as environmental and food antigens encountered mainly at the vast surface areas of mucosal membranes, has resulted during evolution in a strategic distribution of specialized cells involved in the uptake, processing, and presentation of antigens, the production of antibodies, and cell-mediated immunity at the front line of host defense. Furthermore, the great majority of infectious diseases and potential agents of bioterrorism directly afflicts or is acquired through the mucosal surfaces of the respiratory, gastrointestinal, and genitourinary tracts. In addition to the induction of protective responses to infectious agents, the unique immunoregulatory mechanisms involved in the parallel induction of mucosal tolerance efficiently prevent the overstimulation of the systemic compartment of the immune system. Exploitation of the principles of mucosal immunology has not only had a profound impact on theoretical immunology, but has also captured the attention of investigators working in applied fields including autoimmunity, allergy, infectious diseases of gastrointestinal and respiratory tract, sexually transmitted diseases, human immunodeficiency virus infections, and the development of vaccines for human and veterinary medicine. The publication of three editions of Mucosal Immunology within 10 years reflects the impressive expansion of new information and the impact the discipline has had on basic immunologic principles and their practical implications. Thus, the third updated and expanded edition of Mucosal Immunology will provide essential information and an invaluable source of inspiration for investigators in this field as well as related research endeavors.

The foundations of modern mucosal immunology were laid in the early 1960s. The authors of the chapters in this volume have been important contributors and witnesses of the remarkable progress in mucosal immunology. We hope that their unique insights, together with the enthusiasm of younger colleagues of the next generation, have resulted in a volume that provides inspiration and broadens the application of the principles of mucosal immunology to other biomedical disciplines.

The assembly of a volume of this size and scope required dedicated effort of many individuals who provided invaluable contributions at various stages of production. The editors of the third edition of Mucosal Immunology would like to thank the founding editor, Dr Pearay L. Ogra, for his leadership in the first and second editions; therefore, we dedicate this book to him. We also thank the authors of the individual chapters for their excellent contributions and cooperation. We gratefully acknowledge the efforts of our administrative co-workers, namely, Maria D. Crenshaw, Lydia Lopez, Sandra Martinez, Sheila D. Turner, Kelly R. Stinson, and Susan Brill, for their contributions in the completion and assembly of this book. Finally, we thank Margaret MacDonald and Victoria Lebedeva of Elsevier for their exemplary dedication, invaluable help, and deeply appreciated patience.

Jiri Mestecky
John Bienenstock
Michael E. Lamm
Lloyd Mayer
Jerry R. McGhee
Warren Strober

Preface to the Fourth Edition

Remarkable advances in the discipline of mucosal immunology since 2004 and its impact on the physiology of the entire immune system are reflected in this fourth edition of Mucosal Immunology. Publication of this comprehensive treatise is further justified by the increasing acceptance of mucosal immunity as an essential aspect of the immune system, as evidenced by the existence of the journal *Mucosal Immunology* and sections devoted to it in other prestigious journals, as well as by the Society of Mucosal Immunology with an impressive worldwide membership and the organization of now 17 International Conferences of Mucosal Immunology.

With an essentially new set of chapters, the fourth edition of Mucosal Immunology responds to an expanded interest in exciting novel findings concerning the mucosal microbiota, and its interactions with highly diverse populations of epithelial cells that cover the large surface areas of various mucosal membranes, and with their underlying lymphoid cells, dendritic cells, and macrophages of characteristic mucosal phenotypes. In 117 chapters and three appendices, prominent mucosal immunologists from all over the world present the most current and detailed information on the mucosal immune system, its organization and development, its component cells and tissues, its response to infection, the development of mucosal vaccines, and immunity in the various mucosal tracts and exocrine glands. We are grateful to many experienced mucosal immunologists and particularly to our younger colleagues who have been inspired by the intricacies of mucosal immunology and its potential in regulating humoral and cellular immune responses, as well as their exploitation in the design of novel vaccines administered by mucosal routes using a variety of delivery systems.

The assembly of a greatly expanded fourth edition of Mucosal Immunology required the concerted efforts of many individuals at various stages of production. We thank all authors and co-authors of the chapters, members of our administrative staffs, especially Ms Patricia V. Grayson (UAB), and Ms Sara Kaul (NIH). We are grateful to the exemplary efforts and deeply appreciated patience of Ms Mary Preap of Elsevier in guiding us through this effort.

Jiri Mestecky
Warren Strober
Michael W. Russell
Brian L. Kelsall
Hilde Cheroutre
Bart N. Lambrecht

Historical Aspects of Mucosal Immunology

Jiri Mestecky
Departments of Microbiology and Medicine, University of Alabama, Birmingham, AL, USA; Institute of Microbiology, Czech Academy of Sciences and Department of Immunology and Microbiology, School of Medicine, Charles University, Prague, Czech Republic

Jerry R. McGhee
University of Alabama at Birmingham, Birmingham, AL, USA

John Bienenstock
McMaster University, Hamilton, ON, Canada

Michael E. Lamm
Case Western Reserve University, Cleveland, OH, USA

Warren Strober
Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

John J. Cebra
University of Pennsylvania, Philadelphia, PA, USA

Lloyd Mayer[†]
Immunology Center, Mount Sinai Medical Center, New York, NY, USA

Pearay L. Ogra
University at Buffalo, Buffalo, NY, USA

Michael W. Russell
Departments of Microbiology/Immunology and Oral Biology, University at Buffalo, Buffalo, NY, USA

Historia est testis temporum, lux veritatis, vita memoriae, magistra vitae, nuntia vetustatis. (History is the witness of time, the light of truth, the essence of remembrance, the teacher of life, the messenger from times past.)

Marcus Tullius Cicero (106–43 BC)

Who controls the past controls the future…

George Orwell (1903–1950)

For millennia, the empirical experience of past generations suggested that those who survived certain diseases became resistant to repeated attacks. For example, plague survivors could attend to the needs of the sick and deceased without becoming sick again (Thucydides, fifth century BC; translated complete works of Thucydides, 1951). The earliest recorded and surprisingly successful attempt to enhance resistance to a harmful substance—in this case, a plant poison—was described in great detail by the king of Pontus (a territory on the Black Sea Coast of Turkey), Mithridates VI–Eupator (about 132–63 BC) (Reinach, 1890). To protect himself against a highly probable attempt on his life by numerous adversaries to his rather despotic rule, Mithridates invented a universal antidote to the then commonly used plant-derived poisons. The formula found in his archives in his own handwriting consisted of two dried nuts, two figs, and 20 leaves of rue (an aromatic Eurasian plant, the "herb-of-grace" from which volatile oil used in ancient medicine can be expressed), which were crushed and mixed with salt. More importantly, the blood of ducks fed unspecified poisonous weeds was added before ritual ingestion of this mixture every morning. In fear of being captured by his enemies, the king always carried in the hilt of his scimitar a lethal dose of poison extracted from the plants given to the ducks. The protective effect of everyday ingestion of trace amounts of plant poisons apparently present in the ducks' blood was soon to be demonstrated under the most dramatic circumstances. Mithridates had successively added to his kingdom of Pontus other provinces (Cappadocia and lands extending as far as the Crimea); his territorial conquest brought him into conflict with Rome. After his last and fateful battle of the third Mithridatian War with the Romans and betrayal by his own

[†] Deceased.

son Farnaces II, who instigated an army revolt against his father, the desperate Mithridates attempted suicide by ingesting the poison hidden in his sword. Although the poison from the same vial was lethal for his daughters Mithridatis and Nysa, the king survived. Whether the dose was insufficient (he shared it with two additional persons) or Mithridates was "immune" to the poison remains disputable. In desperation, the unlucky king ordered his Gallic mercenary Bituit to stab him shortly before being captured by mutinous soldiers. These dramatic events captured the attention of the prolific French playwright Jean Racine (1639–1699) and inspired him to write the famous tragedy *Mithridate* (1673). A century later, Wolfgang Amadeus Mozart (1756–1791) composed at the age of 14 his highly successful youthful opera seria *Mitridate, Re di Ponto*, which premiered in December 1770 in Milan. Thus, the story of Mithridates, understandably devoid of its immunologic undertones, survives for posterity.

In the fifth century AD, wise men highly venerated for their experience, judgment, and wisdom—called sages—recommended in the Babylonian Talmud for the treatment of rabies that "if one is bitten by a mad dog, he may eat his liver and be cured" (section Moed, tractat Yoma, Chapter 8, segment 84). According to other sources, the diaphragm of a rabid dog should also have been ingested. Although there are no reports suggesting the success of such treatment, based on the current knowledge, it is not surprising that this recommended practice was not widely accepted and remained of historical interest.

The roots of mucosal immunity also can be traced to documents dated around 900 AD. The Chinese developed a secret ritual to ward off the dreaded scourge of their time, smallpox, which we now know was caused by the variola virus. As part of this Chinese ritual, the scabs of healed pustules were ground up and used as an inhalant. In many instances, this earliest form of nasal immunization worked so well that the practice made its way into India. However, in some instances, this risky practice resulted in a fatal infection. Nevertheless, modifications of the practice spread from India to Turkey, where in 1717 Lady Mary Wortley Montagu (1689–1762) learned of it and brought the practice of variolation back to England. Her adaptation, although still risky, worked in many instances. Later in that century, Dr Edward Jenner (1749–1823), who knew of and practiced this method of treatment, worried about the inherent risks of spread of the disease. He astutely recognized that milkmaids often developed handsores closely resembling smallpox pustules; however, the lesions healed and in all cases they were immune to smallpox. As we now appreciate, the cowpox lesions were caused by Vaccinia virus, which, although related to the smallpox virus, was much less virulent for humans. The infection, however, did induce an immunity to smallpox (Jenner, 1798). The actual practice of using Vaccinia (from the Latin *vacca*, meaning "cow") was adapted to describe use of attenuated bacteria or viruses, or inactivated bacterial toxins or recombinant proteins as vaccines, which of course is the accepted terminology today. Interestingly, 1996 was proclaimed the year of the vaccine in recognition of Jenner's contributions 200 years earlier. A complete worldwide vaccination program by the World Health Organization and other health agencies resulted in eradication of smallpox in 1979.

MUCOSAL MICROBIOTA

Based on Pasteur's work on the microbial nature of fermentation, it was widely believed that the presence of bacteria in the intestine was essential for the life of the host (Leidy, 1849). However, Metchnikoff (1903, 1908) tended to regard the intestinal "flora" as hostile, inducing toxemia in the host, and proposed that the process of premature aging could be prevented by altering the intestinal microbiota. Surprisingly, this doctrine found a fertile ground in the early twentieth century and drastic forms of treatment, including high enemas or even therapeutic colectomies, were used to prevent intestinal autointoxication (Lane, 1926). On the other hand, many workers devoted themselves to determining whether life could be maintained with a sterile intestinal tract. One of the first was Schottelius (1899), who was able to rear chicks under sterile conditions. Nuttal and Thierfelder (1895) achieved some success with mammals: they removed embryonic guinea pigs by cesarean section and maintained them uncontaminated for several weeks. The conclusion was that bacteria in the intestinal tract were not necessary for mammalian life, when an appropriate diet was provided. Cohendy (1912) finally showed that "prolonged" life was possible in the absence of gut bacteria by rearing chicks for up to 40 days under germ-free (GF) conditions.

Contemporary approaches were motivated by the belief that GF animals were invaluable tools for discrimination of genetically determined immune mechanisms, spontaneously available, from those induced by environmental antigens, especially intestinal microflora (for review see Sterzl et al., 1987). This belief was supported by experiments done in many countries using guinea pigs: Glimstedt in Sweden (1932); Reyniers (1932) at the Lobund Institute, United States; and Miyakawa et al. (1958) in Japan. It was found that the wasting syndrome, which developed in thymectomized newborns, could be ameliorated by raising the altered animals under conditions that prevented intestinal colonization.

It has also been known for decades that gut commensal microbes colonizing the neonatal mammal affect the activation and development of the systemic immune system, especially to increase circulating specific and "natural" antimicrobial antibodies (Tlaskalová et al., 1970; Carter and Pollard, 1971; Berg and Savage, 1975; Kim, 1979; Tlaskalová-Hogenová and Stepánková, 1980). Piglets were

chosen because they displayed considerable fetal insulation, provided by a six-layered epithelial-chorial placenta. This barrier is impermeable not only to cells but also to larger protein molecules such as immunoglobulins (Ig) (Sterzl and Silverstein, 1967), and passive maternal antibodies are obtained after birth with early suckling of colostrum. Newborns were delivered into sterile bags and transferred into a laminar flow room containing sterilized cages (Trávnícek et al., 1975). Similar approaches have proved effective for obtaining and maintaining GF rats, mice, and rabbits (Gustafsson, 1948; Carter and Pollard, 1971; Berg and Savage, 1975; Tlaskalová-Hogenová and Stepánková, 1980). Of these GF mammalian models, only piglets can be deprived of colostrums and milk and denied any passive immunity via maternal antibodies. Without the passive protection provided by the colostrum, piglets exposed to normal environmental microbes or artificially colonized with a "nonpathogenic" *Escherichia coli* die within 48–72 h of bacterial septicemia (Trnka et al., 1959). However, such colostrum-deprived sterile piglets can be maintained under GF conditions with an appropriate diet.

Joseph Leidy (1849) wrote that "from the opinion so frequently expressed that contagious diseases and some others might have their origin and reproductive character through the agency of cryptogamic spores… I was led to reflect upon the possibility of plants of this description existing in healthy animals, as a natural condition; or at least, apparently so, as in the case of Entozoa." Leidy reasoned that the wet epithelial surfaces of the body could provide a rich culture medium for commensal microbes. Perhaps the first systematic analyses of these commensal microbes were provided by Schaedler, Dubos, and their coworkers (Schaedler et al., 1965a,b; Dubos et al., 1965). They stated that "mice and other mammals normally harbor an extensive bacterial flora, not only in the large intestine, but also in the stomach and small intestine. Although this flora plays an essential role in the development and well being of its host, its exact composition is not known" (Schaedler et al., 1965a). Unfortunately, their final lament is still true, although great strides have been made recently in elucidating the gut microbiome. However, the three seminal papers of Schaedler, Dubos, and coworkers offered the first comprehensive characterization of a portion of the gut microbiota (using both aerobic and anaerobic in vitro culture) and employing the very models used until recently to assess the interactions of gut microbes with the gut-associated lymphoid tissue (GALT)—the natural colonization of neonates and the deliberate colonization of axenic (GF) mice with particular gut commensal bacteria. Interactions between the mucosal immune system and the microbiota have now become a topic of prime interest.

Shortly after the gut lamina propria of several mammalian species (humans, rabbits, rats, and mice) was found to contain an abundance of secretory plasma cells (Crabbé et al., 1965; Crandall et al., 1967; Pierce and Gowans, 1975; Cebra et al., 1977), most of which made IgA, it was noted that both GF adult mice (Crabbé et al., 1970) and neonatal mice (for review, see Parrott and MacDonald, 1990) had a paucity of such cells. Thus, the absence of gut microbes seemed to forestall the natural development of the abundant population of IgA plasma cells normally present in gut lamina propria. As early as 1968, Crabbé et al. (1968) were able to demonstrate that colonization of formerly GF mice with normal intestinal flora could stimulate the development of IgA plasma cells to normal levels within 4 weeks; furthermore, they showed that oral administration of the protein antigen ferritin to GF mice led to the appearance of antigen-specific IgA plasma cells in gut lamina propria (Crabbé et al., 1969). Pollard made the significant observations that Peyer's patches of GF mice contained mainly "primary" (quiescent) B-lymphoid follicles, but that some enteric bacteria could activate germinal center (GC) reactions, whereas others were less effective (Pollard and Sharon, 1970; Carter and Pollard, 1971). Pollard and Sharon (1970), Foo and Lee (1972), and Berg and Savage (1975) all agreed that some enteric bacteria were more effective than others in stimulating the development of specific circulating antibodies. Thus, they tend to support the notion of autochthonous versus normal gut microbiota.

Coincident with these observations, in 1971, Peyer's patches were found to be sites for the preferred generation and accumulation of precursors for IgA plasma cells (Craig and Cebra, 1971), which could immigrate to and selectively populate all mucosal tissues (Cebra et al., 1977). Thus, it became relevant to link the development of specific, IgA-committed B cells in Peyer's patches to the appearance and accumulation of specific IgA plasmablasts in the gut lamina propria or elsewhere in mucosal tissues and to implicate particular gut microbes as effective stimuli of these perturbations. Most notably, the still uncultivable and unclassified "segmented filamentous bacterium" was first shown to stimulate the maturation of mucosal immunity by Klaasen et al. (1993).

HEALING POWERS OF SECRETIONS: HISTORY OF BREASTFEEDING

Injured animals lick their wounds to clean them and also to hasten their healing. In many ancient cultures, squirting milk in the nose or conjunctiva of sick children and the application of urine or saliva to skin injuries were common medical practices. Lactational products of human and other mammalian species have long been associated with unique healing powers. Human milk, especially mother's own milk, has been considered a complete food for infants of all mammals in many ancient scriptures. More than 2500 years ago, with the evolution of agricultural civilization and domestication of mammals, it was proposed by Charak Sutrasthana that milk obtained from buffalo, cow, sheep, camel, donkey, horse, elephant, and goat, when fed to humans, can improve insomnia, appetite, sexual drive, ascites, piles, infestations by worms, skin disorders, muscle weakness, and a variety

of other ailments (Athavale, 1977). As investigations by Koch began to establish research methods to study the etiology and pathogenesis of infectious disease in the 1800s, classic observations by Escherich (1888) provided, for the first time, evidence that intestinal flora of the human neonate is exquisitely sensitive to human milk. In studies carried out with coliform bacteria, he observed that bacteria isolated from the feces of persons on a meat diet possess a very intense ability to solubilize and split complex nitrogen compounds (egg albumin, casein), whereas bacteria from the feces of milk-fed babies utilize only small amounts of such compounds. Escherich stated: "It is noteworthy in connection with this latter property that it must be more than coincidence that if one spreads the feces of milk-fed babies on gelatin plates, not a single colony capable of liquefying the gelatin is found. However, most of the types from the feces of meat-fed persons will liquefy gelatin to a glue-like peptone. Further, both types show a particular effect on different types of sugar that are fermented with the production of acid. They give extensive growth on potato and finally in animal experiments demonstrate pathogenic properties" (Escherich, 1888).

Empirical experience supporting the notion that breast milk may protect against diarrheal diseases of children was reviewed by Hanson et al. (1988): "Analyses of infant mortality in diarrhoea from Sweden and Finland in the early nineteenth century showed that there was a peak during the summer. This increased mortality was related to the frequent 'summer diarrhoea' during the warm months of July and August. But this peak of mortality was primarily seen in areas where mothers did not breast-feed. In nearby areas where breast-feeding was the rule, there was no increase, or only a minor increase, in the infant mortality in diarrhoea during the summer. The difference did not relate primarily to socioeconomic factors since breast-feeding could be seen in very poor populations, whereas in the same area the farmers' wives had to leave their babies at home to be fed cow's milk through an unhygienic cow horn while working in the fields during the harvest."

The earliest scientifically documented contribution to our knowledge of milk as an important source of mucosal immunity and its functions in in vivo settings is based on studies by Paul Ehrlich (1854–1915) who, in 1892, demonstrated that maternal immunization and subsequent breastfeeding induced protection against the toxic substances ricin and abrin in suckling mice (Ehrlich, 1892). Based on his studies on transfer of immunity through milk, he clearly emphasized the natural breastfeeding of children and raised his voice against artificial feeding. His studies attempted to document the benefit of breastfeeding in mumps, typhus, and measles. Furthermore, he discussed the possible protective role of breastfeeding on congenital syphilis.

It is now well established that bifidobacteria predominate in the feces of the breast-milk-fed infant. Human milk, but not cow's milk, contains factors that stimulate colonization with bifidobacteria. This observation was largely possible because of the discovery in 1953 of *Bifidobacterium bifidum*, a subspecies of bifidobacteria that requires human milk for its growth. Over the years, bifidobacterium has been used anecdotally and more recently under controlled conditions as a therapeutic modality to prevent and treat diarrheal disease, induce immunomodulation, detoxify the gastrointestinal tract, and restore normal intestinal flora (for review, see Bezkorovainy and Miller-Catchpole, 1989). Clinical experience in many countries over the past two centuries has suggested a strong link between breastfeeding and protection against diarrheal diseases and against fertility and childbearing (Jelliffe and Jelliffe, 1977).

Biologic linkage of the milk and mammary glands to the mucosal immune system was recognized initially by identification of immunoglobulins in milk by Gugler and von Muralt (1959) and Hanson (1961). Subsequent studies led to the identification of secretory IgA (S-IgA) in human milk (Hanson and Johansson, 1961). As these studies were being carried out in Europe, the presence of IgA was demonstrated in other external mucosal secretions by Tomasi and his coworkers (Chodirker and Tomasi, 1963; Tomasi and Zeigelbaum, 1963; Bienenstock and Tomasi, 1968) in the United States. These observations were followed by the definition of antiviral, antibacterial, and antiparasitic activity in S-IgA and other milk immunoglobulins; demonstration of a diverse spectrum of cellular elements and antigen-specific, cell-mediated immune responses; recovery of cytokines; and identification of other nonimmunologic defense factors in mammalian products of lactation.

Several studies have identified intestinal and respiratory tract axes in the homing of IgA-committed, antibody-forming cells from the intestinal Peyer's patches and bronchus-associated lymphoid tissues (BALTs) to the mammary glands (Montgomery et al., 1974, 1978; Goldblum et al., 1975; Roux et al., 1977; Fishaut et al., 1981; also see subsequent discussion). During the past 20 years, it has become clear that many of the observations made by our predecessors have been proved to be accurate. These include the effects of breastfeeding on mucosal infections, childhood allergy, birth spacing, childhood survival, as well as effects on modulation of immune response and its regulation in autoimmune diseases.

Although "innate immunity" was given renewed impetus as well as a new meaning by Charles Janeway (1943–2003) in the 1990s, and the discovery of Toll-like receptors revealed the importance of innate recognition mechanisms in the initiation of immune responses, the topic has a much longer history. The presence of antibacterial factors in secretions, such as milk and saliva, has been known for many decades (see Chapter 15). Lysozyme, for example, was discovered as a bacteriolytic agent in tears and nasal secretions by Alexander Fleming in 1922 (Fleming, 1922), and subsequently

found in most biological fluids. Lactoferrin was originally discovered as a red protein in milk (Sørensen and Sørensen, 1939), and its properties were more extensively explored by Masson and Heremans during the 1960s (Masson and Heremans, 1971). Its capacity to bind iron with very high affinity, even at low pH, led to the concept that it can exert antibacterial activity by depriving bacteria of this essential element (Arnold et al., 1977), although most pathogens have evolved other strategies for obtaining iron. Lactoperoxidase was found to exert antibacterial activities in milk, and also saliva, by oxidizing thiocyanate to hypothiocyanite (Oram and Reiter, 1966; Pruitt and Adamson, 1977). Numerous other antimicrobial proteins, including defensins, have now been identified in secretions, as described in Chapters 15 and 16.

It is of interest that lysozyme was shown to interact with colostral IgA antibody and complement to bring about lysis of bacteria (Adinolfi et al., 1966), although this finding proved difficult to repeat (Hill and Porter, 1974). Subsequent opinion has held that other unidentified components in the preparations used were responsible for the observations, as it is now known that IgA lacks the C1q-binding site that initiates the classical complement pathway and its originally reported ability to activate the alternative complement pathway depended on artificial aggregation (Götze and Müller-Eberhard, 1971; see also Chapter 22).

ANTIBODIES OF EXTERNAL SECRETIONS

This discovery of antibodies in external secretions, or more specifically in gastrointestinal tract secretions, should be credited to Russian pathologist Alexandre Besredka (1870–1940), who initiated studies at the Pasteur Institute (which he headed) with two species of bacteria (Besredka, 1919). He used toxin-producing *Shigella dysenteriae* for work on enteric infections, and *Bacillus anthracis*, which Pasteur himself had used earlier in studies to develop an anthrax vaccine. Besredka clearly showed that oral immunization of rabbits with *S. dysenteriae* led to a solid immunity in the gastrointestinal tract that was unrelated to titers of serum antibodies. He also showed that cutaneous immunization with anthrax toxoid resulted in serum antibodies associated with resistance to challenge. Besredka (1919, 1927) deduced that both types of bacteria caused disease in part by production of exotoxins; in the case of dysentery, a local antibody response was protective.

The first direct demonstration of antibodies in stool was provided by Davies (1922), who studied fecal extracts from patients recovering from *S. dysenteriae* infection. Agglutinin titers as high as 1:80 were noted; however, peak titers occurred in bloody mucus, and he failed to see antibodies in normal, immunized subjects. Even so, he correctly deduced that most of the antibody activity present was derived by local synthesis and secretion into the gastrointestinal tract.

Studies of local antibody responses in the gastrointestinal tract and their role in protection from infection require a more appropriate infection agent and animal disease model than the dysentery-induced fatal infection of rabbits used by Besredka (1919). One can trace the first successful model, the guinea pig, to Robert Koch (1843–1910), who showed that the disease cholera could be reproduced by direct injection of *Vibrio cholerae* into the duodenum or by oral dosing with vibrios in 5% carbonate to neutralize stomach acidity, a method still in use for oral immunization to the present time. Nevertheless, opium was required to reduce intestinal peristalsis and to allow growth of *V. cholerae* in the small intestine, with subsequent diarrhea and death from dehydration (Koch, 1885). The model for cholera was more suitable for definitive demonstration that antibodies were produced locally in the gastrointestinal tract, because the diarrhea results from an intoxication (by cholera toxin), which is produced by noninvasive *V. cholerae*, as opposed to the dysentery model of Besredka (1919), in which *S. dysenteriae* actually invades the epithelium and causes a bloody diarrhea with sloughing of intestinal mucosa with possible plasma antibody transudates. Nevertheless, both Besredka (1919) and Davies (1922) reached the correct conclusion that gut mucosal antibodies were locally produced and not serum derived using the dysentery model in rabbits or convalescing humans, respectively.

The studies of Burrows, Havens, Koshland, and their coworkers were the first definitive evidence that antibodies to cholera in feces, termed coproantibodies, were indeed induced in guinea pigs after deliberate vaccination or oral infection with *V. cholerae*. In fact, in an elegant series of studies it was clearly established that either intraperitoneal vaccination or suboptimal oral infection led to the presence of coproantibodies, which preceded the development of serum antibodies (Burrows et al., 1947; Burrows and Ware, 1953; Burrows and Havens, 1948). Furthermore, the presence of these coproantibodies correlated with protection from oral challenge with live vibrios. Additional work using prior irradiation and intraperitoneal immunization led to the induction of fecal antibodies in the absence of serum antibody responses, and again protection from challenge was achieved (Koshland and Burrows, 1950).

The essential protective role of intestinal antibodies in survival was demonstrated 35 years ago in a unique model of GF, colostrum-deprived newborn piglets (Rejnek et al., 1968). Because of the absent transplacental transport of antibodies, when deprived of milk, these animals die of septicemia with environmental bacteria, as described earlier. However, after ~2–3 days, the intestinal absorption of antibodies from milk into the circulation ceases and they remain in the gut lumen. Although all control animals given *E. coli* orally succumbed to infection, piglets that also orally received immune milk or serum (or isolated antibodies) survived irrespective of the source of Ig isotype. These experiments convincingly demonstrated that antibodies function

locally within the intestinal lumen and prevent otherwise fatal infection with commensal microbiota.

Studies on the experimental infection of mice with influenza virus have also provided evidence for a local protection. For example, immunity to influenza correlated directly with the presence of antibodies in tracheal–bronchial washes, and not in serum. Furthermore, the presence of antibodies in the murine respiratory tract actually prevented experimental influenza infection (Fazekas de St. Groth and Donnelly, 1950a,b). Thus, studies performed in animals provided strong evidence that secretory antibodies were not mere transudates of antibodies from plasma and that resistance to mucosal infections correlated better with the titers of antibodies in the relevant secretion than in serum. However, the importance of secretory antibodies and their accumulation, as a response to the influenza virus infection, was predicted by Francis and Brightman (1941) and Francis et al. (1943). Although the original publications are unavailable, Shvartsman and Zykov (1976) reviewed a large number of papers from 1938 to 1972, generated by Russian and East European investigators (Soloviev, Parnes, Zakstelskaya, Smorodintsev, Zhdanov, Sokolov, Slepushkin, Ikic, Sarateanu, Cajal, and their coworkers). These studies concerned the immunobiologic properties of secretory antibodies induced in animals by systemic and mucosal immunizations, immunologic memory, designs of live vaccines, and differences in immune responses induced by various influenza virus vaccines (live or inactivated) given to tens of thousands of vaccinees by the oral, nasal, or systemic routes. Retrospectively, we must regret that results of these studies were either not published or printed in journals unavailable (or not read) in other countries.

When IgA was shown to be the major isotype in the secretions (see subsequent discussion), it was logical to infer that such protection was mediated by IgA antibodies. In the ensuing years, a number of laboratories went on to demonstrate, both clinically and experimentally, the ability of IgA antibodies to confer protection against a variety of infectious agents that affect mucosal membranes. In some studies, protection was shown directly; in others, resistance to infection best correlated with specific IgA antibody content in the particular secretion. Some of the key observations were made by Smith et al. (1967), Ogra et al. (1968), and Fubara and Freter (1973). From such studies, together with the knowledge that IgA antibodies in milk are not absorbed from the gut of the suckling infant (Ammann and Stiehm, 1966), it could be concluded that IgA antibodies in mucosal secretions can act as a luminal barrier to inhibit the attachment and penetration of antigens, including intact microbes. A consistent observation was that IgA-deficient individuals have increased serum antibodies to food antigens (Buckley and Dees, 1969), indicative of a deficient intestinal barrier. Further support for the immune barrier concept came from work by Williams and Gibbons (1972), who showed that S-IgA antibodies can prevent bacteria from adhering to epithelial cells, and Walker et al. (1972), who showed that oral immunization can reduce the subsequent absorption of the same antigen. Moreover, the barrier function of S-IgA could be aided by its relative resistance to degradation by proteases (Brown et al., 1970).

The discovery of IgA, with its unique properties and predominance in almost all external secretions, deserves a closer examination. In the early 1950s, many investigators studying the properties of human myeloma proteins noted that not all "γ-globulins" fulfilled the criteria as then defined by low-molecular-weight and carbohydrate-poor (7S), or high-molecular-weight and carbohydrate-rich (19S) antibodies (for review, see Heremans, 1974). It appeared that some proteins with an electrophoretic mobility in the β-region differed from both 7 and 19S antibodies in their precipitability with inorganic salts (e.g., $ZnSO_4$) and displayed antigenic properties distinct from both 7 and 19S antibodies (Slater et al., 1955). Although some of these myeloma proteins with β electrophoretic mobility shared the same molecular weight with 7S antibodies, their carbohydrate content was remarkably higher. These findings prompted Slater et al. (1955) to postulate the existence of an additional class of antibodies.

Drawing on this knowledge and exploiting novel immunochemical techniques, Joseph Heremans (1927–1975) and his coworkers demonstrated in a series of papers (Heremans, 1959; Heremans et al., 1959, 1963; Carbonara and Heremans, 1963) that the carbohydrate-rich, serologically peculiar β-globulin constituted a type of antibody that was distinct from 7 to 19S globulins and was also present in normal human serum. The designation as IgA was accepted in 1964, and all previous synonyms, such as βx, β_{2A}-globulin, γ_{1A}, and γA, were abandoned.

Antibodies were found in milk by Gugler and von Muralt (1959), as mentioned earlier. In parotid saliva, immunoglobulins were identified by Ellison et al. (1960) and by Kraus and Sirisinha (1962). The presence of additional antigenic determinants on milk compared with serum antibodies was noted by Hanson (1961) and Hanson and Johansson (1961). In several outstanding papers, Tomasi and coworkers (Chodirker and Tomasi, 1963; Tomasi and Zeigelbaum, 1963; Tomasi et al., 1965) demonstrated the predominance of IgA in a polymeric (p) form (11S) in many external secretions of human origin and provided a structural explanation for additional antigenic determinants on S-IgA by the discovery of a novel, IgA-associated polypeptide–secretory piece, later renamed secretory component (SC). The independent identification of a previously observed polypeptide, thought to be an aberrant L chain, as a novel component, joining (J) chain, in pIgA from the serum of myeloma patients (Halpern and Koshland, 1970) and in polymeric serum IgM and S-IgA from human colostrum (Mestecky et al., 1971), resulted in the proposal of a

molecular formula for rabbit (O'Daly and Cebra, 1971) and human (Mestecky et al., 1972) dimeric S-IgA as $(\alpha_2L_2)_2$. $SC_1.J_1$. By then, the well-established preponderance of IgA in external secretions had prompted numerous studies that resulted in several models of selectivity of IgA transport (for review, see Brandtzaeg, 1981). Some investigators speculated that monomeric IgA was assembled into polymers within epithelial cells through incorporation of SC (Tomasi et al., 1965; South et al., 1966). Infusion of IgA-containing plasma to children with low serum levels of IgA led to the appearance of IgA in the saliva. Contrary to the SC-dependent polymerization of IgA, Lawton and Mage (1969) and Bienenstock and Strauss (1970) convincingly demonstrated by immunochemical studies of rabbit and human S-IgA that the polymerization is SC independent and must occur within IgA-secreting plasma cells before their product is taken up by epithelial cells. The importance of the polymeric configuration and the presence of J chain for efficient SC binding was predicted by Mach (1970, addendum) and documented by Radl et al. (1971, 1974). Based on previous extensive histochemical studies of the distribution of component chains of S-IgA in tissue sections performed in several laboratories (e.g., Tomasi et al., 1965; Tourville et al., 1969; Poger and Lamm, 1974), but mainly his own, Brandtzaeg (1974) proposed that SC on epithelial cells functions as a receptor for J chain-containing pIgA, which is transported into external secretions. Further evidence to support this contention was provided by in vitro studies of pIgA- or IgM-binding live human adenocarcinoma epithelial cell lines of intestinal origin (Crago et al., 1978; Nagura et al., 1979; Brandtzaeg and Prydz, 1984) and costaining for SC and IgA on isolated intestinal epithelial cells (Brandtzaeg, 1978).

The structural–cellular interactions responsible for an extremely effective transport of pIgA from the circulation into the bile of mice and rats, the so-called "liver pump" (Jackson et al., 1977; Lemaitre-Coelho et al., 1977), were explored in the late 1970s and early 1980s in several laboratories. Immunohistochemical and functional studies of liver cells from many vertebrate species convincingly demonstrated that the murine, rat, and rabbit but not human hepatocytes express SC (see Chapter 19) responsible for the binding and selective transport of pIgA present in a free form, as well as in the form of low-molecular-weight immune complexes.

Extensive studies concerning the interaction of Igs with various cell populations resulted in the discovery of cellular receptors specific for the Fc fragment of Igs of some but not all isotypes. The binding of radiolabeled IgA of various molecular forms, including S-IgA, to human neutrophils (Spiegelberg et al., 1974) and monocytes (Fanger et al., 1980) led to the discovery of Fcα receptors and their participation in cell activation and promotion of phagocytosis. Importantly for many subsequent studies of biologic functions of IgA (e.g., activation of complement and opsonization), aggregated myeloma IgA proteins of both subclasses and polyclonal S-IgA bound better than free IgA and the Fcα receptors differed remarkably in their specificities from the Fcγ receptors (Spiegelberg et al., 1974; Lawrence et al., 1975). The human Fcα receptor (CD89) was characterized and cloned in 1990 (Maliszewski et al., 1990; Monteiro et al., 1990).

Antigenic differences among various human IgA myeloma proteins resulted in the discovery, in 1966, of IgA subgroups, later reclassified as subclasses, by four independent groups of investigators (Feinstein and Franklin, 1966; Kunkel and Prendergast, 1966; Vaerman and Heremans, 1966; Terry and Roberts, 1966). Their structural uniqueness, including differences in heavy–light chain covalent bonding (Grey et al., 1968), carbohydrate structures, existence of genetic variants (allotypes) that are restricted to the IgA2 subclass (Kunkel et al., 1969), characteristic distribution in systemic and mucosal compartments, and medical importance such as sensitivity to proteases produced by bacterial pathogens and their association with diseases (e.g., IgA nephropathy), are described in pertinent chapters of this book.

Although an increased resistance of S-IgA compared with serum immunoglobulins to proteolytic enzymes was observed by Brown et al. (1970), the presence of an Fc fragment of IgA in stools indicated that at least a fraction of intestinal IgA is cleaved in vivo by enzyme(s) of enteric microbial origin (Mehta et al., 1973). This finding resulted in the discovery of bacterial proteases that cleave IgA1 into Fab and Fc fragments: *Streptococcus sanguis, Neisseria gonorrhoeae, Neisseria meningitidis* (Plaut et al., 1974, 1975), *Streptococcus pneumoniae,* and *Haemophilus influenzae* (Kilian et al., 1979; Male, 1979) were initially identified as producers of unique IgA1 proteases.

ANATOMIC STUDIES OF MUCOSAL ORGANS AND THEIR FUNCTIONAL IMPLICATIONS

Current extensive studies of lymphoid cell trafficking as related to the induction of mucosal immune responses or tolerance have emphasized the role of unique lymphoepithelial structures associated with the intestinal and respiratory tract. These structures were described in the intestine by Johannes Conrad Peyer (1653–1712), a Swiss anatomist and naturalist who lived most of his life in what is now known as Schaffhausen in the vicinity of Lake Constance. It was here that he first noted in 1673 the structures that subsequently bear his name: Peyer's patches. He published his observations in 1677 in a treatise entitled "Exercitatio anatomico—medica de glandulis intestinorum, earumque usu et affectionibus. Cui subjungitur anatome ventriculi galliinacei" (Peyer, 1677, 1681). It is noteworthy that

Peyer's original treatise of 1677 was published in exactly the same form for a second time in 1681 by H. Wetstenium in Amsterdam. This accounts for the confusion as to when Peyer originally published his work. At that time, it was common to publish the same work in more than one place. In Garrison and Morton's medical bibliography, it is stated that these same structures were first described by Johannes Nicolau Pechlin (1672) in his treatise titled "De purgantium medicamentorum facultatibus exercitatio nova" (Pechlin, 1672; Norman, 1991). Additionally, it has been suggested that the same structures had already been noted in 1645 by Marco Aurelio Severino (1580–1656), who was a professor of anatomy and surgery in Naples (Schmidt, 1959).

As indicated in his title, Peyer actually described these structures as glands, because when he squeezed them, he saw a milky fluid (chyle) emerging from what he thought were these structures. It was only with the advent of microscopes and histology that it became clear that these structures contained mononuclear cells (lymphocytes) and were lymphoid nodules, not secretory glands.

Peyer was a physician who studied medicine in Basel and subsequently Paris and Montpellier. After his studies he returned to his birthplace, where he became the pupil of Johannes Jakob Wepfer, whose son-in-law was Johannes Conrad Brunner, who first described the duodenal glands that now carry his name. Considering Peyer's close association with Wepfer and Brunner, it is not surprising that Peyer thought that the lymphatic nodules were secreting glands.

The role and function of Peyer's patches remained obscure for 160 years, when William Wood Gerhard published a classic study of patients dying from typhus (Gerhard, 1837). Louis, the great Parisian clinician under whom Gerhard studied, had originally described a triad that involved enlargement of Peyer's patches, mesenteric glands, and spleen in what was at that time termed dothinenteritis or typhoid fever, which also included typhus, these two diseases not having yet been differentiated. Gerhard differentiated them and pointed out the lack of involvement of Peyer's patches in typhus, quoted in *A Bibliographical History of Medicine* (Talbott, 1970).

It was widely thought that the lymphatic glands or nodules were involved in some way in defense mechanisms of the intestinal tract, especially because they were clearly enlarged in various intestinal infections such as typhoid fever. However, relatively little new significant information on these structures became available until the twentieth century. Jolly (1913) coined the name lympho-epithelial organs and applied it to Peyer's patches and the bursa of Fabricius because of the close relationship between lymphatic tissue and the mucosal epithelium. Aschoff (1926), quoted by Ehrich (1929), classified them with lymphatic tissue of the mucous membranes of the digestive, respiratory, reproductive, and urinary systems, in a group distinct from lymph glands and nodes. However, it was not until 1935 that Hummel stated that Peyer's patches "are located at the beginnings of lymph channels rather than in their course" (Hummel, 1935). She did a careful study of intestinal lymphoid tissue and classified it according to the presence or absence of well-formed germinal centers, which were dependent on age and exposure to intestinal contents. Sanders and Florey (1940) published a paper on the effects of the removal of lymphatic tissue. They coined the name *peyerectomy* and successfully carried out the surgical removal of visible Peyer's patches and other lymphoid tissue, including the spleen, to study the effect on the hypertrophy of residual lymphoid tissue and numbers of circulating lymphocytes. Clearly, peyerectomy caused a decrease in the number of circulating lymphocytes and, interestingly, hypertrophy of intrahepatic lymphoid tissue. Jacobson et al. (1961) showed that mice could be protected from otherwise fatal total body irradiation by the shielding of a single Peyer's patch.

In a light and dissecting microscopic study of human Peyer's patches, Cornes (1965) showed that the number of patches increase with age to about 12 years and then decline rapidly to age 20, followed by a slower but steady decrease in number of visible lymphoid aggregates up to old age. All these observations led eventually to the experiments of Cooper et al. (1966), who showed in rabbits that the surgical removal of the sacculus rotundus, appendix, and Peyer's patches followed by x-irradiation, led to a selective deficiency of antibody-producing capacity to a range of antigens while leaving delayed hypersensitivity and rejection of skin allografts intact.

Joel et al. (1970) observed that the lymphoid epithelium of mouse Peyer's patches had the capacity to take up and retain India ink particles selectively. The first description of follicle-associated epithelium overlying GALT was provided by Bockman and Cooper (1973). As they clearly state in their review (Bockman and Cooper, 1973; Bockman and Stevens, 1977) of the previous literature, others had described micropinocytotic activity in such lymphoepithelium before them as well as the unusual nature of this epithelium (Faulk et al., 1970). However, Bockman and Cooper showed selective pinocytosis by this epithelium in the chicken bursa, rabbit appendix, and mouse Peyer's patches. This work was expanded by Owen and Jones (1974) in an ultrastructural study of human Peyer's patch tissue. It was in this last paper that these authors first used the term M for microfold cell, a name that appears to have been retained and is now in general use. Experiments by Schaffner et al. (1974) and Sorvari et al. (1975) clearly showed the selective pinocytotic capacity of chicken bursal lymphoepithelium in regard to environmental and luminal antigens. Owen (1977), as well as Bockman and Stevens (1977), published their separate papers describing a specific and selective uptake of tracer molecules.

Because of similarities in appearance between rabbit intestinal lymphoid tissue in the appendix and sacculus rotundus, Archer et al. (1963) suggested that the lymphoid

tissue in the intestine might be analogous to the avian bursa of Fabricius. The differentiation of the role of the bursa of Fabricius in its regulation of B-cell development from that of the thymus and its role in T-cell development had occurred as a result of the pioneering work of Glick et al. (1956), in a now classic paper on the role of the bursa in antibody production. A series of papers by Cooper, Percy, Good, and associates supported this view of GALT, which was subsequently overtaken by the concept that in mammals the bone marrow may serve as the actual bursal equivalent (for review, see Waksman and Ozer, 1976).

In their paper on the route of recirculation of lymphocytes in the rat, Gowans and Knight (1964) clearly showed for the first time that lymphocytes circulated from blood to lymph and back again, and that this migration from blood to lymph occurred as a result of a special affinity by lymphocytes for the endothelium of postcapillary venules. The same paper showed that thoracic duct lymphoblasts were retained primarily in the bowel and that Peyer's patches were a major site for localization of recirculating small lymphocytes obtained from rat thoracic duct lymph. Griscelli et al. (1969), Hall and Smith (1970), and Hall et al. (1972) showed that lymphoblasts from the thoracic duct were localized or homed primarily to the intestine. The latter authors postulated that they might be "particularly concerned with furnishing the IgA antibodies that protect mucous surfaces in general and the gut in particular." Coincident with these publications from Hall's group, Craig and Cebra (1971) directly demonstrated that Peyer's patches were enriched in lymphoid precursors for IgA plasmablasts, by cell transfer into sublethally, x-irradiated allogeneic rabbits or congenic, IgA-allotype distinct mice (see Cebra et al., 1977). Copious, predominantly IgA, plasmablasts were observed in the spleens of recipient allogeneic rabbits and in the intestinal lamina propria of recipient congenic mice following such cell transfers. These studies were closely followed by findings that Peyer's patches were required for efficient uptake of antigen from the gut lumen and dissemination of specifically stimulated IgA antibody-forming cells throughout the gut lamina propria, using pairs of Thiry-Vella intestinal loops (Robertson and Cebra, 1976; Cebra et al., 1977). Further relevant studies showed that the enhanced potential of Peyer's patch B cells to generate specific IgA plasmablasts in vitro and in vivo was correlated with their markedly higher content of IgA memory B cells compared with systemic lymphoid organs (Cebra et al., 1977; Gearhart and Cebra, 1979; Fuhrman and Cebra, 1981). It was subsequently shown (Rudzik et al., 1975) that the localization of IgA-containing cells in the spleen was due to an allogeneic effect.

Bienenstock et al. (1973a,b) published definitive papers on what they termed BALT and deemed it analogous to the GALT described originally by Good, Cooper, and coworkers (see previous discussion). It may be pertinent that Klein (1875) had noted morphologic similarities between the bronchial and intestinal lymphoid follicles and stated remarkably that "these lymphoid follicles in the bronchial walls are therefore in every respect analogous to the lymph follicles found in other mucous membranes, e.g. tonsils and in the intestine…" Bienenstock (1974) concluded that "this lymphoid tissue might be part of a more universal mucosal lymphoid system" and in 1974 coined the term common mucosal immunological system in the Proceedings of the Second International Congress of Immunology.

Studies of the origin, migration, and homing of lymphoid cells from GALT and BALT to other mucosal sites were of basic importance for parallel attempts to induce specific immune responses in external secretions. Montgomery et al. (1974, 1978) found that specific IgA antibody could be induced in the mammary secretions of rabbits by oral and bronchial immunization. This observation was confirmed and extended by the experiments of Goldblum et al. (1975) in the human. In the same year, Rudzik et al. (1975) and McWilliams et al. (1975, 1977) offered further data suggesting a common mucosal immunologic system when they showed that cells derived from GALT, BALT, or mesenteric lymph nodes repopulated bronchial and intestinal lamina propria with IgA-containing cells. This general concept that cells migrate from one mucosal site to another and there provide protection against the immunizing antigen received considerable support and development as a result of the experiments of many investigators. Thus, Michalek et al. (1976) demonstrated that oral immunization with *Streptococcus mutans* induces salivary IgA antibodies that protected rats from the development of dental caries. Roux et al. (1977) and Weisz-Carrington et al. (1978, 1979) showed that mesenteric lymph node blasts homed to the murine lactating mammary gland and that this homing was under hormonal influence. The parallel induction of specific IgA antibodies in saliva and tears of orally immunized individuals extended the commonality of the mucosal immune system to the oral cavity and ocular system in humans (Mestecky et al., 1978). Finally, this work was extended to the female reproductive tract of experimental animals (McDermott and Bienenstock, 1979; McDermott et al., 1980).

Many investigators have contributed to the development of the concept of the common mucosal immune system from its first proposal. It is now thought that the mucosal immune system may be more generalized even than originally thought. Although mucosal-associated lymphoid tissue (MALT) has been used to describe this concept (Bienenstock et al., 1979), it is widely assumed that the solitary lymphoid follicles or nodules often found in mucosal tissues may be part of this generalized system. Indeed, Ham (1969), in his classic textbook of histology, suggested that these isolated mucosal lymphoid follicles were "a characteristic of wet epithelial surfaces." Thus, the concept of a

common mucosal immune system may well comprise all mucosal surfaces including the nasal, lacrimal, mammary, and salivary glands, the mucosal part of the bronchial tract, intestine, and both female and male reproductive tracts. The extent to which the skin is involved in this system remains to be determined.

In addition to lymphocytes in Peyer's patches and lamina propria, the epithelial lining of some mucosal surfaces, intestines in particular, contains large numbers of unique cells—intraepithelial lymphocytes (see Chapter 30). The discovery and initial characterization of the "round cells" (*runde Zellen*) as leukocytes were made by Weber (1847) and Eberth (1864) and were followed by numerous papers from other German investigators (for reviews see Wolf-Heidegger, 1939; Ferguson, 1977). Considering the rather limited repertoire of available methodologies to study the function of epithelial lymphocytes, several researchers predicted with an admirable foresight that these lymphocytes may rejuvenate the aging epithelial cells. For example, Guieysse-Pellissier (1912), Goldner (1929), and Bunting and Huston (1921) proposed that the gut was the graveyard of lymphocytes. Based on extensive studies concerning the presence of lymphocytes within the intestinal epithelium of many species, Fichtelius (1967, 1970) speculated that these cells, called theliolymphocytes, represented a mammalian analog of avian bursa of Fabricius (a "bursa-equivalent") that influences the maturation of B cells. Extensive studies carried out from the early 1960s to the late 1970s (for review, see Ferguson, 1977) provided detailed morphologic description and characterization of intraepithelial T cells at the light and electron microscopic levels, their distribution throughout the gut, precise localization and relationship to the villous epithelium, initial quantitative data, and their proliferative potential. Furthermore, it was demonstrated that the numbers and phenotypes of these cells display marked variations in patients with celiac disease, dermatitis herpetiformis, tropical sprue, giardiasis, and other gastrointestinal disorders, but not in Crohn's disease or ulcerative colitis (Ferguson, 1977).

MUCOSAL VACCINATION

The seminal contributions of Paul Ehrlich to the field of mucosal immunity are rarely appreciated in modern literature, yet his outstanding studies performed more than 100 years ago must impress current researchers with their simplicity, perfection of execution, and impact on the future development of mucosal immunology. The protective effect of oral immunization with highly toxic substances of plant origin (ricin, abrin, and robin) was demonstrated in three animal species (mainly mice, but also guinea pigs and rabbits) (Ehrlich, 1891a,b). After having determined the precise lethal dose (for both systemic and oral administration) of abrin and ricin, Ehrlich immunized these animals by the oral route with initially minute but subsequently increasing doses given for up to 40 consecutive days. After such treatment, animals became immune to a subcutaneous challenge with ricin at doses that were 400- to 800-fold greater than the normally lethal amount. Furthermore, the blood from immunized animals contained protective antiricin "matter" (*ein antitoxischer Körper*) transferable to unimmunized animals. For historical precision, we should remind ourselves that the terms *Antikörper* in German and antibody in English were used for the first time by an Austrian-born American pathologist, Karl Landsteiner (1868–1943), in 1900. The discoverers of these "matters," Emil von Behring (1854–1917) and Shibasaburo Kitasato (1852–1931), did not use such terminology in their landmark paper (Behring and Kitasato, 1890) on the passive protection of animals with sera from tetanus toxin-immunized animals, published in 1890, only 1 year before Ehrlich's studies. In contrast to systemic immunization with ricin or abrin, which may cause severe local reactions even at small doses, oral immunization was much safer, although higher doses were required to achieve immunity. However, the most astounding finding of Ehrlich's studies concerned the immunity induced in the eyes of orally immunized animals. Ricin, and especially abrin, are extremely toxic when given in the conjunctival sac: panophthalmia with subsequent necrosis of the eye follows. Surprisingly, orally immunized animals tolerated intraconjunctival application of concentrated ricin or abrin ointments or solutions without any ill effects! The exquisite specificity of the protective "matters" was convincingly demonstrated by the lack of immunity to ricin in abrin-immunized animals and vice versa. Therefore, without knowledge of the existence of antibodies in external secretions or the migratory patterns of antibody-secreting cells, Paul Ehrlich inadvertently demonstrated in 1891 the concept of the common mucosal immune system! Only 1 year later, in 1892, Ehrlich documented the protective property of milk taken from immunized dams and given to suckling pups (see earlier discussion).

The first attempts at vaccination against bacterial diseases through the intestinal tract were carried out in Pasteur's laboratory before 1880 (for reviews, see Calmette, 1923; Gay, 1924). Pasteur (1880), Roux, and Chamberland protected chickens against chicken cholera by ingestion of food containing *Bacillus* avisepticus (now *Yersinia multocida*), although the feeding of anthrax spores to sheep was less effective than the subcutaneous injection of attenuated vaccines (Table 1).

A series of attempts followed between 1892 and 1903, when many scientists (e.g., Klemperer, 1892) induced immunity to *V. cholerae* and *Salmonella typhi* by the ingestion of killed or living bacteria by animals and even humans (see in Calmette, 1923). Interestingly, serum agglutinins were considered the indicators of protection. This contention met with considerable criticism from Besredka, Calmette, and Gay. The last author wrote in 1924: "It is particularly true that a general reaction as evidenced by serum antibodies is no indication of a superior local protection, for example in the intestine, if we admit that

Table 1 Selected List of Bacterial and Food Antigens Used in Mucosal Immunization Studies in Humans and Animals

Antigen	Results and Comments	Author
Yersinia multocida (chicken cholera)	Oral immunization; protection induced	Pasteur (1880)
Vibrio cholerae	Oral immunization, moderate protection	Klemperer (1892) and Metchnikoff (1903)[a]
Mycobacterium tuberculosis	Serum antibodies induced by oral immunization	Calmette and Guérin (1906–1923)[a]
Yersinia pestis		
Corynebacterium diphtheria		Dserzgowdky (1910) and Enlows (1925)
Shigella dysenteriae	Limited protection	Besredka (1919, 1927)
Salmonella typhi	Oral immunization preferable to systemic	Vaillant (1922)[a], Besredka (1919, 1927) and Combiesco et al., (1923)
Streptococcus pneumoniae	Protection achieved by nasal immunization	Bull and McKee, 1929
Staphylococcus pyogenes	Protection achieved by oral immunization	Ross (1930)
Abrin, ricin, robin	Partial protection Oral immunization results in systemic and mucosal protection	Combiesco and Calab (1924) and Ehrlich (1891a, 1891b)
Cow's milk and whey	Prevention of anaphylaxis by feeding	Besredka (1909)
Cow's milk, ox blood, egg white, zein, oats	Decrease in systemic reactivity after prolonged but not short ingestion of these antigens	Wells and Osborne (1911)
Dinitrochlorobenzene	Inhibition of systemic (skin) reactivity after hapten feeding; inability to suppress skin sensitivity by oral immunization in previously sensitized animals[b]	Chase (1946)
Poison ivy	Oral ingestion results in decreased skin reactivity in a few studies; discouraged for lack of efficacy	Stevens (1945)
Horse serum and meat	Sensitization for anaphylaxis	Rosenau and Anderson (1907)[a]
Proteins from rice, corn, and oat flour	Precipitins in serum	Magnus, 1906[a]

[a]*Data from Bull and McKee (1929), Chase (1946), Gay (1924), Stevens (1945), Klingman (1958), Wells and Osborne (1911), and Calmette (1923).*
[b]*See Table 3.*

this exists." (!) Various other bacteria, such as *Mycobacterium tuberculosis, Yersinia pestis,* and *Corynebacterium diphtheria*, were also given orally with some degree of success (Calmette, 1923). However, bacteria that infect through the intestinal tract, where their pathogenic effects are manifest, remained in focus "to protect certain areas of increased susceptibility by the process of local immunization or to close certain 'portals of entry' by the same process" (Gay, 1924). The efficacy of oral immunization in the protection against intraperitoneal challenge with *S. pneumoniae* in a rat model was demonstrated in a series of papers by Ross (1930). A single—or even better, repeated—ingestion of heat-killed, desiccated, mechanically disrupted, or bile acid–dissolved pneumococci induced a high degree of protection against 10^3–10^4 lethal doses, curiously, as short as 48 h after immunization. Feeding of rat tissues of animals killed by pneumococci or living or acid-killed pneumococci was also protective. Sera of orally immunized animals did not contain any agglutinins or precipitins; external secretions were not examined.

As described in a previous section, "Antibodies of External Secretions," the concept of oral or intestinal immunization was brought to prominence by Besredka (1919, 1927). Although his immunization studies with *S. typhi* in a rabbit model met with considerable skepticism (Gay, 1924), his later reports using *S. dysenteriae*, again in a rabbit model, demonstrated that when killed cultures were given per os, protection was local in that antibodies were found first locally in the intestine rather than in the general circulation (Besredka, 1919). Similar results were obtained by Masaki (1922) with *V. cholerae* in a rabbit model. In several studies, Besredka stressed the importance of giving bile before or with the administration of oral vaccines.

The validity of results obtained in animal models was soon tested in humans. Vaillant (1922) used Besredka's vaccine during an outbreak of typhoid fever: among 1236 subjects immunized orally, there were only 2 cases of typhoid (0.17%); in 173 who received subcutaneous vaccine, 4 cases were recorded (2.3%); and among 600 unvaccinated individuals, 50 cases were observed (8.3%)! Later, Besredka (1927) reported the results of immunization of students in a military academy: among 253 students immunized subcutaneously, 10 cases of typhoid occurred (4%), whereas in those who received the oral vaccine with bile (268 subjects), 5 cases of infection were recorded (1.9%).

The current revival of interest in intranasal immunization initialized by Waldman et al. (1968) should also be viewed from a historical perspective. Bull and McKee (1929) immunized rabbits intranasally with a suspension of killed pneumococci before challenge with the live pathogen. A single intranasal immunization performed 11 days before challenge protected all animals from death (Table 2), whereas 83% of rabbits immunized once 8 days before challenge, and 57% of unimmunized controls, succumbed. To detect antibody responses, the authors used the complement-fixation test performed with sera from these animals. In the absence of such antibodies, the authors concluded that the protection was independent of serum antibodies. In light of our current knowledge, it is likely that IgA-mediated protective responses were induced but were not detectable in serum because of the extremely rapid elimination of IgA from the circulation of rabbits. Moreover, complement fixation is inappropriate to test for the presence of IgA antibodies. Antibodies in nasal secretions were not examined. In the authors' defense, antibody isotypes and their different complement-binding properties were unknown, and the discovery of antibodies in nasal secretions was 40 years away.

Table 2 Protection Achieved by Intranasal Immunization of Rabbits with *Streptococcus pneumoniae*

No. of Treatments	Died (%)	Infected but Recovered (%)	Escaped Infection (%)
4	7.7	7.7	84.6
2	14.3	14.3	71.4
1 (11 days before infection)	0	83.4	16.6
1 (8 days before infection)	83.4	0	16.6
Normal rabbits (10 series of 6)	57	27.0	16.0

Modified from Bull and McKee (1929).

The seminal role of inductive sites of MALT in the generation of immune responses at the site of vaccination as well as in secretions and tissues of anatomically remote mucosae and glands has been exploited with increasing frequency in the design of vaccines that can be administered by mucosal routes. Although many such vaccines and unique delivery systems are currently being explored, it may be useful to illustrate the "mucosal" history of vaccination against poliomyelitis and the role of the oral polio vaccine in continuing efforts to rid the world of this dreadful disease.

Efforts to develop immunoprophylaxis against polioviruses began immediately after the first isolation of the virus. Both killed and live virus vaccine candidates were developed as early as 1910, although at that time information on the existence of three distinct poliovirus types was not available. During the early 1930s, studies were undertaken to vaccinate humans with infected monkey spinal cord suspensions inactivated with formalin or sodium ricinoleate (Kolmer et al., 1935; Brodie and Park, 1936). However, these trials failed for lack of adequate controls, failure or inability to standardize vaccine preparations, and lack of reproducible quantitative methodologies for virus titration. The battle against polio began seriously at the national level in the United States with the establishment of the National Foundation for Infantile Paralysis—March of Dimes organization in 1938 with the first Franklin D. Roosevelt Birthday Ball to support clinical research aid, at Georgia Warm Springs Foundation in 1939 (Smith, 1991). Furthermore, during World War II, information became available regarding the distinct antigenic types of the virus, their ability to induce specific antibody responses after inactivation, the ability of inactivated virus to induce protection against intracerebral challenge (Morgan, 1948; Bodian, 1949), and the capacity of polioviruses to replicate in vitro in human and primate tissue culture cells (Enders et al., 1949; Enders, 1952). Other wartime efforts directed toward the control of epidemics of influenza with an inactivated vaccine resulted in a renewed interest in the development of formalin-inactivated poliovaccines (Salk, 1953). The introduction of tissue culture techniques and the characterization of the poliovirus passage in tissue culture is a landmark and represents the cornerstone of our current knowledge of cell–virus interaction. These observations significantly facilitated the development of other live attenuated or inactivated vaccine candidates (Koprowski et al., 1952; Jervis et al., 1956; Sabin, 1955). The inactivated type of polio vaccine (Salk IPV) was licensed in 1955 and the Sabin oral live attenuated polio vaccine (Sabin OPV) was licensed in 1961–1962 (Report of the Commission on the Cost of Medical Care, 1964). The concept of alimentary resistance induced following naturally acquired wild poliovirus infection was proposed by Koprowski et al. (1956) and the presence of poliovirus-specific antibodies was demonstrated as coproantibodies (Lipman and Seligman, 1963). However, the

development of S-IgA antibody responses following oral immunization with Sabin polio vaccine was first demonstrated in the late 1960s (Ogra et al., 1968). In additional investigations, it was observed that Salk IPV in general did not induce a consistent level of mucosal immunity to reinfection, although the induced circulating antibodies were found to be highly effective in the prevention of viremia and systemic infection (Ogra and Karzon, 1969, 1971).

Following licensing of the Salk vaccine, a mass vaccination campaign was initiated under the auspices of the March of Dimes. The incidence of poliomyelitis fell precipitously as more and more children were immunized.

By 1965, the incidence of paralytic disease displayed a low incidence not recorded in the previous 6 decades. From 1950 to 1954, the average number of poliomyelitis cases reported per year was 38,727. On the other hand, in 1961 the total number of poliomyelitis cases had declined to 1312; of these, 988 cases were reported to be paralytic. This represented a more than 95% decline in the incidence of disease from the previous 5-year period (Berkovich et al., 1961). It is estimated that almost a 90% reduction in the number of poliomyelitis cases was attained with Salk IPV alone before the introduction of OPV. The death rate from poliomyelitis had declined to 0.1 in 100,000 in 1961 compared with an annual death rate of 1.9 in 1915–1924, and 1.2 in 1945–1954. The 1961 figures represent the lowest death rate observed for any reporting period in the United States during the previous 5 decades (Report of the Commission on the Cost of Medical Care, 1964). However, a relative increase in the incidence of type 3 virus outbreaks was observed in 1959 and 1960. In these clusters, more than 50% of the subjects had received three or more doses of IPV (Berkovich et al., 1961; Gresham et al., 1962). Other investigations carried out during that time also suggested that the reduction in the incidence of paralytic polio could not be entirely accounted for by the known efficacy of the IPV or the number of persons immunized with IPV (Bodian, 1961). From a historical perspective, it is gratifying to note that poliomyelitis has been eradicated in the Americas, Europe, and indeed most parts of the world. This success is largely the result of orally administered vaccines associated with the development of effective serum and secretory antibody responses to the virus. In retrospect it is remarkable that, as late as the 1950s, experts in infectious diseases opined that "neither passive immunization by human immune or animal serum nor active immunization by vaccines can be advised" against poliovirus infection (Harries and Mitman, 1951).

ORAL TOLERANCE

The precise origin of the frequently claimed beneficial effect of eating plants in the prevention and treatment of skin rashes caused by repeated exposure to certain plants is shrouded in mystery. Although Dakin (1829) states, "Some good meaning, mystical, marvelous physicians, or favored ladies with knowledge inherent, say the bane will prove the best antidote, and hence advise the forbidden leaves to be eaten, both as a preventive and cure to the external disease," we have no information as to the historical basis for this traditional treatment. Gilmore (1911) refers to a practice of chewing plant leaves used by some tribes of American Indians. The "bane" or leaves consumed belong to representatives of some 50 species of plants called "poison vine, ivy, or oak" of the genus *Rhus*, later reclassified as *Toxicodendron*, with species *toxicodendron, radicans, diversilobum,* and *vernix*, which are native to North America. In Japan, the sap of the lac tree (*Rhus vernicifera*), called kiurushi, displays a skin-sensitizing ability, as well as chemical structures and physical properties, analogous to its American counterpart (for reviews, see Stevens, 1945; Klingman, 1958). The effectiveness of inducing systemic tolerance, in this case to skin delayed-type hypersensitivity reactions, by feeding fresh or dried plants (or their ether, alcohol, or oil extracts) was controversial despite extensive studies performed in hypersensitive patients and experimental animals (Kligman, 1958). Oral therapy with ether extracts of fresh leaves, sometimes combined with systemic hyposensitization, was successful in the hands of several investigators (e.g., Duncan, 1916; Shelmire, 1941), whereas others reported no improvement, as summarized in detail by Stevens (1945) and Klingman (1958). Although the skin, anal orifice, and oral mucosa are in that order excellent sensitizing sites that become inflamed on reexposure to poisoning, the intestinal mucosa is apparently refractory (Silvers, 1941).

Extensive systematic studies of immune responses, and anaphylaxis in particular, to plant and animal proteins were carried out at the beginning of this century in many laboratories, but the experiments reported by A. Besredka and H.G. Wells (1875–1943) should attract the attention of mucosal immunologists. It appears that Besredka (1909) was the first investigator to make several observations that were relevant to the concept of anaphylaxis and its prevention by ingestion or rectal administration of protein antigens, in this case milk and milk whey. In an extensive series of experiments, Besredka demonstrated that the injection of milk to previously systemically sensitized animals resulted in fatal anaphylaxis within a few minutes. However, no sensitization occurred when milk was administered rectally or orally. Most importantly, the administration of whole milk or milk whey by the oral or rectal route prevented "sensitization" to the subsequent injection of milk and thus provided a safe and good way for "anti-anaphylactic vaccination." These studies were shortly followed by those of Wells and Osborne (1911), who contributed enormously in this now classical paper to our comprehension of oral tolerance: (1) guinea pigs fed on animal (cow's milk, egg white, ox blood) or plant (corn or oats) proteins are at first rendered sensitive to these proteins, as demonstrated by anaphylactic reactions

when the proteins are injected systemically; (2) feeding of plant proteins extended to a few weeks or months makes experimental animals refractory to anaphylaxis; (3) this refractory condition seems to be reached more easily with vegetable proteins of natural food than with animal proteins, perhaps because of their presence in the diet from the time of weaning; and (4) young animals fed vegetable proteins immediately after weaning became completely refractory to any reaction against injected proteins. Analogous studies of cutaneous hypersensitivity and the induction of serum precipitins in marasmic and normal infants fed cow's milk proteins, egg white, sheep serum, or almond flour were performed by Du Bois et al. (1925). The authors concluded that the ingestion of these antigens leads to the appearance of specific "precipitins" in blood and, in many cases, to cutaneous hypersensitiveness in both marasmic and normal infants. Because the results of the intracutaneous test were obtained within 1 h after the injection, it is likely that they reflect the presence of IgE antibodies, whereas the serum "precipitins" were represented mainly by IgG antibodies.

Inhibition of skin manifestations of delayed-type hypersensitivity to a hapten, 2,4-dinitrochlorobenzene, by prior feeding was reported by Chase (1946). Hypersensitivity reactions in the small intestine to dietary antigens due to the induction of cell-mediated responses was reported by Mowat and Ferguson (1981). A cursory look at one simple table in this rather brief paper of such basic importance tells the story without need for involved statistical analysis (Table 3). Guinea pigs given, by the oral route, 1% solution of the hapten in olive oil for six consecutive days and again two to three times after an 8-day rest displayed "…a very considerable diminution [of skin reactions upon challenge] in groups that had received prior feeding of the chemical." To demonstrate the specificity of unresponsiveness induced by feeding hapten (2,4-dinitrochlorobenzene),

Table 3 Inhibition of Hypersensitivity Reactions by Hapten Feeding

Hypersensitivity Rated as	Prior Feeding of Allergen (%) (93 Animals)	Controls (77 Animals)
High	3.2	74.0
Good	0.0	16.9
Moderate	8.6	5.2
Weak	20.4	3.9
Low	46.2	0.0
Very faint, or Entirely negative	21.5	0.0

Modified from Chase (1946).

tolerized animals were systemically sensitized with a second, unrelated hapten (*o*-chlorobenzoyl chloride). When such animals received a simultaneous series of intracutaneous injections with both haptens, they reacted only to the second hapten. Other findings in this landmark paper concern the longevity of tolerance (at least 31 weeks) induced by feeding, and the failure of oral treatment to diminish the degree of hypersensitivity in animals with established sensitivity to the hapten induced by the systemic route! The potential for inhibiting the development of skin sensitization by giving antigens through oral (Chase, 1946) or systemic (Sulzberger, 1930) routes is sometimes referred to as the Chase-Sulzberger phenomenon. Detailed examination of Sulzberger's and Chase's papers, however, leads to the conclusion that the single feature common to both studies is that inhibition of skin sensitization can be achieved when the hapten is first given by another route. To inhibit skin sensitization, in contrast to Chase's oral route, Sulzberger injected the hapten (arsphenamine) into the heart, muscles, tongue, peritoneal cavity, lungs, or testes of experimental animals. Therefore, it is likely that different mechanisms of prevention of subsequent skin sensitization were involved.

Attempts to induce systemic unresponsiveness by prior feeding of hapten or antigen in humans had been reported infrequently until the recent revival of interest in oral tolerance. Poison ivy or oak extracts were used with variable results, as summarized in admirable completeness by Stevens (1945). In more recent literature, oral desensitization with the same allergen was largely unsuccessful and therefore discouraged (Klingman, 1958). Sulfonamides, introduced into medicine before World War II, are known inducers of allergic reactions when given per os or applied to the skin: eczematous dermatitis ensues in some patients. In a limited study, Park (1944) administered small doses of sulfanilamide orally to desensitize allergic patients with success. Grolnick (1951) used another known skin sensitizer—krameria—by the oral route in an attempt to achieve inhibition of subsequent skin sensitization, although unsuccessfully. These experiments were of considerable medical importance because krameria, an extract of the roots of Brazilian or Peruvian rhatany (shrubs or herbs of the family Leguminosae) was used frequently as an astringent and listed as an official tincture in the US Pharmacopoeia. Grolnick (1951) administered large doses three times daily for 2 weeks, followed by a double dose (for up to 8 weeks) of the diluted tincture before the skin sensitization regimen. However, no difference in the frequency or intensity of skin reactions was observed between orally "desensitized" subjects and controls (no oral ingestion) when skin sensitization with krameria was induced. Using the same hapten, 2,4-dinitrochlorobenzene, as Chase (1946), Lowney (1968, 1973) observed reduced incidence and intensity of cutaneous sensitization in individuals given this hapten orally by application of a 2% solution in acetone

on the buccal mucosa: eight of 17 (47%) individuals were tolerant in the experimental group, compared with one of 26 (4%) subjects in the control group. The efficacy of feeding this hapten in capsules on the suppression of subsequent contact sensitization was dose dependent: small amounts (<20 mg) had no effect, whereas higher doses (>20 mg) induced a significant decrease in reactivity.

Induction of systemic unresponsiveness to an ingested antigen—bovine serum albumin (BSA)—was studied in adults by Korenblat et al. (1968). Observing that the sera from more than 80% of normal children but only 7% of adults older than 40 years of age contained anti-BSA or anti-α-lactalbumin antibodies, the authors tested for possible oral tolerance by systemic immunization with BSA. Indeed, those individuals who had no anti-BSA antibodies did not respond to a systemic or oral challenge. By contrast, serum anti-BSA titers were increased by systemic immunization in individuals with preexisting antibodies. In retrospect, these results are reminiscent of the previously mentioned studies of Wells and Osborne (1911), indicating that ingestion of proteins led first to the induction of responses that decreased on prolonged feeding of the antigen.

IMMUNOPATHOLOGY

IgA Deficiency

Selective deficiency of IgA (or β_2A or γ1A according to previous terminology) was described by Giedion and Scheidegger (1957), Fudenberg et al. (1962), and West et al. (1962). Interestingly, these initial reports contained descriptions of patients whose symptoms presaged the clinical profiles of patients with IgA deficiency as defined in later, more extensive studies of the condition. Some of the patients, for instance, had respiratory infections and thus predicted the major clinical manifestation of IgA deficiency, that is, chronic upper and lower respiratory infections leading in the untreated state to bronchiectasis and respiratory failure. In addition, one patient had steatorrhea and malabsorption and was therefore representative of another symptom complex in IgA deficiency, a non-gluten-sensitive sprue-like syndrome marked by villous atrophy, malabsorption, and at times, intestinal nodular lymphoid hyperplasia. The origin of this symptom complex, initially described in depth by Crabbé and Heremans (1966), is still poorly understood, although most students of IgA deficiency consider it to be an autoimmune manifestation of the disease (McCarthy et al., 1978). On this basis, it must be differentiated from gastrointestinal problems due to infections of the gastrointestinal tract such as giardia or *Salmonella* infection, which have been shown to occur more frequently in IgA deficiency than in normals by Ammann and Hong (1971). Finally, one patient in the West series had a lupus-like syndrome and was thus indicative of the rather strong association of IgA deficiency with autoimmunity, as later shown by the increased incidence of "silent" IgA deficiency in autoimmune diseases and the increased incidence of antibodies against self-proteins or food proteins and frank autoimmunity in IgA-deficient patients themselves (Buckley and Dees, 1969; Ammann and Hong, 1971; Cassidy et al., 1973). The basis of this association was later investigated by Cunningham-Rundles et al. (1978), who showed that IgA-deficient patients absorb an increased amount of intact macromolecules from the food into the bloodstream and, in addition, manifest high levels of circulating antigen–antibody complexes following food ingestion that presumably arise as a result of prior antibody responses to the absorbed food protein. In addition, these investigators showed that the presence of absorbed food molecules and antigen–antibody complexes in the circulation correlated with the presence of autoantibodies or autoimmune disease. Thus, they postulated that in the absence of IgA, the gastrointestinal tract manifests reduced barrier function and permits entry of macromolecules, some of which cross-react with self-antigen and give rise to autoantibody responses (Cunningham-Rundles et al., 1981).

Another early milestone in the history of IgA deficiency was the discovery by Rockey et al. (1964) that IgA deficiency can occur in ostensibly healthy individuals. This finding was later expanded by epidemiologic studies of blood bank donors, which established that IgA deficiency is mainly a "submerged" immunodeficiency occurring in 1/300–1/2000 individuals in various Caucasian populations (Hanson et al., 1983). The existence of such seemingly silent IgA deficiency has prompted studies to determine the factors that result in increased susceptibility to infection. One factor, first identified by Oxelius et al. (1981), relates to the finding that a subset of patients with IgA deficiency also have IgG subclass deficiency, and thus are at further risk for infection. Indeed, as subsequently shown by Björkander et al. (1985), many, but not all, patients with associated IgG subclass deficiency had a greater frequency of infections than patients with IgA deficiency alone. Another factor, identified by Mellander et al. (1986), relates to the ability of IgA-deficient patients to manifest compensatory IgM or IgG antibody responses that then presumably provide sufficient protection at mucosal surfaces to prevent infections; it should be noted here that in humans, IgM like IgA can be transported to the mucosal surface via the polymeric Ig receptor. Finally, the level of IgA produced in patients may be a factor in the occurrence of infection. Thus, patients whose immune systems produce virtually no IgA may be at greater risk than those that produce reduced amounts of IgA. Two caveats concerning silent IgA deficiency are in order. First, as emphasized by Cunningham-Rundles et al. (1980), such unidentified immunodeficiency may in fact be a risk factor for gastrointestinal neoplasia or, as mentioned earlier, autoimmunity. Second, although silent IgA deficiency may be silent in the relatively clean environments

of Western, industrialized countries, it may lead to disease in less developed countries that have environments more closely approximating those that led to the evolution of the immune system.

The finding that some patients with IgA deficiency do produce some IgA and thus have what might be called a partial IgA deficiency relates to the important studies of Savilahti and Pelkonen (1979) showing that a sizable group of IgA-deficient patients, mostly those who have partial IgA deficiency, exhibit transient IgA deficiency that eventually reverts to normal. The causes of such transient deficiency are presently unclear. Among the possibilities that have been suggested is exposure to certain viruses and drugs (particularly anticonvulsants) as well as certain insults to the immune system such as graft-versus host disease, all of which have been associated with IgA deficiency in one way or another (Savilahti and Pelkonen, 1979; Elfenbein et al., 1976). Whether such transient IgA deficiency is qualitatively different from complete IgA deficiency remains to be explored, as does the question of whether all forms of IgA deficiency require an environmental trigger.

Yet another observation concerning IgA deficiency that was made in the early years following its discovery was that of LaPlane et al. (1962) showing that the deficiency occurs in relatives of patients with common variable immunodeficiency (CVI). This observation was the first to suggest that IgA deficiency and CVI are related diseases and to suggest that these immunodeficiencies have a common genetic basis. These possibilities were later put on a firmer footing by the discovery that the two diseases share a common set of HLA haplotypes and that IgA deficiency occasionally evolves into frank panhypogammaglobulinemia (see Chapter 64). In addition, it eventually became apparent that the immunologic abnormalities found in IgA deficiency and CVI were fundamentally similar and thus the two deficiencies represented two ends of the same disease spectrum. As for genetic studies of IgA deficiency (and CVI), these begin with the studies of Koistinen (1975), who noted familial clustering of IgA deficiency and the studies of Van Thiel et al. (1977) showing the occurrence of kindreds with IgA deficiency and various autoimmune diseases. Ambrus et al. (1977) showed that IgA deficiency was associated with HLA-B8 and thus ushered in a series of studies of MHC genes in IgA deficiency and CVI.

The previous considerations bring us to studies of the immunopathogenesis of IgA deficiency (and CVI). In the late 1970s and throughout the 1980s, evidence was accumulated that established that although IgA deficiency (and CVI) may sometimes be associated with class-specific suppressor T cells, the more constant and more basic deficit resides in the B cells. In particular, it was shown by Mitsuya et al. (1981) and Pereira et al. (1982) that IgA B cells in IgA deficiency (and all B cells in CVI) manifest defective class switching and terminal differentiation. Interestingly, this defect in patients with CVI appears to be hierarchical in the sense that upon in vitro stimulation, IgA differentiation is most affected, IgG differentiation is next most affected, and IgM differentiation is least affected.

Genitourinary Diseases

A detailed review of the immunology of the genital tract (Georgieva, 2012) revealed several remarkable findings, particularly related to the immunological basis of infertility. The antigenicity of sperm was independently reported by Landsteiner (1899) and Metchnikoff (1899); these studies of fundamental importance provided a rational basis for many ensuing investigations performed by Levin, Baskin and others (for review see Georgieva, 2012). Of particular importance were experiments performed in farm animals by Bratanov et al. (1949) who demonstrated the presence of high titers of anti-sperm antibodies in sera of repeatedly inseminated but infertile cows and also in rabbits and sheep immunized with allogeneic spermatozoa. The authors concluded that such anti-sperm antibodies blocked fertilization Voisin et al. (1951) reported that immunization with extracts of guinea pig testis induced pathological alterations manifested in the presence of anti-sperm antibodies and aspermatogenesis in the other testicle of the same animal and created an experimental model of autoimmune aspermogenic orchitis. Independently Rümke (1954) and Wilson (1954) demonstrated anti-sperm antibodies in semen and plasma of infertile male patients with oligo-or azoospermia, which effectively agglutinated and immobilized spermatozoa, thus preventing their penetration through cervical mucus. Sterility in female guinea pigs was induced by immunization with an emulsion of testis due to the formation of anti-testicular antibodies, including against sperm, in sera and vaginal fluids (Isojima et al., 1959). Extension of these principles to infertile women revealed the presence of anti-sperm antibodies in their sera and vaginal fluids (Franklin and Dukes, 1964a,b; Isojima et al., 1968). Subsequent extensive studies of the presence, level, specificity, and isotype of anti-sperm antibodies demonstrated their essential role as mediators of antibody-dependent infertility in females (Isojima et al., 1968; Parish and Ward, 1968; Sudo et al., 1977; Ingerslev, 1980; Moghissi et al., 1980; Ingerslev et al., 1982).

Mucosal infections of the respiratory tract and diseases of the gastrointestinal tract or liver may result in the alteration of IgA metabolism and the deposition of IgA1 in the glomerular mesangium and skin. Berger and Hinglais (1968) and Berger (1969) described a new form of glomerulonephritis characterized by prominent codeposits of IgA and IgG in the glomerular mesangium. The disease, now termed IgA nephropathy, or according to its discoverer, *Berger's disease*, is the most common cause of glomerulonephritis in the world. Subsequent studies indicated that the

mesangial deposition of IgA1 may also occur in other diseases including Henoch-Schönlein purpura (HSP), systemic lupus erythematosus, dermatitis herpetiformis, alcoholic liver cirrhosis, and inflammatory bowel disease. Although HSP in children was first described by Heberden (1801), and then by Schönlein (1837) and Henoch (1874), its relationship to IgA nephropathy was elucidated relatively recently. Interestingly, based on careful review of historical records of the symptoms and duration of the disease, Davies (1991) speculated that the kidney failure and ultimate death of W. A. Mozart was due to HSP.

Gluten-Sensitive Enteropathy (GSE)-Celiac Disease and Celiac Sprue

The discovery of GSE is credited to W. K. Dicke, a Dutch pediatrician, who noted during the mid-1930s that one of his patients repeatedly developed diarrhea and rash soon after the ingestion of bread (Dicke, 1941). Notwithstanding the fact that, in retrospect, the clinical syndrome in this patient is better classified as allergy to wheat protein rather than GSE (which is a nonallergic immunologic hypersensitivity and does not result in immediate symptoms), Dicke generalized this observation to a larger group of children with chronic diarrhea and wasting who probably did have GSE. On this basis, in a 1940 meeting of the Dutch Pediatric Society, he proposed a wheat-free diet for children with GSE (then called Gee-Herter syndrome). There is a persistent anecdote that Dicke subsequently became convinced of his theory in the early 1940s during the German occupation of Holland when he noted that the children with GSE actually improved in spite of the general food shortage (which of course included a wheat shortage) and suffered relapses at one point when wheat was air-lifted into Holland by the Allies (Smits, 1989). Finally, in the late 1940s, Dicke teamed up with several Dutch scientists, particularly J. H. Van de Kamer, who had devised a method of measuring fat excretion in the stool to formally show that feeding of certain cereal grains to patients with GSE led to increased fat excretion (i.e., fat malabsorption) (Van de Kamer et al., 1953). This result, published as a Ph.D. thesis by Dicke in 1950, was rapidly reproduced in other parts of Europe and GSE was thus uniquely defined as a diarrheal syndrome due to cereal grain protein hypersensitivity (Dicke, 1950).

In the approximately 65 years that have elapsed since this singular discovery, there have been many important additional landmarks in the study of GSE. In the 1950s, it was shown that GSE is characterized by the presence of villous atrophy and that the loss of absorptive surface that results from such atrophy is responsible for the main clinical manifestation of the disease—malabsorption and nutrient deficiency (Paulley, 1954; Shiner and Doniach, 1960). This discovery also enabled clinicians in the 1960s to link the skin disease, dermatitis herpetiformis, to GSE because patients with dermatitis herpetiformis could also be shown to have various degrees of villous atrophy and to have amelioration of disease with a gluten-free diet (Shuster et al., 1968). The existence of two clinical forms of gluten sensitivity led to the increasing use of the term gluten-sensitive enteropathy rather than celiac disease as the more inclusive name for the disease. Finally, during this early period of the study of GSE, it was also established that the offending protein in gluten causing GSE was the wheat prolamin known as gliadin; as shown later, similar components of rye and oat grains also cause GSE (Dicke et al., 1950).

In the 1960s and early 1970s, the first evidence that GSE was associated with gluten-specific immune dysfunction appeared in studies showing that patient mucosal tissue displayed evidence of increased immunologic activity, including increased numbers of plasma cells in lamina propria and increased intraepithelial cells (Eidelman et al., 1966; Ferguson and Murray, 1971). In addition, it was shown that high serum IgA levels prevalent in GSE tend to fall after the institution of a gluten-free diet (Asquith et al., 1969), and feeding of gluten to patients with quiescent disease leads to a prompt increase in IgA and IgM synthesis, some of which is gluten specific (Loeb et al., 1971). Finally, the first evidence that T-cell immunity might be involved in GSE appeared with a report from Ferguson et al. (1975) showing that lymphocytes from patients produce a cytokine upon exposure to gluten (migration-inhibition factor). At this point, strong evidence that the disease was in fact due to an immunologic abnormality was then provided by Falchuk et al. (1974) and Katz et al. (1976), who used organ culture techniques to show that gliadin was not directly toxic to patient tissue, but instead required the stimulation of an "endogenous mechanism," which results in the secretion of soluble mediators of villous atrophy and which is inhibitable by steroids. The "endogenous mechanism" was at that time assumed to be and was later proven to be an immunologic reaction resulting in the production of IFN-γ (Przemioslo et al., 1995).

These developments were now expanded, beginning in the early 1970s and extending into the 1980s, by the discovery that GSE is strongly associated with a particular set of MHC genes. The initial finding here was made by Falchuk et al. (1972), who showed that GSE is associated with the MHC class I gene encoding HLA-B8. This observation was later followed by those of Keuning et al. (1976) and Tosi et al. (1983), who demonstrated that GSE was associated with the MHC class II genes encoding HLA-DR3, HLA-DR7, and most importantly, HLA-DQ2.

Inflammatory Bowel Disease

The inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis, are commonly thought to have been

"discovered" relatively recently, that is, in the twentieth century. Review of the historical record, however, quickly discloses that although the prevalence of these diseases may have vastly increased during this period, the first cases were recognized hundreds of years ago and numerous cases were described in the British medical literature in the last half of the nineteenth century. Thus, as far as ulcerative colitis is concerned, the first clearly reported case can be traced back to Wilks and Moxon (1875), who described a young woman with ulcerations involving the entire colon and who ultimately died of the complications of bloody diarrhea. Over the next 25–40 years hundreds of cases of ulcerative diseases of the colon were reported, not only in Britain, but also in other European countries and ulcerative colitis was a major gastrointestinal disease at the time of the Congress of Medicine held in Paris in 1913. Similarly, with respect to Crohn's disease, the first case was reported by Wilks (1859) who described a 42-year-old woman with inflammation of both the colon and terminal ileum who died after several months with diarrhea and fever; this patient was initially said to have ulcerative colitis, but on reevaluation of the findings much later was found to have had Crohn's disease. Similar cases were reported by Fenwick (1889) and Dalziel (1913) on 13 patients with more or less classic findings of Crohn's disease, which were attributed to a mycobacterial agent other than *M. tuberculosis* (Tietze, 1920; Moschcowitz and Wilensky, 1923). In the ensuing 20 years, numerous instances of gastrointestinal disease resembling Crohn's disease were reported that finally crystallized the idea that Crohn's disease is a separate and unique disease entity. In 1932, two young physicians, an internist and a surgeon, presented findings related to what they proposed was a new clinical and pathologic entity: terminal ileitis with granulomatous inflammation. Ginzburg and Oppenheimer (1932) reported on 51 cases of granulomatous inflammation of the bowel that were not tuberculous, amebic, or syphilitic. They proposed six categories, including one with isolated terminal ileitis characterized by fissuring, longitudinal ulcers, granulomatous inflammation, stenotic bowel, and the propensity to fistulize. This series was published in 1932 in the Transactions of the American Gastroenterological Association with only Ginzburg and Oppenheimer as authors. One month later, Burrill B. Crohn presented 14 cases of pure ileitis and published a landmark paper describing the clinical, pathologic, radiographic, and therapeutic features of the disease. Cases from both studies were from the service of Dr A. A. Berg, a noted Mount Sinai surgeon (Chief of Service). Dr Crohn's paper, published in the Journal of the American Medical Association, received the critical acclaim and notice, hence the disease designation Crohn's disease. The initial presentation by Dr Ginzburg did not include Crohn's name and this has led to some debate regarding the appropriate naming of the disease. The Scots refer to terminal ileitis as Dalziel's disease, the world as Crohn's disease, and Ginzburg, until his death in the 1990s, as Ginzburg's disease. The Crohn et al. (1932) publication was able to establish a new disease entity and ultimately to provide its eponymous name, not because it contained a more extensive series of cases of chronic intestinal inflammation than earlier reports, but rather because it provided specific evidence that the inflammation was not due to a known infectious agent, particularly *M. tuberculosis*, and was therefore a new type of inflammatory bowel disease. Thus, it justifiably stands as a landmark in the history of gastrointestinal disease and mucosal immunopathology.

More complete clinical and pathologic characterization of ulcerative colitis and Crohn's disease followed the initial definition of these diseases as outlined previously. Ulcerative colitis was characterized as a relatively superficial disease usually beginning in the rectum and then extending proximally to involve the descending colon in some patients and the entire colon in others; in addition, the characteristic microscopic findings of the disease were identified including epithelial cell hyperplasia and goblet cell depletion, the presence of crypt abscesses, and a mixed lamina propria infiltrate of lymphocytes and eosinophils (Warren and Sommers, 1954). In contrast, Crohn's disease was defined by the presence of focal lesions of the small intestine, most commonly involving the terminal ileum but also frequently involving the ascending colon; furthermore, the lesions themselves were shown to be characterized by transmural thickening, luminal narrowing, fistula formation, and fibrosis (Warren and Sommers, 1948). Finally, Crohn's disease, on microscopic examination, was shown to be a granulomatous inflammation sometimes associated with the presence of giant cells, and although crypt abscesses were also present in Crohn's inflammation, overall granulocytic infiltration was far less prominent than in ulcerative colitis (Warren and Sommers, 1948; Rappaport et al., 1951). On the basis of these distinctive morphologic features, ulcerative colitis and Crohn's disease could clearly be defined as different pathologic entities. Nevertheless, they remained grouped as members of the inflammatory bowel disease spectrum because they both were idiopathic inflammations of the intestine without an obvious infectious etiology. In addition, they were found to be genetically related diseases in that patients with one of the forms of inflammatory bowel disease frequently had family members with the other form (Jackman and Bargen, 1942).

For many years, the cause of both ulcerative colitis and Crohn's disease was assumed to be infectious in nature and one after another candidate organism was championed as the causative agent. In the 1920s, for instance, diplostreptococci, organisms ordinarily found in the oral cavity, were considered the cause of ulcerative colitis, and in the ensuing decades, *Pseudomonas aeruginosa, E. coli, Entamoeba histolytica*, and *Chlamydia* were likewise considered. Later in the 1950s and 1960s, these bacterial and parasitic

candidate organisms lost favor—instead, various viruses were believed to be the etiologic agent. A similar pattern emerged for Crohn's disease beginning in the era before Crohn's report with the assumption that the disease was due to a mycobacterial infection; it was in fact the exclusion of mycobacterial infection by animal inoculation, syphilis by serologic testing, and actinomycosis by histologic findings, that allowed Crohn's disease to emerge as a separate entity (Crohn et al., 1932). This initial exclusion of an infectious etiology, however, did not stop the search for an infectious agent and in the period extending from 1952 to 1985, numerous organisms were proposed as causes of Crohn's disease including various bacterial, chlamydial, and viral organisms. The last enjoyed a particular vogue throughout the 1970s and into the 1980s, but was all but eliminated as a possibility by the inability to culture viral organisms from lesions (Phillpots et al., 1980). Of note, interest in the mycobacterial etiology of Crohn's disease resurfaced in the late 1970s and 1980s with the emergence of evidence that the disease was caused by an atypical cell wall–deficient mycobacterial species (Chiodini et al., 1984). Ultimately, however, this idea also failed because the putative organism could not be found in lesional tissues by sophisticated immunologic and culture techniques and because there was no evidence that the putative organism caused an immune response. The latter fact was particularly influential in light of the emerging belief among many students of the disease that IBD is basically an immunologic dysfunction.

The concept that IBD might be due to a nonallergic immunologic dysfunction was first seriously considered by Kirsner (1961), who conducted the first series of studies of a possible immunologic dysfunction in IBD, taking the approach of creating animal models of bowel inflammation that resembled IBD. One such model was created in rabbits and was based on the "Auer" procedure, which consisted of stimulating antibody responses to a given antigen and then inducing mucosal deposition of antigen–antibody complexes by subsequently applying the antigen to the colon that had been preexposed to formalin (Kraft et al., 1963). An inflammation was thereby achieved that resembled ulcerative colitis histologically, but which differed from ulcerative colitis in that it was self-limited. In later studies by Mee et al. (1979), a similar rabbit model was created, except for the fact that investigators preimmunized the animals with *E. coli*, a member of the normal mucosal microflora (Mee et al., 1979), and the ulcerative colitis-like disease obtained was persistent. Taken together, these experiments suggested that IBD may result from an initial insult, followed by an inappropriate and sustained immunologic response to normal flora. A similar conclusion can be drawn from the almost forgotten studies of Halpern et al. (1967), who induced chronic ulcerative colitis-like lesions in rats by injecting the latter with strains of live or dead *E. coli* in Freund's adjuvant. Interestingly, in this case, the colitis could be prevented by prefeeding with *E. coli*, which in retrospect suggests that induction of tolerance with the inducing antigen (by feeding) affected colitis production and that colitis was a result of a failure of mucosal immunoregulation.

Later studies of animal models, conducted in the 1970s, enlarged on the previous themes. In one model studied during this period, the contactant dinitrochlorobenzene was used to induce colitis, providing an early suggestion that T cells rather than B cells might be the key elements in the inflammatory response of IBD (Onderdonk et al., 1978). In another model, it was shown that in mice and other animals wherein colitis had been induced by carrageenan, the coadministration of metronidazol prevented colitis induction (Broberger and Perelman, 1959). This again suggested a role of intestinal microflora. Overall, these early animal studies presaged current concepts of IBD that hold that both ulcerative colitis and Crohn's disease are due to an abnormality of immunoregulation and an inappropriate response to antigens in the mucosa environment.

The 1950s and 1960s, in addition to the above described animal model work, saw the advent of the first studies of human IBD from an immunologic point of view. The pioneering work that was conducted by Broberger and Perlmann (1959) and their various colleagues provided evidence that patients with IBD, particularly those with ulcerative colitis, developed antibodies to gut constituents, either bacterial antigens or cross-reactive self-antigens present in epithelial cells. Later it became apparent that these "autoantibodies" were most likely not disease specific and probably occurred secondary to tissue injury; nevertheless, they paved the way to future studies showing that ulcerative colitis is associated with the production of particular autoantibodies such as antineutrophil cytoplasmic antibody (ANCA) and antitropomyosin.

Perlmann and Broberger (1963) and their colleagues also introduced the idea that IBD was characterized by the development of cytotoxic cells, which were ultimately shown by Shorter et al. (1970) to be natural killer (NK) cells capable of mediating antibody-dependent cell-mediated cytotoxic reactions against epithelial cells, perhaps in conjunction with the antiepithelial cell antibodies alluded to earlier (Perlmann and Broberger, 1963; Shorter et al., 1970). This cytotoxicity phenomenon also proved to be disease nonspecific, but was nevertheless important because it focused attention on cell-mediated immunologic processes as a cause of IBD. With these studies, the stage was now set for studies of T cells, first at the cellular level and later at the cytokine level (Hodgson et al., 1978; Elson et al., 1981). These, together with the newer animal models that have come along in the past decade, strongly suggest that ulcerative colitis and Crohn's disease represent different kinds of dysregulated mucosal immune responses induced by antigens in the normal microbiota.

CODA

As authors of this treatise, we are well aware of dangers inherent in writing historical reviews: inadvertent omission of some important articles, overemphasis of some but underappreciation of other contributions, and subjective differences in the perception or interpretation of published data. Nevertheless, we hope that ultimately this review, which covers relevant topics from ancient past to the late 1980s and early 1990s, will provide interesting and stimulating background information. We sincerely hope that the outstanding accomplishments of our predecessors and still active contemporaries who initiated research in this area and published their work more than 30 years ago will find appreciative readers.

REFERENCES

Adinolfi, M., Glynn, A.A., Lindsay, M., Milne, C.M., 1966. Serological properties of γA antibodies to *Escherichia coli* present in human colostrum. Immunology 10, 517–526.

Ambrus, M., Hernádi, E., Bajtai, G., 1977. Prevalence of HLA-A1 and HLA-B8 antigens in selective IgA deficiency. Clin. Immunol. Immunopathol. 7, 311–314.

Ammann, A.J., Hong, R., 1971. Selective IgA deficiency: presentation of 30 cases and a review of the literature. Medicine 50, 223–236.

Ammann, A.J., Stiehm, E.R., 1966. Immune globulin levels in colostrum and breast milk, and serum from formula- and breast-fed newborns. Proc. Soc. Exp. Biol. Med. 122, 1098–1100.

Archer, O.K., Sutherland, D.E.R., Good, R.A., 1963. Appendix of the rabbit: a homologue of the bursa in the chicken. Nature 200, 337–339.

Arnold, R.R., Cole, M.F., McGhee, J.R., 1977. A bactericidal effect for human lactoferrin. Science 197, 263–265.

Aschoff, L., 1926. Die lymphatischen Organe. Beihefte zur Medizinischen Klinik XXII Vol. I, 1–22 Berlin.

Asquith, P., Thompson, R.A., Cooke, W.T., 1969. Serum-immunoglobulins in adult coeliac disease. Lancet 2, 129–131.

Athavale, B., 1977. Bala-veda pediatrics and ayurveda. New Delhi. In: Proceedings of the XV International Congress of Pediatrics. Shilp Associates, Bombay, pp. 1–190.

Behring, von E., Kitasato, S., 1890. Ueber das zustandekommen der diphtheric immunität und der tetanus-immunität bei thieren. Dtsch. Med. Wochenschr. 16, 1113–1114.

Berg, R.D., Savage, D.C., 1975. Immune responses of specific pathogen-free and gnotobiotic mice to antigens of indigenous and nonindigenous microorganisms. Infect. Immun. 11, 320–329.

Berger, J., 1969. IgA glomerular deposits in renal disease. Transplant. Proc. 1, 939–944.

Berger, J., Hinglais, N., 1968. Les dépôts intercapillaires d'IgA-IgG. J. Urol. Néphrol. 74, 694–695.

Berkovich, S., Picketing, J.E., Kibrick, S., 1961. Paralytic poliomyelitis in Massachusetts, 1959. A study of the disease in a well vaccinated population. N. Engl. J. Med. 264, 1323–1326.

Besredka, A., 1909. De l'anaphylaxie. De l'anaphylaxie lactique. Ann. Inst. Pasteur 23, 166–176.

Besredka, A., 1919. De la vaccination contre les états typhoides par la voie buccale. Ann. Inst. Pasteur 33, 882–903.

Besredka, A., 1927. Local Immunization. Williams & Wilkins, Baltimore.

Bezkorovainy, A., Miller-Catchpole, R., 1989. Biochemistry and Physiology of Bifidobacteria. CRC Press, Boca Raton, FL, pp. 1–192.

Bienenstock, J., 1974. The physiology of the local immune response and the gastrointestinal tract. In: Brent, L., Holborow, J. (Eds.), Clinical Aspects I. Progress in Immunology, vol. 4. North Holland American Elsevier, Amsterdam, pp. 197–207.

Bienenstock, J., Johnston, N., Perey, D.Y.E., 1973a. Bronchial lymphoid tissue. I. Morphologic characteristics. Lab. Invest. 28, 686–692.

Bienenstock, J., Johnston, N., Perey, D.Y.E., 1973b. Bronchial lymphoid tissue. II. Functional characteristics. Lab. Invest. 28, 693–698.

Bienenstock, J., McDermott, M., Befus, D., 1979. A common mucosal immune system. In: Ogra, P.L., Dayton, D.L. (Eds.), Immunology of Breast Milk. Raven Press, New York, pp. 91–104.

Bienenstock, J., Strauss, H., 1970. Evidence for synthesis of human colostral IgA as 11S dimer. J. Immunol. 105, 274–277.

Bienenstock, J., Tomasi Jr., T.B., 1968. Secretory IgA in normal urine. J. Clin. Invest. 47, 1162–1171.

Björkander, J., Bake, B., Oxelius, V.A., Hanson, L.Å., 1985. Impaired lung function in patients with IgA deficiency and low levels of IgG2 or IgG3. N. Engl. J. Med. 313, 720–724.

Bockman, D.E., Cooper, M.D., 1973. Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study. Am. J. Anat. 136, 455–477.

Bockman, D.E., Stevens, W., 1977. Gut-associated lymphoepithelial tissue: bidirectional transport of tracer by specialized epithelial cells associated with lymphoid follicles. J. Reticuloendothel. Soc. 21, 245–254.

Bodian, D., 1949. Differentiation of types of poliomyelitis viruses. I. Reinfection experiments in monkeys (second attacks). Am. J. Hyg. 49, 200–224.

Bodian, D., 1961. Poliomyelitis immunization. Mass use of oral vaccine in the United States might prevent definitive evaluation of either vaccine. Science 134, 819–822.

Brandtzaeg, P., 1974. Mucosal and glandular distribution of immunoglobulin components: differential localization of free and bound SC in secretory epithelial cells. J. Immunol. 112, 1553–1559.

Brandtzaeg, P., 1978. Polymeric IgA is complexed with secretory component (SC) on the surface of human intestinal epithelial cells. Scand. J. Immunol. 8, 39–52.

Brandtzaeg, P., 1981. Transport models for secretory IgA and secretory IgM. Clin. Exp. Immunol. 44, 221–232.

Brandtzaeg, P., Prydz, H., 1984. Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. Nature 311, 71–73.

Bratanov, K., Geroff, K., Todorov, T., 1949. Sur la formation des spermoanticorps dans l'organisme des animaux domestiques. Ann. Acad. Rurale "G. Dimitrov" Fal. Med. Vet. 26, 321–334, Bulg. Res. Fr.

Broberger, O., Perlmann, P., 1959. Autoantibodies in human ulcerative colitis. J. Exp. Med. 110, 657–674.

Brodie, M., Park, W.H., 1936. Active immunization against poliomyelitis. Am. J. Public Health 26, 119–125.

Brown, W.R., Newcomb, R.W., Ishizaka, K., 1970. Proteolytic degradation of exocrine and serum immunoglobulins. J. Clin. Invest. 49, 1374–1380.

Buckley, R.H., Dees, S.C., 1969. Correlation of milk precipitins with IgA deficiency. N. Engl. J. Med. 281, 465–469.

Bull, C.G., McKee, C.M., 1929. Respiratory immunity in rabbits. VII. Resistance to intranasal infection in the absence of demonstrable antibodies. Am. J. Hyg. 9, 490–499.

Bunting, C.H., Huston, J., 1921. Fate of the lymphocyte. J. Exp. Med. 33, 593–600.

Burrows, W., Elliott, M., Havens, I., 1947. Studies on immunity in Asiatic cholera. The excretion of coproantibody in experimental enteric cholera in the guinea pig. J. Infect. Dis. 81, 261–281.

Burrows, W., Havens, I., 1948. Studies on immunity to Asiatic cholera. V. The absorption of immune globulin from the bowel and its excretion in the urine and feces of experimental animals and human volunteers. J. Infect. Dis. 82, 231–250.

Burrows, W., Ware, L.L., 1953. Studies on immunity to Asiatic cholera. VII. Prophylactic immunity to experimental enteric cholera. J. Infect. Dis. 92, 164–174.

Calmette, A., 1923. Les vaccinations microbiennes par voie buccale. Ann. Inst. Pasteur 37, 900–920.

Carbonara, A.O., Heremans, J.F., 1963. Subunits of normal and pathological g1A-globulins b2A-globulins. Arch. Biochem. Biophys. 102, 137–143.

Carter, P.B., Pollard, M., 1971. Host responses to "normal" microbial flora in germ-free mice. J. Reticuloendothel. Soc. 9, 580–587.

Cassidy, J.T., Petty, R.E., Sullivan, D.B., 1973. Abnormalities in the distribution of serum immunoglobulin concentrations in juvenile rheumatoid arthritis. J. Clin. Invest. 52, 1931–1936.

Cebra, J.J., Gearhart, P.J., Kamat, R., Robertson, S.M., Tseng, J., 1977. Origin and differentiation of lymphocytes involved in the secretory IgA response. In: Origins of Lymphocyte Diversity, Cold Spring Harbor Symp. Quant. Biol., vol. 41, pp. 201–215.

Chase, M.V., 1946. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc. Soc. Exp. Biol. Med. 61, 257–259.

Chiodini, R.J., Van Kruiningen, H.J., Thayer, W.R., Merkal, R.S., Coutu, J.A., 1984. Possible role of mycobacteria in inflammatory bowel disease. I. An unclassified *Mycobacterium* species isolated from patients with Crohn's disease. Dig. Dis. Sci. 29, 1073–1079.

Chodirker, W.B., Tomasi Jr., T.B., 1963. Gamma-globulins: quantitative relationships in human serum and nonvascular fluids. Science 142, 1080–1081.

Cohendy, M., 1912. Expériences sur la vie sans microbes. Compt. Rend. Acad. Sci. 154, 533–536.

Combiesco, D., Calab, G., 1924. De l'immunisation contre Staphylocoque pyogéne par la voie buccale, chez le lapin. Comp. Rend. Seanc. Soc. Biol. Fil. 91, 734–735.

Combiesco, D., Magheru, A., Calab, G., 1923. Vaccination preventive contre la dysentérie par la voie digestive, chez le lapin. Compt. Rend. Seanc. Soc. Biol. Fil. 88, 904–906.

Cooper, M.D., Perey, D.Y., McKneally, M.F., Gabrielsen, A.E., Sutherland, D.E.R., Good, R.A., 1966. A mammalian equivalent of the avian bursa of Fabricius. Lancet 1, 1388–1391.

Cornes, J.S., 1965. Number, size, and distribution of Peyer's patches in the human small intestine. Part I. The development of Peyer's patches. Gut 6, 225–233.

Crabbé, P.A., Bazin, H., Eyssen, H., Heremans, J.F., 1968. The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. Int. Arch. Allergy 34, 362–375.

Crabbé, P.A., Carbonara, A.O., Heremans, J.E., 1965. The normal human intestinal mucosa as a major source of plasma cells containing gA-immunoglobulin. Lab. Invest. 14, 235–248.

Crabbé, P.A., Heremans, J.F., 1966. Lack of gamma A-immunoglobulin in serum of patients with steatorrhoea. Gut 7, 119–127.

Crabbé, P.A., Nash, D.R., Bazin, H., Eyssen, H., Heremans, J.F., 1969. Antibodies of the IgA type in intestinal plasma cells of germfree mice after oral or parenteral immunization with ferritin. J. Exp. Med. 130, 723–744.

Crabbé, P.A., Nash, D.R., Bazin, H., Eyssen, H., Heremans, J.F., 1970. Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Lab. Invest. 22, 448–457.

Crago, S.S., Kulhavy, R., Prince, S.J., Mestecky, J., 1978. Secretory component on epithelial cells is a surface receptor for polymeric immunoglobulins. J. Exp. Med. 147, 1832–1837.

Craig, S.W., Cebra, J.J., 1971. Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. J. Exp. Med. 134, 188–200.

Crandall, R.B., Cebra, J.J., Crandall, C.A., 1967. The relative proportions of IgG-, IgA-, and IgM-containing cells in rabbit tissues during experimental trichinosis. Immunology 12, 147–158.

Crohn, B.B., Ginzburg, L., Oppenheimer, G.D., 1932. Regional ileitis: a pathologic and clinical entity. J. Am. Med. Assoc. 99, 1323–1329.

Cunningham-Rundles, C., Brandeis, W.E., Good, R.A., Day, N.K., 1978. Milk precipitins, circulating immune complexes, and IgA deficiency. Proc. Natl. Acad. Sci. U.S.A. 75, 3387–3389.

Cunningham-Rundles, C., Brandeis, W.E., Pudifin, D.J., Day, N.K., Good, R.A., 1981. Autoimmunity in selective IgA deficiency: relationship to anti-bovine protein antibodies, circulating immune complexes and clinical diseases. Clin. Exp. Immunol. 45, 299–304.

Cunningham-Rundles, C., Pudifin, D.J., Armstrong, D., Good, R.A., 1980. Selective IgA deficiency and neoplasia. Vox Sang. 38, 61–67.

Dakin, R., 1829. Remarks on a cutaneous affliction, produced by certain poisonous vegetables. Am. J. Med. Sci. 4, 98–100.

Dalziel, T.K., 1913. Chronic interstitial enteritis. Br. Med. J. 2, 1068–1070.

Davies, A., 1922. An investigation into the serological properties of dysentery stools. Lancet 2, 1009–1012.

Davies, P.J., 1991. Mozart's death: a rebuttal of Karhausen. Further evidence for Schönlein-Henoch syndrome. J. R. Soc. Med. 84, 737–740.

Dicke, W.K., 1941. Eenvoudig dieet bij het syndroom van Gee-Herter. N. T. Geneesk 85, 1715–1721.

Dicke, W.K., 1950. Coeliac Disease. Investigation of the Harmful Effects of Certain Types of Cereal on Patients with Coeliac Disease (Thesis). Utrecht, The Netherlands.

Dserzgowdky, S.K., 1910. Ueber die aktive Immunisierung des Menschen gegen Diphtherie. Zeitschr. Immunitätsforsh. Exp. Ther. 3, 602.

Du Bois, R.O., Schloss, O.M., Anderson, A.F., 1925. The development of cutaneous hypersensitivities following the intestinal absorption of antigenic protein. Proc. Soc. Exp. Biol. Med. 23, 176–180.

Dubos, R., Schaedler, R.W., Costello, R., Hoet, P., 1965. Indigenous, normal, and autochthonous flora of the gastrointestinal tract. J. Exp. Med. 12, 67–75.

Duncan, C.H., 1916. Autotherapy in ivy poisoning. N.Y. Med. J. 104, 901.

Eberth, C.J., 1864. Ueber den feineren Bau der Darmschteinhaut. Würzburger Natur. Wissenschaft. Zeitschr. 5, 22–33.

Ehrich, W., 1929. Studies of the lymphatic tissue. Am. J. Anat. 43, 347–371.

Ehrlich, P., 1891a. Experimentelle Untersuchungen über Immunität. I. Ueber Ricin. Dtsch. Med. Wochenschr. 17, 976–979.

Ehrlich, P., 1891b. Experimentelle Untersuchungen über Immunität. II. Ueber Abrin. Dtsch. Med. Wochenschr. 17, 1218–1219.

Ehrlich, P., 1892. Ueber Immunität durch Vererbung und Säugung. Zeitschr. Hyg. Infekt. Krankh. 12, 183–203.

Eidelman, S., Davis, S.D., Lagunoff, D., Rubin, C.E., 1966. The relationship between intestinal plasma cells and serum immunoglobulin A (IgA) in man. J. Clin. Invest. 45, 1003.

Elfenbein, G.J., Anderson, P.N., Humphrey, R.L., Mullins, G.M., Sensenbrenner, L.L., Wands, J.R., Santos, G.W., 1976. Immune system reconstitution following allogeneic bone marrow transplantation in man: a multiparameter analysis. Transplant. Proc. 8, 641–646.

Ellison, S.A., Mashimo, P.A., Mandel, I.D., 1960. Immunochemical studies of human saliva. I. The demonstration of serum proteins in whole and parotid saliva. J. Dent. Res. 39, 892–898.

Elson, C.O., Graeff, A.S., James, S.P., Strober, W., 1981. Covert suppressor T cells in Crohn's disease. Gastroenterology 80, 1513–1521.

Enders, J.F., 1952. General preface to studies on the cultivation of poliomyelitis viruses in tissue culture. J. Immunol. 69, 639–643.

Enders, J.F., Weller, T.H., Robbins, F.C., 1949. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. Science 109, 85–87.

Enlows, E.M.A., 1925. Vaccination by mouth against bacillary dysentery. Publ. Health Rep. 40, 639–649.

Escherich, T., 1888. The intestinal bacteria of the neonate and breast-fed infant. Rev. Infect. Dis. 10, 1220–1225.

Falchuk, Z.M., Gebhard, R.L., Sessoms, C., Strober, W., 1974. An in vitro model of gluten sensitive enteropathy: effect of gliadin on intestinal epithelial cells of patients with gluten sensitive enteropathy in organ culture. J. Clin. Invest. 53, 487–500.

Falchuk, Z.M., Rogentine, G.N., Strober, W., 1972. Predominance of histocompatibility antigen HLA-A8 in patients with gluten-sensitive enteropathy. J. Clin. Invest. 51, 1602–1606.

Fanger, M.W., Shen, L., Pugh, J., Bernier, G.M., 1980. Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. Proc. Natl. Acad. Sci. U.S.A. 77, 3640–3644.

Faulk, W.P., McCormick, J.N., Goodman, J.R., Yoffey, J.M., Fudenberg, H.H., 1970. Peyer's patches: morphologic studies. Cell. Immunol. 1, 500–520.

Fazekas de St. Groth, S., Donelley, M., 1950a. Studies in experimental immunology of influenza. III. Antibody response. Aust. J. Exp. Biol. Med. Sci. 28, 45–60.

Fazekas de St. Groth, S., Donelley, M., 1950b. Studies in experimental immunology of influenza IV. The protective value of active immunization. Aust. J. Exp. Biol. Med. Sci. 28, 61–75.

Feinstein, D., Franklin, E.C., 1966. Two antigenically distinguishable subclasses of human gA myeloma proteins differing in their heavy chains. Nature 212, 1496–1498.

Fenwick, S., 1889. Clinical Lectures on Some Obscure Disease of the Abdomen. T & A Churchill, London.

Ferguson, A., 1977. Intraepithelial lymphocytes of the small intestine. Gut 18, 921–937.

Ferguson, A., MacDonald, T.T., McClure, J.P., Holden, R.J., 1975. Cell-mediated immunity to gliadin within the small-intestinal mucosa in coeliac disease. Lancet 1, 895–897.

Ferguson, A., Murray, D., 1971. Quantitation of intraepithelial lymphocytes in human jejunum. Gut 12, 988–994.

Fichtelius, K.-E., 1967. The mammalian equivalent to bursa Fabricii of birds. Exp. Cell Res. 46, 231–234.

Fichtelius, K.-E., 1970. Cellular aspects on the phylogeny of immunity. Lymphology 3, 50–59.

Fishaut, M., Murphy, D., Neifert, M., McIntosh, K., Ogra, P.L., 1981. Bronchomammary axis in the immune response to respiratory syncytial virus. J. Pediatr. 99, 186–191.

Fleming, A., 1922. On a remarkable bacteriolytic element found in tissues and secretions. Proc. Roy. Soc. London Ser. B 39, 306–317.

Foo, M.C., Lee, A., 1972. Immunological response of mice to members of the autochthonous intestinal microflora. Infect. Immun. 6, 525–532.

Francis Jr., T., Brightman, I.J., 1941. Virus-inactivating capacity of nasal secretions in the acute and convalescent stages of influenza. Proc. Soc. Exp. Boil. Med. 48, 116–117.

Francis Jr., T., Pearson, H.E., Sullivan, E.R., Brown, P.M., 1943. The effect of subcutaneous vaccination with influenza virus upon the virus-inactivating capacity of nasal secretions. Am. J. Hyg. 37, 294–300.

Franklin, R.R., Dukes, C.D., 1964a. Further studies on sperm-agglutinating antibody and unexplained infertility. J. Am. Med. Assoc. 190, 682–683.

Franklin, R.R., Dukes, C.D., 1964b. Anti-spermatozoal antibody and unexplained infertility. Am. J. Obstet. Gynecol. 89, 6–9.

Fubara, E.S., Freter, R., 1973. Protection against enteric bacterial infection by secretory IgA antibodies. J. Immunol. 111, 395–403.

Fudenberg, H., German III, J.L., Kunkel, H.G., 1962. The occurrence of rheumatoid factor and other abnormalities in families of patients with agammaglobulinemia. Arthritis Rheumatol. 5, 565–588.

Fuhrman, J.A., Cebra, J.J., 1981. Special features of the priming process for a secretory IgA response: B cell priming with cholera toxin. J. Exp. Med. 153, 534–544.

Gay, F.P., 1924. Local resistance and local immunity to bacteria. Physiol. Rev. 4, 191–214.

Gerhard, W.W., 1837. On the typhus fever which occurred in Philadelphia in the Spring and Summer of 1836. Am. J. Med. Sci. 18, 289–322.

Gearhart, P.J., Cebra, J.J., 1979. Differentiated B lymphocytes: potential to express particular antibody variable and constant regions depends on site of lymphoid tissue and antigen load. J. Exp. Med. 149, 216–227.

Georgieva, R., 2012. History of Reproductive Immunology and the Contribution of Bulgaria. International Coordination Committee for Immunology of Reproduction, Sofia, Bulgaria.

Giedion, A., Scheidegger, J.J., 1957. Kongenitale Immunparese bei Fehlen spezifischer β_2-Globuline und quantitativ normalen γ-Globulinen. Helvet. Paediatr. Acta 12, 241–259.

Gilmore, R.M., 1911. Uses of plants by Indians. In: Thirty-third Annual Report. Department of Agriculture, Entomology and Plant Quarantine Review, 100.

Ginzburg, L., Oppenheimer, G.D., 1932. Non-specific granulomata of the intestine (Inflammatory tumors and structures of bowel). Trans. Am. Gastroenterol. Assoc. 35, 241–283.

Glick, B., Chang, T.S., Jaap, R.G., 1956. The bursa of Fabricius and antibody production. Poult. Sci. 35, 224–225.

Glimstedt, G., 1932. Das Leben ohne Bakterien. Sterile Aufziehnung von Meerschweinchen. Vehandl. Anat. Ges. Anat. Anz. 75, 78–89.

Goldblum, R.M., Ahlstedt, S., Carlsson, B., Hanson, L.Å., Jodal, U., Lidin-Janson, G., Sohl-Åkerlund, A., 1975. Antibody-forming cells in human colostrum after oral immunization. Nature 257, 797–799.

Goldner, J., 1929. Le probléme de la régénération de l'epithélium intestinal. Bull. Histol. Appliq. Physiol. 6, 79–95.

Götze, O., Müller-Eberhard, H.J., 1971. The C3-activator system: an alternative pathway of complement activation. J. Exp. Med. 134, 90s–108s.

Gowans, J.L., Knight, E.J., 1964. The route of re-circulation of lymphocytes in the rat. Proc. R. Soc. London Biol. 159, 257–282.

Gresham, G.E., Joseph, J.M., Farber, R.E., Silverman, C., 1962. Epidemic of type 3 paralytic poliomyelitis in Baltimore, Maryland, 1960. Public Health Rep. 77, 349–355.

Grey, H.M., Abel, C.A., Yount, W.J., Kunkel, H.G., 1968. A subclass of human gA-globulins (gA2) which lacks the disulfide bonds linking heavy and light chains. J. Exp. Med. 128, 1223–1236.

Griscelli, C., Vassalli, P., McCluskey, R.T., 1969. The distribution of large dividing lymph node cells in syngeneic recipient rats after intravenous injection. J. Exp. Med. 130, 1427–1451.

Grolnick, M., 1951. Studies on contact-dermatitis. VIII. The effect of feeding of antigen on the subsequent development of skin sensitization. J. Allergy 22, 170–174.

Gugler, E., von Muralt, G., 1959. Ueber immuno-elektrophoretische Untersuchungen an Frauenmilch-Proteinen. 2. Mitt. Schweiz. Med. Wochenschr 89, 925–929.

Guieysse-Pellissier, A., 1912. Caryanabiose et greffe nucleaire. Arch. Anat. Microscop. 13, 1–94.

Gustafsson, B., 1948. Germfree rearing of rats. Acta Pathol. Microbiol. Scand. 22, 1–132.

Hall, J.G., Parry, D.M., Smith, M.E., 1972. The distribution and differentiation of lymph-borne immunoblasts after intravenous injection into syngeneic recipients. Cell Tissue Kinet. 5, 269–281.

Hall, J.G., Smith, M.E., 1970. Homing of lymph-borne immunoblasts to the gut. Nature 226, 262–263.

Halpern, B., Zweibaum, A., Oriol Palau, R., Morard, J.C., 1967. Experimental immune ulcerative colitis. In: Miescher, P., Grabar, P. (Eds.), Immunopathology. International Symposium. Schwabe and Co, Basel, Switzerland, pp. 161–178.

Halpern, M.S., Koshland, M.E., 1970. Novel subunit in secretory IgA. Nature 228, 1276–1278.

Ham, A.W., 1969. Histology. JP Lippincott, Philadelphia. 313.

Hanson, L.Å., 1961. Comparative immunological studies of the immune globulins of human milk and of blood serum. Int. Arch. Allergy Appl. Immunol. 18, 241–267.

Hanson, L.Å., Adlerberth, I., Carlsson, B., Coffman, K., Dahlgren, U., Nilsson, K., Jalil, F., Roberton, D., 1988. Mucosal immunity—from past to present. Monogr. Allergy 24, 1–8.

Hanson, L.Å., Björkander, J., Oxelius, V.A., 1983. Selective IgA deficiency. In: Chandra, R. (Ed.), Primary and Secondary Immunodeficiency Disorders. Churchill-Livingston, Edinburgh, Scotland, pp. 62–84.

Hanson, L.Å., Johansson, B., 1961. Immunological characterization of chromatographically separated protein fractions from human colostrum. Int. Arch. Allergy Appl. Immunol. 20, 65–79.

Harries, E.H.R., Mitman, M., with collaboration of Taylor, I, 1951. Clinical Practice in Infectious Diseases, 448. Williams & Wilkins, Baltimore.

Heberden, W., 1801. Commertarii di Marlbaun. Historia et Curatione. Payne, London.

Henoch, E.H.H., 1874. Ueber eine eigenthümliche Form von Purpura. Berl. Klin. Wochenschr. 51, 641–643.

Heremans, J.F., 1959. Immunochemical studies on protein pathology. The immunoglobulin concept. Clin. Chim. Acta 4, 639–646.

Heremans, J.F., 1974. Immunoglobulin A. In: Sela, M. (Ed.), The Antigens, vol. 2. Academic Press, New York, pp. 365–522.

Heremans, J.F., Heremans, M.T., Schultze, H.E., 1959. Isolation and description of a few properties of the b_2A-globulin of human serum. Clin. Chim. Acta 4, 96–102.

Heremans, J.F., Vaerman, J.-P., Vaerman, C., 1963. Studies on the immune globulins of human serum. II. A study of the distribution of anti-Brucella and anti-diphtheria antibody activities among g_{ss}- g_{1M}- and g_{1A}-globulin fractions. J. Immunol. 91, 11–17.

Hill, I.R., Porter, P., 1974. Studies of bactericidal activity to *Escherichia coli* of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. Immunology 26, 1239–1250.

Hodgson, H.J., Wands, J.R., Isselbacher, K.J., 1978. Decreased suppressor cell activity in inflammatory bowel disease. Clin. Exp. Immunol. 32, 451–458.

Hummel, K.P., 1935. The structure and development of the lymphatic tissue in the intestine of the albino rat. Am. J. Anat. 57, 351–383.

Ingerslev, H.J., 1980. Sperm agglutinating antibodies and sperm penetration in cervical mucus from infertile women with sperm agglutinating antibodies in serum. Fertil. Steril. 34, 561–568.

Ingerslev, H.J., Møller, N.P., Jager, S., Kremer, J., 1982. Immunoglobulin class of sperm antibodies in cervical mucus from infertile women. Am. J. Reprod. Immunol. 2, 296–300.

Isojima, S., Li, T.S., Ashitaka, Y., 1968. Immunologic analysis of sperm-immobilizing factor found in sera of women with unexplained sterility. Am. J. Obstet. Gynecol. 101, 677–683.

Isojima, S., Graham, R.M., Graham, J.B., 1959. Sterility in female guinea pigs induced by injection with testis. Science 129, 44.

Jackman, R.J., Bargen, J.A., 1942. Familial occurrence of chronic ulcerative colitis (thrombo ulcerative colitis). Am. J. Dig. Dis. Nutr. 9, 147–149.

Jackson, G.D.F., Lemaitre-Coelho, I., Vaerman, J.-P., 1977. Transfer of MOPC-315 IgA to secretions in MOPC-315 tumor-bearing and normal BALB/c mice. Protides Biol. Fluids 25, 919–922.

Jacobson, L.O., Marks, E.K., Simmons, E.L., Gaston, E.O., 1961. Immune response in irradiated mice with Peyer's patch shielding. Proc. Soc. Exp. Biol. Med. 108, 487–493.

Jelliffe, D.B., Jelliffe, E.F.P., 1977. Current concepts in nutrition. "Breast is best": modern meanings. N. Engl. J. Med. 297, 912–915.

Jenner, E., 1798. An Inquiry into the Causes and Effects of the Variolae Vaccinae, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire, and Known by the Name of the Cow Pox. Sampson Low, London.

Jervis, G.A., Koprowski, H., McGee, E.L., Nelsen, D.J., Norton, T.W., Stokes Jr., J., 1956. Immunization of children by the feeding of living attentuated type I and type II poliomyelitis virus and the intramuscular injection of immune serum globulin. Am. J. Med. Sci. 232, 378–388.

Joel, D.D., Sordat, B., Hess, M.W., Cottier, H., 1970. Uptake and retention of particles from the intestine by Peyer's patches in mice. Experientia 26, 694.

Jolly, J., 1913. Sur les organes lymphoepitheliaux. Comp. Rend. Soc. Biol. T 74, 540–543.

Katz, A.J., Falchuk, Z.M., Strober, W., Shwachman, H., 1976. Gluten-sensitive enteropathy. Inhibition by cortisol of the effect of gluten protein in vitro. N. Engl. J. Med. 295, 131–135.

Keuning, J.J., Pena, A.S., van Leeuwen, A., van Hooff, J.P., van Rood, J.J., 1976. HLA-DW3 associated with coeliac disease. Lancet 1, 506–508.

Kilian, M., Mestecky, J., Schrohenloher, R.E., 1979. Pathogenic species of the genus *Haemophilus* and *Streptococcus pneumoniae* produce immunoglobulin A1 protease. Infect. Immun. 26, 143–149.

Kim, Y.B., 1979. Role of antigen in ontogeny of the immune response. In: Schlessinger, D. (Ed.), Microbiology 1979. American Society for Microbiology, Washington, D.C., pp. 343–348.

Kirsner, J.B., 1961. Experimental "colitis" with particular reference to hypersensitivity reactions in the colon. Gastroenterology 40, 307–312.

Klaasen, H.L., Van der Heijden, P.J., Stok, W., Poelma, F.G., Koopman, J.P., Van den Brink, M.E., Bakker, M.H., Eling, W.M., Beynen, A.C., 1993. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. Infect. Immun. 61, 303–306.

Klein, E., 1875. The Anatomy of the Lymphatic System. II. The Lung. Smith and Elder, London.

Klemperer, G., 1892. Untersuchungen über künstlichen Impfschutz gegen Choleraintoxication. Berl. Klin. Wochenschr. 29, 789–793.

Klingman, A.M., 1958. Poison ivy (Rhus) dermatitis. Arch. Dermatol. 77, 149–180.

Koch, R., 1885. Zweiter Konferenz zur Erörterung der Cholerafrage. Dtsch. Med. Wochenschr. 37A, 1–60.

Koistinen, J., 1975. Selective IgA deficiency in blood donors. Vox Sang. 29, 192–202.

Kolmer, J.A., Klugh Jr., G., Rule, A.M., 1935. A successful method for vaccination against acute anterior poliomyelitis. J. Am. Med. Assoc. 104, 456–460.

Koprowski, H., Jervis, G.A., Norton, T.W., 1952. Immune responses in human volunteers upon oral administration of a rodent-adapted strain of poliomyelitis virus. Am. J. Hyg. 55, 108–126.

Koprowski, H., 1956. Immunization against poliomyelitis with living attenuated virus. Am. J. Trop. Med. Hyg 5, 440–452.

Korenblat, P.E., Rothberg, R.M., Minden, P., Farr, R.S., 1968. Immune responses of human adults after oral and parenteral exposure to bovine serum albumin. J. Allergy 41, 226–235.

Koshland, M.E., Burrows, W., 1950. Quantitative studies of the relationship between fecal and serum antibody. J. Immunol. 65, 93–103.

Kraft, S.C., Fitch, F.W., Kirsner, J.B., 1963. Histologic and immunohistochemical features of the Auer "colitis" in rabbits. Am. J. Pathol. 43, 913–927.

Kraus, F.W., Sirisinha, S., 1962. Gamma globulin in saliva. Arch. Oral. Biol. 7, 221–233.

Kunkel, H.G., Prendergast, R.A., 1966. Subgroups of γA immune globulins. Proc. Soc. Exp. Biol. Med. 122, 910–913.

Kunkel, H.G., Smith, W.K., Joslin, F.G., Natvig, J.B., Litwin, S.D., 1969. Genetic marker of the gA2 subgroup of gA immunmoglobulins. Nature 223, 1247–1248.

Landsteiner, K., 1899. Zur Kenntnisse der spezifisch auf Blutkörperchen wirkenden Sera. Zbl. Bakt. 25, 546–549.

LaPlane, R., Burtin, P., Curioni, S., Houet, C., Graveleau, D., LeBalle, J.C., 1962. Sur un cas d'agammaglobulinemie atypique. Etude biologique et génétique. Ann. Pediat. 38, 281–290.

Lane, W.A., 1926. The prevention of the disease. Surg. Gynecol. Obstet. 42, 196–208.

Lawrence, D.A., Weigle, W.O., Spiegelberg, H.L., 1975. Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. J. Clin. Invest. 55, 368–376.

Lawton 3rd, A.R., Mage, R.G., 1969. The synthesis of secretory IgA in the rabbit. I. Evidence for synthesis as an 11S dimer. J. Immunol. 102, 693–697.

Leidy, J., 1849. On the existence of entophyta in healthy animals as a natural condition. Proc. Acad. Natl. Sci. Philadelphia 4, 225–233.

Lemaitre-Coelho, I., Jackson, G.D.F., Vaerman, J.-P., 1977. Rat bile as a convenient source of secretory IgA and free secretory component. Eur. J. Immunol. 7, 588–590.

Loeb, P.M., Strober, W., Falchuk, Z.M., Laster, L., 1971. Incorporation of L-leucine-^{14}C into immunoglobulins by jejunal biopsies of patients with celiac sprue and other gastrointestinal diseases. J. Clin. Invest. 50, 559–569.

Lowney, E.D., 1968. Immunologic unresponsiveness to a contact sensitizer in man. J. Invest. Dermatol. 51, 411–417.

Lowney, E.D., 1973. Suppression of contact sensitization in man by prior feeding of antigen. J. Invest. Dermatol. 61, 90–93.

Mach, J.P., 1970. In vitro combination of human and bovine free secretory component with IgA of various species. Nature 228, 1278–1282.

Magnus, W., 1906. Weitere Ergebnisse der Serum-Diagnostik fur die Theoretische und angewandte Botanik. Ber. Deut. Bot. Gesell 26a, 532–539.

Male, C., 1979. Immunoglobulin A1 protease production by *Haemophilus influenzae* and *Streptococcus pneumoniae*. Infect. Immun. 26, 254–261.

Maliszewski, C.R., March, C.J., Schoenborn, M.A., Gimpel, S., Shen, L., 1990. Expression cloning of a human Fc receptor for IgA. J. Exp. Med. 172, 1665–1672.

Masaki, S., 1922. Du mécanisme de l'infection cholérique et de la vaccination contré le choléra par voie buccale. Compt. Rend. Seanc. Soc. Biol. Filial. 86, 532–534.

Masson, P.L., Heremans, J.F., 1971. Lactoferrin in milk from different species. Comp. Biochem. Physiol. B 39, 119–129.

McCarthy, D.M., Katz, S.I., Gazze, L., Waldmann, T.A., Nelson, D.L., Strober, W., 1978. Selective IgA deficiency associated with total villous atrophy of the small intestine and an organ-specific anti-epithelial cell antibody. J. Immunol. 120, 932–938.

McDermott, M.R., Bienenstock, J., 1979. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. J. Immunol. 122, 1892–1898.

McDermott, M.R., Clark, D.A., Bienenstock, J., 1980. Evidence for a common mucosal immunologic system. II. Influence of the estrous cycle on B immunoblast migration into genital and intestinal tissues. J. Immunol. 124, 2536–2539.

McWilliams, M., Phillips-Quagliata, J.M., Lamm, M.E., 1975. Characteristics of mesenteric lymph node cells homing to gut-associated lymphoid tissue in syngeneic mice. J. Immunol. 115, 54–58.

McWilliams, M., Phillips-Quagliata, J.M., Lamm, M.E., 1977. Mesenteric lymph node B lymphoblasts which home to the small intestine are precommitted to IgA synthesis. J. Exp. Med. 145, 866–875.

Mee, A.S., McLaughlin, J.E., Hodgson, H.J.F., Jewell, D.P., 1979. Chronic immune colitis in rabbits. Gut 20, 1–5.

Mehta, S.K., Plaut, A.G., Calvanico, N.J., Tomasi Jr., T.B., 1973. Human immunoglobulin A: production of an Fc fragment by an enteric microbial proteolytic enzyme. J. Immunol. 111, 1274–1276.

Mellander, L., Björkander, J., Carlsson, B., Hanson, L.Å., 1986. Secretory antibodies in IgA deficient and immunosuppressed individuals. J. Clin. Immunol. 6, 284–291.

Mestecky, J., Kulhavy, R., Kraus, F.W., 1972. Studies on human secretory immunoglobulin A. II. Subunit structure. J. Immunol. 108, 738–747.

Mestecky, J., McGhee, J.R., Arnold, R.R., Michalek, S.M., Prince, S.J., Babb, J.L., 1978. Selective induction of an immune response in human external secretions by ingestion of bacterial antigen. J. Clin. Invest. 61, 731–737.

Mestecky, J., Zikan, J., Butler, W.T., 1971. Immunoglobulin M and secretory immunoglobulin A: presence of a common polypeptide chain different from light chains. Science 171, 1163–1165.

Metchnikoff, E., 1899. Études sur la résorption des cellules. Ann. Inst. Pasteur. 13, 737–769.

Metchnikoff, E., 1903. In: Mitchell, P.C. (Ed.), The Nature of Man. G. P. Putman, New York, pp. 72–73.

Metchnikoff, E., 1908. In: Mitchell, P.C. (Ed.), The Prolongation of Life. G. P. Putman, New York, pp. 58–83, 150–183.

Michalek, S.M., McGhee, J.R., Mestecky, J., Arnold, R.R., Bozzo, L., 1976. Ingestion of *Streptococcus mutans* induces secretory IgA and caries immunity. Science 192, 1238–1240.

Mitsuya, H., Osaki, K., Tomino, S., Katsuki, T., Kishimoto, S., 1981. Pathophysiologic analysis of peripheral blood lymphocytes from patients with primary immunodeficiency. I. Ig synthesis by peripheral blood lymphocytes stimulated with either pokeweed mitogen or Epstein-Barr virus in vitro. J. Immunol. 127, 311–315.

Miyakawa, M., Iijima, S., Kishimoto, H., Kobayashi, R., Tajima, M., Isomura, N., Asano, M., Hong, C., 1958. Rearing germfree guinea pigs. Acta Pathol. Jpn. 8, 55.

Moghissi, K.S., Sacco, A.G., Borin, K., 1980. Immunologic infertility. I. Cervical mucosa antibodies and postcoital test. Am. J. Obstet. Gynecol. 136, 941–950.

Monteiro, R.C., Kubagawa, H., Cooper, M.D., 1990. Cellular distribution, regulation, and biochemical nature of an Fcα receptor in humans. J. Exp. Med. 171, 597–613.

Montgomery, P.C., Connelly, K.M., Cohn, J., Skandera, C.A., 1978. Remote-site stimulation of secretory IgA antibodies following bronchial and gastric stimulation. Adv. Exp. Med. Biol. 107, 113–122.

Montgomery, P.C., Rosner, B.R., Cohn, J., 1974. The secretory antibody response. Anti-DNP antibodies induced by dinitrophenylated type 3 pneumococcus. Immunol. Commun. 3, 143–156.

Morgan, I.M., 1948. Immunization of monkeys with formalin-inactivated poliomyelitis viruses. Am. J. Hyg. 48, 394–406.

Moschcowitz, E., Wilensky, A.D., 1923. Non-specific granulomata of the intestine. Am. J. Med. Sci. 166, 48–66.

Mowat, A.M., Ferguson, A., 1981. Hypersensitivity in the small intestinal mucosa. V. Induction of cell-mediated immunity to a dietary antigen. Clin. Exp. Immunol. 43, 574–582.

Nagura, H., Nakane, P.K., Brown, W.R., 1979. Translocation of dimeric IgA through neoplastic colon cells in vitro. J. Immunol. 123, 2359–2368.

Norman, J.M., 1991. Garrison and Morton's Medical Bibliography. Scolar Press, Menston, England.

Nuttall, G.H.F., Thierfelder, H., 1895. Thierisches Leben ohne Bakterien im Verdauungskanal. Z. Physiol. Chem. 21, 109–121.

O'Daly, J.A., Cebra, J.J., 1971. Chemical and physicochemical studies of the component polypeptide chains of rabbit secretory immunoglobulin A. Biochemistry 10, 3843–3850.

Ogra, P.L., Karzon, D.T., 1969. Poliovirus antibody response in serum and nasal secretions following intranasal inoculation with inactivated poliovaccine. J. Immunol. 102, 15–23.

Ogra, P., Karzon, D., 1971. Formation and function of poliovirus antibody in different tissues. Prog. Med. Virol. 13, 156–193.

Ogra, P.L., Karzon, D.T., Righthand, F., MacGillivray, M., 1968. Immunoglobulin response in serum and secretions after immunization with live and inactivated poliovaccine and natural infection. N. Engl. J. Med. 279, 893–900.

Onderdonk, A.B., Hermos, J.A., Dzink, J.L., Bartlett, J.G., 1978. Protective effect of metronidazole in experimental ulcerative colitis. Gastroenterology 74, 521–526.

Oram, J.D., Reiter, B., 1966. The inhibition of streptococci by lactoperoxidase, thiocyanate and hydrogen peroxide. The effect of the inhibitory system on susceptible and resistant strains of group N streptococci. Biochem. J. 100, 373–381.

Owen, R.L., 1977. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. Gastroenterology 72, 440–451.

Owen, R.L., Jones, A.L., 1974. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology 66, 189–203.

Oxelius, V.A., Laurell, A.B., Lindquist, B., Golebiowska, H., Axelsson, U., Bjorkander, J., Hanson, L.Å., 1981. IgG subclasses in selective IgA deficiency: importance of IgG2-IgA deficiency. N. Engl. J. Med. 304, 1476–1477.

Parish, W.E., Ward, A., 1968. Studies of cervical mucus and serum from infertile women. J. Obstet. Gynecol. Br. Commonw. 75, 1089–1100.

Park, R.G., 1944. Sulphonamide allergy. The persistence and desensitization. BMJ 2, 816–817.

Parrott, D., MacDonald, T.T., 1990. The ontogeny of the mucosal immune system in rodents. In: MacDonald, T.T. (Ed.), Ontogeny of the Immune System of the Gut. CRC Press, Boca Raton, FL, pp. 51–67.

Pasteur, L., 1880. De l'attenuation du virus du choléra des poules. Compt. Rend. Acad. Sci. 91, 673–680.

Paulley, L.W., 1954. Observation on the aetiology of idiopathic steatorrhoea jejunal and lymph-node biopsies. Br. Med. J. 2, 1318–1321.

Pechlin, J.N., 1672. De Purgantium Medicamentorvm Facultatibus Exercitatio Nova. Apud Danielem, Abrahamum & Adrianum a Gaasbeek. Leyden, Amsterdam, The Netherlands.

Pereira, S., Webster, D., Platts-Mills, T., 1982. Immature B cells in fetal development and immunodeficiency: studies of IgM, IgG, IgA, and IgD production in vitro using Epstein-Barr virus activation. Eur. J. Immunol. 12, 540–546.

Perlmann, P., Broberger, O., 1963. In vitro studies in ulcerative colitis. II. Cytotoxic action of white blood cells from patients on human fetal colon cells. J. Exp. Med. 117, 717–733.

Peyer, J.C., 1677. Exercitatio Anatomico-Medica de Usu et Affectionibus. Cui Subjungitur Anatome Ventriculi Galliinacei. Onophrius et Waldkirch, Schaffhausen.

Peyer, J.C., 1681. Exercitatio Anatomico-Medica de Glandulis Intestinorum, Earumque Usu et Affectionibus. Cui Subjungitur Anatome Ventriculi Gallinacei. H. Wetstenium, Amsterdam.

Phillpots, R.J., Hermon-Taylor, J., Teich, N.M., Brooke, B.N., 1980. A search for persistent virus infection in Crohn's disease. Gut 21, 202–207.

Pierce, N.F., Gowans, J.L., 1975. Cellular kinetics of the intestinal immune response to cholera toxoid in rats. J. Exp. Med. 142, 1550–1563.

Plaut, A.G., Gilbert, J.V., Artenstein, M.S., Capra, J.D., 1975. *Neisseria gonorrhoeae* and *Neisseria meningitidis*: extracellular enzyme cleaves human immunoglobulin A. Science 190, 1103–1105.

Plaut, A.G., Wistar Jr., R., Capra, J.D., 1974. Differential susceptibility of human IgA immunoglobulins to streptococcal IgA protease. J. Clin. Invest. 54, 1295–1300.

Poger, M.E., Lamm, M.E., 1974. Localization of free and bound secretory component in human intestinal epithelial cells. A model for the assembly of secretory IgA. J. Exp. Med. 139, 629–642.

Pollard, M., Sharon, S., 1970. Responses of the Peyer's patches in germfree mice to antigenic stimulation. Infect. Immun. 2, 96–100.

Pruitt, K.M., Adamson, M., 1977. Enzyme activity of salivary lactoperoxidase adsorbed to human enamel. Infect. Immun. 17, 112–116.

Przemioslo, R.T., Lundin, K.E., Sollid, L.M., Nelufer, J., Ciclitira, P.J., 1995. Histological changes in small bowel mucosa induced by gliadin sensitive T lymphocytes can be blocked by anti-interferon gamma antibody. Gut 36, 874–879.

Radl, J., Klein, F., van den Berg, P., de Bruyn, A.M., Hijmans, W., 1971. Binding of secretory piece to polymeric IgA and IgM paraproteins in vitro. Immunology 20, 843–852.

Radl, J., Schuit, H.R.E., Mestecky, J., Hijmans, W., 1974. The origin of monomeric and polymeric forms of IgA in man. Adv. Exp. Med. Biol. 45, 57–65.

Rappaport, H., Burgoyne, F.H., Smetana, H.F., 1951. The pathology of regional enteritis. Milit. Surg. 109, 463–488.

Reinach, T., 1890. Mithridate Eupator. Roi de Pont. Firmin-Didot, Paris, pp. 283–285, 409–410.

Rejnek, J., Travnicek, J., Kostka, J., Sterzl, J., Lanc, A., 1968. Study of the effect of antibodies in the intestinal tract of germ-free baby pigs. Folia Microbiol. 13, 36–42.

Report of the Commission on the Cost of Medical Care, 1964. The economic significance of the prevention of paralytic poliomyelitis 1955–1961. J. Am. Med. Assoc. 29–46.

Reyniers, J.A., 1932. The use of germfree guinea pigs in bacteriology. Proc. Indiana Acad. Sci. 42, 35–37.

Robertson, S.M., Cebra, J.J., 1976. A model for local immunity. Ricerca Clin. Lab. 6, 105–119.

Rockey, J.H., Hanson, L.Å., Heremans, J.F., Kunkel, H.G., 1964. Beta-2A-aglobulinemia in two healthy men. J. Lab. Clin. Med. 63, 205–212.

Rosenau, M.J., Anderson, J.F., 1907. Further studies upon hypersusceptibility and immunity. J. Med. Res. 16, 381–418.

Ross, V., 1930. Oral immunization against pneumococcus. Use of bile salt dissolved organisms, etc., time of appearance of immunity and dosage. J. Exp. Med. 51, 585–607.

Roux, M.E., McWilliams, M., Phillips-Quagliata, J.M., Weisz-Carrington, P., Lamm, M.E., 1977. Origin of IgA-secreting plasma cells in the mammary gland. J. Exp. Med. 146, 1311–1322.

Rudzik, O., Clancy, R.L., Perey, D.Y.E., Day, R.P., Bienenstock, J., 1975. Repopulation with IgA-containing cells of bronchial and intestinal lamina propria after transfer of homologous Peyer's patch and bronchial lymphocytes. J. Immunol. 114, 1599–1604.

Rűmke, P., 1954. The presence of sperm antibodies in the serum of two patients with oligospermia. Vox Sang. 4, 135–140.

Sabin, A.B., 1955. Characteristics and genetic potentialities of experimentally produced and naturally occurring variants of poliomyelitis virus. Ann. N. Y. Acad. Sci. 61, 924–938.

Salk, J.E., 1953. Principles of immunization as applied to poliomyelitis and influenza. Am. J. Public Health 43, 1384–1398.

Sanders, A.G., Florey, H.W., 1940. The effects of the removal of lymphoid tissue. Br. J. Exp. Pathol. 21, 275–287.

Savilahti, E., Pelkonen, P., 1979. Clinical findings and intestinal immunoglobulins in children with partial IgA deficiency. Acta Paediatr. Scand. 68, 513–519.

Schaedler, R.W., Dubos, R., Costello, R., 1965a. The development of the bacterial flora in the gastrointestinal tract of mice. J. Exp. Med. 122, 59–66.

Schaedler, R.W., Dubos, R., Costello, R., 1965b. Association of germ-free mice with bacteria isolated from normal mice. J. Exp. Med. 122, 77–83.

Schaffner, T., Mueller, J., Hess, M.W., Cottier, H., Sordat, B., Ropke, C., 1974. The bursa of Fabricius: a central organ providing for contact between the lymphoid system and intestinal content. Cell Immunol. 13, 304–312.

Schmidt, J.E., 1959. Medical Discoveries: Who and When. Charles C. Thomas, Springfield, IL.

Schönlein, J.L., 1837. Allgemeine und specielle Pathologie und Therapie. Herisan, Würzburg, Germany.

Schottelius, M., 1899. Die Bedeutung der Darmbakterien für die Ernahrung. Arch. Hyg. 34, 210–243.

Shelmire, B., 1941. Hyposensitization of poison ivy. Arch. Dermatol. Syphilol. 44, 983–998.

Shiner, M., Doniach, I., 1960. Histopathologic studies in steatorrhea. Gastroenterology 38, 419–440.

Shorter, R.G., Huizenga, K.A., ReMine, S.G., Spencer, R.J., 1970. Effects of preliminary incubation of lymphocytes with serum on their cytotoxicity for colonic epithelial cells. Gastroenterology 58, 843–850.

Shuster, S., Watson, A.J., Marks, J., 1968. Coeliac syndrome in dermatitis herpetiformis. Lancet 2, 1101–1106.

Shvartsman, Ya S., Zykov, M.P., 1976. Secretory anti-influenza immunity. Adv. Immunol. 22, 291–330.

Silvers, S.H., 1941. Stomatitis venenata and dermititis of anal orifice from chewing poison leaves (Rhus toxicodendron). J. Am. Med. Assoc. 116, 2257.

Slater, R.J., Ward, S.M., Kunkel, H.G., 1955. Immunological relationships among the myeloma proteins. J. Exp. Med. 101, 85–108.

Smith, C.B., Bellanti, J.A., Chanock, R.M., 1967. Immunoglobulins in serum and nasal secretions following infection with type 1 parainfluenza virus and injection of inactivated vaccines. J. Immunol. 99, 133–141.

Smith, J.S., 1991. Patenting the Sun: Polio and Salk Vaccine. Anchor Books Doubleday, New York.

Smits, B.J., 1989. History of coeliac disease. Br. Med. J. 298, 387.

Sørensen, M., Sørensen, P.M.L., 1939. The Proteins in Whey. C. R. Lab. Carlsberg. 23, 55.

Sorvari, T., Sorvari, R., Ruotsalainen, P., Toivanen, A., Toivanen, P., 1975. Uptake of environmental antigens by the bursa of Fabricius. Nature 253, 217–219.

South, M.A., Cooper, M.D., Wollheim, F.A., Hong, R., Good, R.A., 1966. The IgA system. I. Studies of the transport and immunochemistry of IgA in the saliva. J. Exp. Med. 123, 615–627.

Spiegelberg, H.L., Lawrence, D.A., Henson, P., 1974. Cytophilic properties of IgA to human neutrophils. Adv. Exp. Med. Biol. 45, 67–74.

Sterzl, J., Mandel, L., Stepankova, R., 1987. The use of gnotobiological models for the studies of immune mechanisms. Nahrung 31, 599–608.

Sterzl, J., Silverstein, A.M., 1967. Developmental aspects of immunity. Adv. Immunol. 6, 337–459.

Stevens, F.A., 1945. Council on pharmacy and chemistry. Report of the council: status of poison ivy extracts. J. Am. Med. Assoc. 127, 912–921.

Sudo, N., Shulman, S., Stone, M.L., 1977. Antibodies to spermatozoa. IX. Sperm-agglutination phenomenon in cervical mucus in vitro: a possible cause of infertility. Am. J. Obstet. Gynecol. 129, 360–367.

Sulzberger, M.B., 1930. Arsphenamine hypersensitivity in guinea-pigs. II. Experiments demonstrating the role of the skin, both as originator and as site of hypersensitiveness. Arch. Dermatol. Syphilol. 22, 839–849.

Talbott, J.H., 1970. A Biographical History of Medicine. Grune & Stratton, New York. 734–736.

Terry, W.D., Roberts, M.S., 1966. Antigenic heterogeneity of human immunoglobulin A proteins. Science 153, 1007–1008.

Thucydides, 1951. The Complete Writings of Thucydides: The Peloponnesian War. The Modern Library, New York (Finley, J.H. Jr., Trans.), 112.

Tietze, A., 1920. Ueber entzundliche Dickdarmgeschwulste. Ergeb. Chir. Orthop. 12, 211–273.

Tlaskalová, H., Stepánková, R., 1980. Development of antibody formation in germ-free and conventionally reared rabbits: the role of intestinal lymphoid tissue in antibody formation to *E. coli* antigens. Folia Biol. 26, 81–93.

Tlaskalová, H., Sterzl, J., Hájek, P., Pospísil, M., Ríha, I., Marvanová, H., Kamarytová, V., Mandel, L., Kruml, J., Kováru, F., 1970. The development of antibody formation during embryonal and postnatal periods. In: Sterzl, J., Ríha, I. (Eds.), Developmental Aspects of Antibody Formation and Structure. Academia Publishing House, Prague, pp. 767–790.

Tomasi Jr., T.B., Tan, E.M., Solomon, A., Prendergast, R.A., 1965. Characteristics of an immune system common to certain external secretions. J. Exp. Med. 121, 101–124.

Tomasi Jr., T.B., Zigelbaum, S.O., 1963. The selective occurrence of g1A globulins in certain body fluids. J. Clin. Invest. 42, 1552–1560.

Tosi, R., Vismara, D., Tanigaki, N., Ferrara, G.B., Cicimarra, F., Buffolano, W., Follo, D., Auricchio, S., 1983. Evidence that celiac disease is primarily associated with a DC locus allelic specificity. Clin. Immunol. Immunopathol. 28, 395–404.

Tourville, D.R., Adler, R.H., Bienenstock, J., Tomasi Jr., T.B., 1969. The human secretory immunoglobulin system: immunohistological localization of gA, secretory "piece," and lactoferrin in normal human tissues. J. Exp. Med. 129, 411–429.

Trávnícek, J., Kovaru, F., Mandel, L., Stozicky, V., Kruml, J., 1975. The delivery of piglets for germfree rearing by caesarean section in a closed system. Acta Vet. 33, 297.

Trnka, Z., Sterzl, J., Lanc, A., Mandel, L., 1959. Natural and experimental infections in agammaglobulinemic piglets. Giorn. Mal. Infet. Parasit. 11, 330.

Vaerman, J.P., Heremans, J.F., 1966. Subclasses of human immunoglobulin A based on differences in the alpha polypeptide chains. Science 153, 647–649.

Vaillant, L., 1922. Note sur l'emploi du vaccine bilié de Besredka par la voie buccale dans quelques foyers épidémiques de fiévre typhoïde. Ann. Inst. Pasteur 36, 149–156.

Van de Kamer, J.H., Weijers, H.A., Dicke, W.K., 1953. Coeliac disease. IV. An investigation into the injurious constituents of wheats in connection with their action on patients with coeliac disease. Acta Paediatr. 42, 223–230.

Van Thiel, D.H., Smith Jr., W.I., Rabin, B.S., Fisher, S.E., Lester, R., 1977. A syndrome of immunoglobulin A deficiency, diabetes mellitus, malabsorption, a common HLA haplotype. Immunologic and genetic studies of forty-three family members. Ann. Intern. Med. 86, 10–19.

Voisin, G., DeLaunay, A., Barber, M., 1951. Sur des lésions testiculaires provoquées chez le cobaye par iso- et auto-sensibilisation. Ann. Inst. Pasteur 81, 48–63.

Waksman, B.H., Ozer, H., 1976. Specialized amplification elements in the immune system. The role of nodular lymphoid organs in the mucosal membranes. Prog. Allergy 21, 1–113.

Waldman, R.H., Kasel, J.A., Fulk, R.V., Mann, J.J., Togo, Y., Hornick, R.B., Heiner, G.T., Dawkins, A.T., 1968. Influenza antibody in human respiratory secretions after subcutaneous or respiratory immunization with inactivated virus. Nature 218, 594–595.

Walker, W.A., Isselbacher, K.J., Bloch, K.J., 1972. Intestinal uptake of macromolecules: effect of oral immunization. Science 177, 608–610.

Warren, S., Sommers, S.C., 1948. Cicatrizing enteritis (regional ileitis) as a pathologic entity: analysis of 120 cases. Am. J. Pathol. 24, 475–497.

Warren, S., Sommers, S.C., 1954. Pathology of regional ileitis and ulcerative colitis. J. Am. Med. Assoc. 154, 189–193.

Weber, E.H., 1847. Ueber den Mechanismus der Einsaugung des Speisesaftes beim Menschen und bei einigen Tieren. Arch. Anat. Physiol. Wissensch. Med. 7, 400–402.

Weisz-Carrington, P., Roux, M.E., McWilliams, M., Phillips-Quagliata, J.M., Lamm, M.E., 1978. Hormonal induction of the secretory immune system in the mammary gland. Proc. Natl. Acad. Sci. U.S.A. 75, 2928–2932.

Weisz-Carrington, P., Roux, M.E., McWilliams, M., Phillips-Quagliata, J.M., Lamm, M.E., 1979. Organ and isotype distribution of plasma cells producing specific antibody after oral immunization: evidence for a generalized secretory immune system. J. Immunol. 123, 1705–1708.

Wells, H.G., Osborne, T.B., 1911. The biological reactions of the vegetable proteins. I. Anaphylaxis. J. Infect. Dis. 8, 66–124.

Wells, G.G., Osborne, T.B., 1911. The biological reactions of the vegetable protein. I. Anaphylaxis. J. Infect. Dis 8, 66–124.

West, C.D., Hong, R., Holland, N.H., 1962. Immunoglobulin levels from the newborn period to adulthood and in immunoglobulin deficiency states. J. Clin. Invest. 41, 2054–2064.

Wilks, S., 1859. Morbid appearances in the intestine of Miss Bankes. London Med. Gazzette 2, 264.

Wilks, S., Moxon, W., 1875. Lectures on Pathological Anatomy. Lindsay & Blakiston.

Williams, R.C., Gibbons, R.J., 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. Science 177, 697–699.

Wilson, L., 1954. Sperm agglutinins in human semen and blood. Proc. Soc. Exp. Biol. Med. 85, 652–655.

Wolf-Heidegger, G., 1939. Zur Frage der Lymphocytenwanderung durch das Darmepithel. Zeitschr. Mikrosc. Anat. Forsch. 45, 90–103.

Chapter 1

Overview: The Mucosal Immune System

Michael W. Russell
Departments of Microbiology/Immunology and Oral Biology, University at Buffalo, Buffalo, NY, USA

Jiri Mestecky
Departments of Microbiology and Medicine, University of Alabama, Birmingham, AL, USA; Institute of Microbiology, Czech Academy of Sciences and Department of Immunology and Microbiology, School of Medicine, Charles University, Prague, Czech Republic

Warren Strober
Chief, Mucosal Immunity Section, Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Bart N. Lambrecht
VIB Inflammation Research Center, Ghent University, and Department of Respiratory Medicine, University Hospital Ghent, Ghent, Belgium

Brian L. Kelsall
Mucosal Immunobiology Section, Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Hilde Cheroutre
Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA

Chapter Outline

INTRODUCTION

The mucosal immune system has been referred to as the local immune system, a term that implies regional restriction and belittles its actual importance. In reality, the primary force that has driven the development and stimulation of the entire immune system during evolution as well as in ontogeny—and continues to do so in everyday life—is the external environment comprising the resident, highly complex mucosal microbiota, antigens of food origin or from inhaled air, environmental xenobiotics, and potential pathogens and their products. The evolutionary selective pressure of these environmental antigens has resulted in the strategic distribution throughout the mucosae of cells involved in the uptake, processing, and presentation of antigens and in the production of humoral and cellular factors of innate and specific immunity. Quantitative evaluation of macrophages, dendritic cells (DC), T and B lymphocytes, innate lymphoid cells (ILCs), and other lineages in the mucosal immune system reveals their superior numbers as well as their distinct phenotypic and functional heterogeneity. These cells work together to contain the vast onslaught of environmental antigens without compromising the integrity of the mucosal barrier while preventing overstimulation of the immune system. This goal is achieved by concerted interactions between the immune cells and phenotypically and functionally diverse epithelial cells that cover the enormous surface areas of the mucosal membranes.

Mucosal Immunology. http://dx.doi.org/10.1016/B978-0-12-415847-4.00001-X

MUCOSAL MICROBIOTA

One of the most significant developments in biomedicine in recent years is the realization that the resident mucosal microbiota has an extensive and vital interaction with the mucosae, especially in the gastrointestinal tract. Because this is an extremely complex system involving huge numbers of organisms and microbiological taxa—most of which have not been cultivated and are known only from metagenomic sequencing studies—much remains to be examined in detail. However, it is already clear that the microbiome, and both quantitative and qualitative variations in its composition between and within individuals over time, are associated with a range of health conditions. In addition, herbivores, which derive a large proportion of their caloric intake from microbial fermentation of cellulose in the foregut or hindgut, are absolutely dependent on a symbiotic microbiota. The mucosal immune system has the primary role in interacting with the microbiota, in a bidirectional manner and on a continuous basis, in the maintenance of homeostasis, health, and survival. It can be argued that this is the pre-eminent function of the mucosal immune system, and indeed of the entire immune system, given that the great majority of immune cells and molecules, including immunoglobulins, cytokines, innate defense proteins, and so forth, are positioned at the mucosal interface with the microbiota. From this perspective, an individual constitutes an ecosystem comprising the complex microbiota within the host body, and the mucosal immune system is responsible for maintaining the interdependence of these two parts.

On top of this, the great majority of infectious agents either directly afflict or gain access to the internal tissues of the body through the mucosal surfaces, by ingestion, inhalation, or sexual contact. Thus, the mucosal immune system has evolved to discriminate between essentially harmless environmental antigens, such as food, commensal or symbiotic microbes, and dangerous pathogens or toxins, and to mount the appropriate responses.

A great deal of recent study on the function of the mucosal immune system has centered on the interaction between the mucosal microbiota (or microbiome) and the normal mucosal immune system, as well as the abnormal immune system present in disease states. In the realm of the normal mucosal immune system, we have the exciting finding that a bacterial species known as the segmented filamentous bacterium (SFB) normally populates the murine small intestine in close physical association with intestinal epithelial cells. At this site, it induces the production of cytokines by lamina propria cells including interleukin (IL)-17, which then affect the host defense and the development of autoimmune disease. SFB is an uncultivable commensal organism related to the genus *Clostridium* that depends on the luminal environment for essential nutrients. It is likely that its interaction with epithelial cells results in the production of a unique set of cellular products that either induce or sustain the IL-17 response. Whether similar or related organisms exist in humans remains to be seen.

In the realm of the diseased mucosal immune system, there is now a large body of data supporting the idea that the microbiome in inflammatory bowel disease contains bacteria that either prevent or, conversely, cause the disease. Perhaps the most striking of the findings in this area is the demonstration that members of the *Clostridium* genus have the capacity to induce regulatory T cells in the lamina propria, most likely through the production of short-chain fatty acids that facilitate the induction of regulatory T cells by immunosuppressive cytokines, such as transforming growth factor (TGF)-β. On the other side of the coin are studies showing that an adherent-invasive biotype of *Escherichia coli* is present in the small intestine of patients with Crohn's disease, and that this organism plays a role in disease pathogenesis. Overall, these studies of the microbiome are providing very solid evidence that commensal bacteria have a profound impact on mucosal immune function and, as such, warrant continued study.

It has long been known that animals raised in a germ-free environment have minimally developed immune systems, in the circulatory (systemic) as well as mucosal compartments. The finding that immune functionality is incompletely restored by the introduction of a conventional microbiota after weaning implies that important interactions occur between the microbiota and immune system during and after the neonatal period. Some of the details of these processes have begun to be elucidated; for example, it was found that a specific capsular polysaccharide of *Bacteroides fragilis* is able to restore immune development in germ-free mice, as well as the role of SFB referred to above. Much anecdotal information, especially in the popular media, ascribes beneficial health effects to the consumption of "probiotic" food products such as yogurt, although the scientific basis for these supposed benefits has been sparse. Further studies may reveal how certain specific microbes present in foods elicit desirable responses from the enteric immune system, or conversely suppress undesirable responses.

EPITHELIAL CELLS AS ESSENTIAL PARTNERS OF THE MUCOSAL IMMUNE SYSTEM

In contrast to secondary lymphoid organs, such as lymph nodes and spleen, a distinctive feature of mucosal tissues is the intimate, extensive, and functionally interdependent collaboration between the immune system and epithelial cells. Epithelial cells used to be regarded merely as passive barrier cells that prevented the transit of antigens and pathogens contained in the lumen of the gut, lung, and genitourinary tract. This barrier function depends on intercellular junctions that maintain epithelial cohesiveness,

and maintenance of this barrier is critical for health. Epithelial cells are heterogenous and some are specialized for producing mucus as a vital physicochemical barrier. In the respiratory and genitourinary tracts, epithelial cells are also endowed with beating cilia, which together with mucus form the mucociliary blanket, which is essential for pulmonary defense and reproduction.

We now know, however, that epithelia are more than just passive barriers, and they express a range of innate pattern recognition receptors that recognize microbe-associated molecular patterns. Interactions of environmental antigens of microbial origin with various epithelial cells and underlying adaptive and innate lymphocytes and DCs are instrumental in both inductive and effector phases of the immune response, manifested by the development of humoral and cellular immunity as well as by oral tolerance. It is noteworthy that lymphocyte–epithelial cell interactions are also fundamental to the development of the T-cell repertoire in the thymus, where T cells are selected through contact with epithelium derived from embryonic pharyngeal pouches.

In the gastrointestinal tract, a single layer of columnar epithelial cells is all that separates the internal milieu from the external environment. Consequently, the immune apparatus associated with mucosal inductive sites must be able to respond effectively to potentially pathogenic challenge, while preventing overstimulation of the entire immune system by the commensal microbiota and the large mass of essentially harmless food antigens—minute but immunologically significant quantities of which are absorbed in an undigested form. This entails noninflammatory defense mechanisms that minimize tissue damage, as well as the capacity to distinguish between nonaggressive antigenic stimuli that are always present and pathogens that may appear suddenly. In the lung, epithelial cells are similarly essential for discriminating inhaled antigens and allergens from potentially dangerous pathogens. Immune and inflammatory events are tightly controlled by anti-inflammatory mechanisms that prevent overt damage to the delicate gas-exchange apparatus of the lung alveolo-capillary membrane, again made up of only a thin layer of alveolar epithelial cells, basement membrane, and thin capillary endothelium.

Epithelial cells are also a source of numerous diverse humoral factors of innate immunity and are an integral component of the regulatory cytokine network. Human intestinal and lung epithelial cell lines have the potential to produce an array of cytokines and chemokines that play essential roles in the influx, activation, and differentiation of myeloid and lymphoid cells; bacterial products or invasion of epithelia by bacteria and viruses enhance their production. Whereas initially they express proinflammatory cytokines, subsequently with the onset of specific immune responses, epithelial cells produce cytokines that are important in the differentiation of B cells toward immunoglobulin (Ig) A synthesis. The differentiation of mucosal B cells toward IgA synthesis may therefore be fostered by epithelial cells acting in concert with local T cells and driven by the microbiota. Immunosuppressive cytokines, such as TGF-β and IL-10, mediate oral tolerance, which is important in preventing overreaction of the entire immune system to the myriad of essentially harmless environmental antigens. Intraepithelial T cells, which in mice constitute ~50% of the entire T-cell population, are in the most intimate contact with epithelial cells. The production of antimicrobial and other factors resulting from these interactions is instrumental in the regulation of ensuing immune responses and in the maintenance of the epithelial barrier, the maturation and differentiation of epithelial cells, and their expression of membrane receptors and antigen-presenting molecules, including the polymeric immunoglobulin receptor (pIgR) and class II major histocompatibility complex (MHC) molecules.

At the effector sites where mucosal immune responses are expressed, cooperation between epithelial and lymphoid cells is essential for the selective transepithelial transport of polymeric IgA. Epithelial cells of mucosal surfaces and secretory glands express pIgR, which is specific for J chain-containing polymeric IgA (and IgM), and is required for the transcytosis of large quantities of IgA produced locally by resident plasma cells (~5 g/day in humans) across the epithelia to form S-IgA. During the transport process, epithelial cells contribute secretory component (SC), the extracellular part of pIgR, to this hybrid S-IgA molecule, reinforcing the intrinsic resistance of IgA to proteolysis and, due to its high content of glycan chains, participating in the inhibition of microbial adherence. The IgA and IgM transport process is of additional functional importance in the elimination of absorbed antigens as a means of noninflammatory antigen disposal.

During their short lifespan (3–4 days) and rapid proliferation, intestinal epithelial cells display a highly dynamic phenotype and radically change their immunological function from the transport and assembly of S-IgA in the crypts to the expression of MHC class II molecules and the processing and presentation of antigens as they mature and migrate up the villi. The level of expression of both pIgR and MHC class II is regulated by cytokines produced by T cells in their vicinity.

The epithelium has a special role in the inductive sites of the mucosal immune system. These sites include the gut-associated lymphoid tissues (GALT), such as Peyer's patches and other similarly organized lymphoid follicles, and the nasopharynx-associated lymphoid tissues (NALT) represented in humans by Waldeyer's ring of tonsils and adenoids.

ANTIGEN UPTAKE AND PRESENTATION IN THE MUCOSAL IMMUNE SYSTEM

The close proximity of the outside world and the microbiota has resulted in the development of a selective

antigen-sampling machinery in the mucosa, while keeping barrier function intact. Specialized M cells in the epithelium overlying the GALT and NALT follicles sample antigens by endocytosing bacteria, viruses, and macromolecules. These are passed to the underlying follicles where immune responses (or tolerance) are initiated and cells are dispersed to both mucosal and systemic immune compartments. Studies in vitro have suggested that absorptive enterocytes can also take up soluble antigens for intracellular processing and presentation in association with surface class I or class II MHC antigens to intraepithelial or subepithelial T cells, thereby eliciting immune responses or, in some cases, tolerance. DCs located within the lamina propria or between basal epithelial cells protrude pseudopods into the lumen to sample antigens, in a process involving activation and regulation by epithelial cells. Epithelial integrity is maintained by the formation of tight junctions with neighboring epithelial cells. After antigen recognition, DCs migrate to the draining regional lymph nodes, Peyer's patches, or to localized tertiary lymphoid follicles. Different types of DC and macrophages occur in mucosal tissues, depending on their location and source from which they are recruited. Especially in the gut, microbiota and other environmental stimuli influence the recruitment and differentiation of these antigen-presenting cells, which in turn impact on the induction of T cells and the type of immune responses that ensue.

MUCOSAL T CELLS AND CELL-MEDIATED IMMUNITY

For a long time, mucosal immunology was dominated by a focus on S-IgA antibodies, and comprehension of cell-mediated immunity at mucosal surfaces was lacking. That situation has changed dramatically in recent years. The Th1/Th2 paradigm that previously governed the biology of CD4+ T-helper (Th) cells was expanded by the description of the Th17 subset in 2005; it has since been further extended with additional lineages or differentiation states of CD4+ T cells. Through the production of their characteristic cytokines, including IL-17 and IL-22, Th17 cells have a major effect on mucosal defense by stimulating the secretion of innate antimicrobial proteins and peptides by epithelial cells, and by recruiting neutrophils through the downstream induction of chemokines. It also appears that Th17 cells may facilitate production and transport of S-IgA by enhancing the differentiation of IgA-secreting B cells and upregulating epithelial cell expression of pIgR.

T cells, including a significant population of T-cell receptor (TCR)-γδ T cells as well as MHC class I- and class II-restricted TCR-αβ T cells, form the main component of cell-mediated immunity at mucosal surfaces. In addition to a role in epithelial repair, TCR-γδ T cells located within the epithelium possess innate and adaptive cytotoxic mechanisms. The majority of CD8αβ$^+$ TCR-αβ epithelial T cells are long-lived, tissue-resident, memory cytotoxic T lymphocytes (CTL) that can be rapidly reactivated to provide immediate cytotoxic responses against infections. MHC class II-restricted CD4+ Th cells are less frequent in the mucosa, although under inflammatory conditions they can increase greatly in number and contribute significantly to the pathology. However, not all CD4$^+$ epithelial T cells are Th cells, as many display a cytotoxic phenotype and constitute an important arm of mucosal defense, especially against virally infected epithelial cells that constitutively express MHC class II. This protective capacity becomes critical when surveillance by conventional CD8αβ+ CTL has been evaded or in chronic infections when the CD8αβ+ CTL become exhausted. A significant population of CD4/CD8 double-negative TCRαβ T cells has been found, but their function and antigen-specificity remain elusive.

MUCOSAL IMMUNITY AND HOMEOSTASIS

The defense of mucosal surfaces must be accomplished without impairment of their structural and functional integrity. The mucosal immune system has therefore evolved to limit the challenge of microorganisms and environmental molecules perpetually present at mucosal surfaces and prevent them from gaining ingress or causing overstimulation of the immune system. Thus, regulation of responses, immunological tolerance, and noninflammatory effector mechanisms are hallmarks of mucosal immunity. Nevertheless, sophisticated and sometimes unique mechanisms exist for the recognition of potential pathogens or transformed cells, and responding to them requires the ability to distinguish not merely between self and nonself (the classical view of immune discrimination), but further between the normal microbiota (which is continually present but is usually nonthreatening) and the sporadic appearance of pathogens that pose a threat. Commensal organisms that do not invade or damage the epithelium, as well as food substances, either evoke minimal immune responses or induce tolerance, or else they redirect the functional differentiation of responding immune cells to differentiate into cytotoxic T cells and become part of the resident protective immune system. Experimental oral administration of large amounts of novel nonviable antigens usually results in modest immune responses that do not persist, or else induces oral tolerance. Potent mucosal immunogens are those such as live organisms that invade the mucosa (e.g., poliovirus, *Salmonella*) or toxins that activate epithelial cells (e.g., cholera and related enterotoxins).

Huge quantities of S-IgA are generated daily in humans: in the gut alone, this has been estimated at up to 5 g per day, making IgA by far the most abundantly produced immunoglobulin isotype. The concept that S-IgA antibodies defend the mucosae by inhibiting the adherence of microorganisms to the epithelial surfaces has developed concomitantly with

recognition that adherence is an essential first step in microbial pathogenesis, and mucosal antibodies are uniquely adapted to fulfill this role. Structural complementarity of the component chains of S-IgA or S-IgM molecules forms the basis of unique cooperation of cells engaged in the assembly of S-IgA. Polymeric IgA (pIgA) with an incorporated J chain produced by large numbers of subepithelial plasma cells is selectively transported through the epithelial cells. The basolateral epithelial receptor, pIgR, specifically interacts with pIgA and becomes covalently bonded to form S-IgA. Characteristic structural features of S-IgA support its biological function. Thus, the four and eight antigen-binding sites in the dimeric and tetrameric molecules, respectively, generate markedly enhanced avidity due to the bonus effect of multivalency despite low intrinsic affinity. Furthermore, S-IgA also exhibits a high degree of polyreactivity toward broadly related antigens. In addition to the specific antibody activity, S-IgA with heavily glycosylated H chains and SC reacts with a broad spectrum of bacteria and viruses through glycan-mediated interactions, leading to the inhibition of microbial adherence to complementary glycan receptors on epithelial cells. This may also contribute to the formation of bacterial biofilms, particularly in the large intestine. Consequently, S-IgA displays concomitant protective and enhancing functions that favor colonization with commensal microbes. Mucosal homeostasis and containment of the abundant microbiota are maintained by the concerted interaction of noninflammatory S-IgA antibodies, which are resistant to proteolytic enzymes in the secretions, with anti-inflammatory cytokines produced by epithelial cells and regulatory T cells.

In addition to IgA antibodies, it is now clear that T regulatory (Treg) cells are induced to differentiate primarily in the GALT and mesenteric lymph nodes (MLN), at least in part in response to commensal bacteria. Their importance is evident from findings that mice lacking sufficient Tregs develop intestinal inflammation. Furthermore, ILCs, a population overlooked until recently, are a major source of intestinal IL-17 and IL-22, which play an important role in epithelial cell growth and restitution, as well as the production of antimicrobial peptides and proteins that control commensal bacteria. Thus, in addition to IgA, Tregs and ILCs have emerged as essential components of the mucosal immune system that maintain immunological homeostasis in the intestine.

UNIQUE FEATURES OF INDIVIDUAL COMPONENTS OF THE MUCOSAL IMMUNE SYSTEM AND THEIR INTEGRATION

The mucosal immune system comprises anatomically remote and physiologically distinct compartments to provide protection relevant at the ocular, nasopharyngeal, respiratory, oral, gastrointestinal, and genitourinary mucosae as well as mammary glands. Although humoral and cellular antigen-specific immune responses can be induced by the direct local application of antigens to selected mucosal membranes, the ensuing responses are usually of low magnitude and remain restricted to the application site. The seminal discovery of mucosal inductive and effector sites with their characteristic cell populations revealed the communication networks that are essential for the induction of generalized immune responses that are manifested in parallel at anatomically distant mucosal tissues and glands. In turn, these concepts have resulted in novel immunization strategies now being exploited with mucosally administered vaccines. Induction of responses in secretory glands that are not directly stimulated by mucosal antigens, such as the mammary gland, is of paramount importance for the survival of the species, because the spectrum of specific antibodies and perhaps T cells in milk reflects maternal exposure to environmental antigens, thereby conferring passive protection to the newborn. The important concept of the common mucosal immune system extends also to the lacrimal, salivary, and genital tract glands, whose secretions contain antibodies to antigens present in the respiratory and intestinal tracts due to the dissemination of cells from inductive to effector mucosal sites.

GLOBAL IMPACT OF MUCOSAL IMMUNITY IN VACCINE INITIATIVES

The magnitude and extent of the mucosal immune system and its strategic location at the most frequent sites of infection offer hitherto underappreciated scope for exploitation to manipulate immune responses with vaccines, or alternatively to induce tolerance. Most current vaccines administered by systemic routes inadequately elicit protective immunity at the mucosae. Yet secretory antibodies and mucosal effector T cells are amenable to the induction of desired responses by utilizing mucosal routes of antigen delivery. The spectrum of vaccines comprises almost all leading communicable diseases (respiratory tract infections, diarrheal diseases, tuberculosis, measles, pertussis, meningitis, and sexually transmitted infections) responsible for high morbidity and mortality, particularly in developing countries, as well as a large number of veterinary vaccines. Furthermore, mucosal immunization that inhibits asymptomatic carriage of pathogens at mucosal surfaces would reduce the incidence of community-acquired disease and benefit even unimmunized individuals on the principle of herd immunity. Goals include eliciting persistent or recallable immunity to infections, not only by means of mucosal or circulating antibodies but also by inducing CTL and other cell-mediated immune defense mechanisms.

However, most nonviable antigens, especially in soluble form, are poorly immunogenic because they are readily digested in the gastrointestinal tract and have little or no tropism for the mucosal inductive sites. Numerous strategies have

therefore been proposed to overcome these limitations. These include a variety of antigen-delivery systems based on nonviable microparticles that are readily taken up by the inductive sites of the mucosal immune system, as well as live attenuated bacteria or viruses that invade through these inductive sites. Such bacteria and viruses can also be genetically engineered to express unrelated antigens, thereby theoretically creating broad-spectrum multivalent vaccines. These strategies also offer the advantage of providing some degree of protection for the vaccine antigens against destruction by gastric acid and digestive enzymes. Considerable effort has been devoted to the investigation of adjuvants suitable for mucosal administration and that enhance mucosal immune responses to coadministered vaccines. Notable among these are cholera toxin and related heat-labile enterotoxins derived from enteric bacteria, all of which demonstrate vigorous immuno-enhancing properties in experimental animals by various routes of administration. Although the inherent toxicity of these materials for humans presents a problem, several effective solutions have been devised by creating nontoxic mutants or subcomponents that retain adjuvant activity. Certain of these approaches have been examined in clinical trials with various degrees of success, so that it is possible that some might become available in vaccines within the foreseeable future. Other interesting strategies have included the development of vaccines expressed in edible plant material. However, despite the immediate appeal of this approach, difficulties include the limited bioavailability of expressed antigens that may be retained within indigestible structures, such as seeds, the low and variable level of antigen expression and hence uncertainty over vaccine dosage, and the destruction of antigens if the food is subjected to cooking or other processing. Nevertheless, further developments in this direction can be anticipated.

THE FUTURE

The enormous potential for exploitation of the mucosal immune system in medicine has recently received increased attention, particularly in the immunoregulation of desired responses. The attractive properties and advantages of mucosal vaccines have resulted in the establishment of several commercial institutions focusing on the development of vaccines against microbial antigens and allergens applied by mucosal routes (nasal, sublingual, and oral) using proprietary vaccine delivery systems and mucosal adjuvants. Anticipated target vaccines include those effective in the prevention of infectious disease of the respiratory, gastrointestinal, and genital tracts (e.g., respiratory syncytial virus, new influenza viruses, pneumococcus, diarrheal diseases, human immunodeficiency virus). Promising results generated in animal models in the prevention or treatment of several autoimmune diseases based on induction of mucosal tolerance, however, have met with limited success in human trials. Such outcomes should not have been unforeseen, as mucosal tolerance can be readily induced in immunologically naïve animals that have not been previously exposed to the relevant antigen, but less effectively in those with an ongoing systemic immune response. However, efforts to exploit mucosal tolerance for clinical benefit are continuing and novel approaches, including the use of immunoregulatory cytokines, delivery systems, and adjuvants, are being pursued.

We now understand in much greater detail how microbiota are recognized and contribute to health and disease. Large-scale microbiome projects are currently underway to relate information on microbial composition and functional characteristics with the genetic makeup of the host and gene expression data in health and disease. These studies will shed greater light on the complex interaction of the mucosal immune system with the environment. Many mucosal diseases, such as asthma and Crohn's disease, are on the rise in the Western industrialized world, possibly due to conditions of increased hygiene, alterations in diet, and diminished infectious pressure from the environment. Understanding how infectious pressure shapes homeostasis in the mucosal immune system holds the key to finding preventive strategies for these common diseases that have assumed epidemic proportions.

Chapter 2

Development and Physiology of the Intestinal Mucosal Defense

Hai Ning Shi and W. Allan Walker

Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Chapter Outline

DEVELOPMENT OF MUCOSAL DEFENSE

Prenatal Development of the Intestinal Mucosa

The form and function of intestine changes throughout our lifetime. This dynamic process can be influenced by genetic and environmental factors. The development and maturation of intestinal mucosal immunity is first initiated in the intrauterine environment. The early growth and development of intestine may have significant consequences on the responsiveness of the gut to physiological and pathogenic challenges later in life. There is good evidence that the fetal mucosal immune system is capable of mounting a response, which may be stimulated through intrauterine infection and possibly as an anti-idiotypic response to maternal antibody. Premature babies older than 28 weeks' gestation are capable of mounting an effective mucosal immune response at birth (Gleeson and Cripps, 2004). At approximately 100 days of gestation, immunoglobulin (Ig)M/IgD/CD5-positive cells can be detected in the human fetal intestine, indicating that the B cell maturation process starts (Spencer et al., 1986; Gleeson and Cripps, 2004). At 120 days of gestation, IgA expression occurs, and at approximately 130–140 days of gestation, primary B cell follicles, T cell zones with high endothelial venules, a dome region, and follicle-associated epithelium can be observed (reviewed in Gleeson and Cripps, 2004). Intestinal T cells have been observed in the human terminal ileum at 100 days of gestation, and by 140 days they are organized around distinct B cell follicular areas (Spencer et al., 1986). The developing T cell repertoire in the lamina propria (LP) includes populations of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells (Russell et al., 1990; Spencer et al., 1986). Fetal intraepithelial lymphocytes (IELs) are $CD3^+/CD4^-/CD8^-$ cells, expressing predominantly γδTCR (Gleeson and Cripps, 2004; Figure 1).

Mucosal Immunology. http://dx.doi.org/10.1016/B978-0-12-415847-4.00002-1

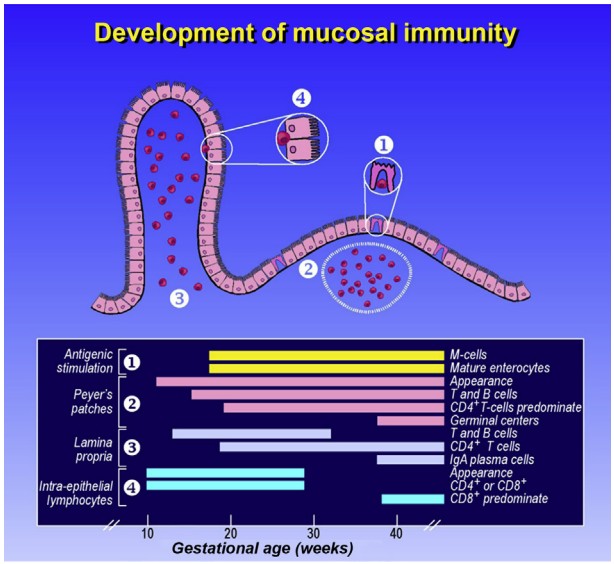

FIGURE 1 Diagram of mucosal immune defense expressed in the human neonate as a function of gestational age. All components of mucosal immune function are mature at birth in the term human infant. (1) Specialized IEC (M cells) overlying PPs (DCs), (2) PPs containing aggregates of lymphoid elements, (3) interstitial lymphocytes, and (4) IELs.

Maternal Immune Status

During pregnancy, the fetus, which is a semiallogeneic tissue, is allowed to grow within the maternal uterus without being rejected by the maternal immune system. It has been postulated that adaptive immune responses skew toward a T helper-2 (Th2) type during pregnancy (Wegmann et al., 1993). Maternal tolerance toward fetal alloantigens may be explained by this predominant Th2-type immunity during pregnancy, which downregulates a T helper-1 (Th1) response, the response that is considered to be hazardous to fetal development, protecting the fetus from maternal Th1 cell attack (Wegmann et al., 1993). Th2 cytokine-induced attenuation of Th1 immunity may contribute to the impairment of the defense against Th1-related pathogens, which also can be deleterious to the fetus.

The Th1/Th2 paradigm appears to be insufficient to explain the mechanism by which maternal immune cells protect/reject the fetus. Th1 and Th2 immunities have been observed in recurrent abortion (Piccinni et al., 1998; Chaouat et al., 2003). Recent advances in understanding immune regulation during pregnancy leads to the expansion of the Th1/Th2 paradigm into the Th1/Th2/Th17 and regulatory T (Treg) cell paradigm (Peck and Mellins, 2010). Although Th1 and Th2 cells have long been known to regulate cellular and humoral immunity and to play an important role in pregnancy, Th17 cells have been identified only recently as a Th lineage that regulates inflammation via production of distinct cytokines such as interleukin (IL)-17A, IL-17F, IL-21, IL-22, IL-6, and tumor necrosis factor-α (TNFα) (Fischer, 2008; Peck and Mellins, 2010). Limited evidence suggests that Th17 cells promote inflammation at the fetal–maternal interface in preterm delivery (Leber et al., 2010).

A unique subpopulation of T cells expanding either during human or murine pregnancy is made up of Treg cells (Leber et al., 2010). These cells are described as $CD4^+CD25^+Foxp3^+$ cells because their characteristic transcription factor is forkhead box P3 (Foxp3) (Zheng et al., 2007). They can exert anti-inflammatory effects and maintain tolerance to self components by contact-dependent suppression or the release of anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGFβ) (Gavin et al., 2006). T cells have emerged in the past few years as a key player in allowing fetal survival within the maternal uterus. T cells can regulate immune cell responses directly at the fetal–maternal interface, creating a tolerant microenvironment (Leber et al., 2010). The accumulation of maternal T cells during pregnancy parallels the need for expanded tolerance to encompass non-self fetal antigens (i.e., parental antigens expressed by the developing fetus). However, one of the potential consequences of sustained Foxp3 Treg may contribute to increased susceptibility to prenatal infection. It has been demonstrated in a mouse model that maternal Treg cells impair host defense, causing susceptibility to pathogens (Rowe et al., 2011). In addition, infection-induced reductions in maternal $Foxp3^+$ Treg suppression have been shown to play a critical role in the pathogenesis of immune-mediated fetal wastage (Rowe et al., 2012a). Recent evidence demonstrates that pregnancy selectively stimulates the development of maternal $Foxp3^+CD4^+$ Treg cells with fetal specificity and that after delivery these cells maintain tolerance to pre-existing fetal antigen and can rapidly re-accumulate during a subsequent pregnancy. These observations suggested that pregnancy may imprint regulatory memory that sustains anergy to fetal antigen (Rowe et al., 2012b).

Fetal Nutrition

In addition to maternal immune status as discussed above, factors such as maternal health, gestation, fetal nutrition, and intrauterine antigen exposures can play a significant role in this process. Maternal health during gestation has a significant effect on the health of the offspring, and nutritional, toxic, genetic, metabolic, and infectious factors all contribute to the eventual newborn phenotype (Kaplan et al., 2011). Intrauterine undernutrition has been shown to result in a shift in the Th1/Th2 cytokine balance toward Th1, contributing to an altered inflammatory response in the airway mucosa of the offspring (Landgraf et al., 2012). Therefore, maternal status may influence the growth and development of her offsprings later in their life.

Epidemiologic studies have shown an association between low birth weight and increased susceptibility to developing one or more components of the metabolic syndrome during adulthood (Hales, 1997). Failure of the

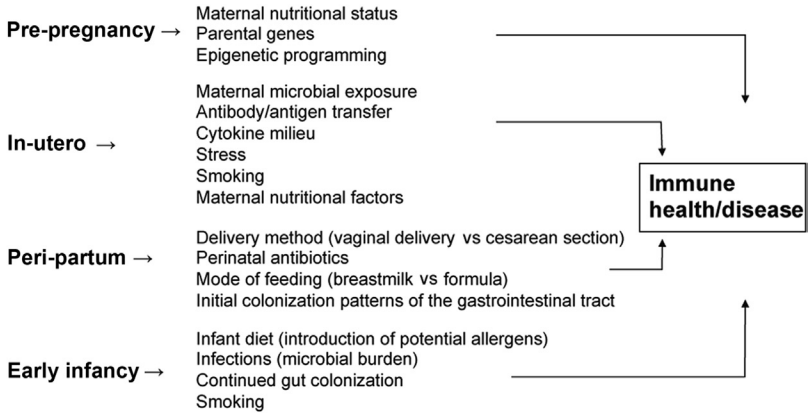

FIGURE 2 Factors that may influence immune development and related health/disease outcomes in the offspring.

maternal–placental nutrient supply to match fetal demand can result in a range of fetal adaptations and developmental changes. Recent evidence suggests that maternal diabetes influences the postnatal development of the intestine and the expression of various brush border enzymes (sucrose, lactase, and sodium glucose co-transporter-1) and transport functions in the rat intestine (Sharma et al., 2009, 2012). Changes in enzyme and transport functions of the intestine can be expected to influence the growth and development of the offspring during postnatal life. A range of maternal and environmental factors that may influence fetal immune development and are implicated in short- and long-term immune health is illustrated in Figure 2.

Maternal Gut Microbiota

During pregnancy, substantial changes (immunological, as discussed previously, hormonal, and metabolic) take place in the body of the mother. The changes in the immune system at the mucosal surface during pregnancy may contribute to the changes in the microbiota (Koren et al., 2012). The type of bacterial initial colonization and changes in infant microbiota may affect mucosal immune development because commensal bacteria possess immunostimulatory activities that can modulate the mucosal immune system, affecting the development and homeostasis of the intestinal mucosal immune system. Therefore, the period of infancy is important for "setting the stage" for immune function and regulation later in life. By 4 years of age, the greatest similarity (child/mother microbiota) to their own mother has been detected (Koren et al., 2012). These patterns are consistent with observations showing similarities in microbiomes within family for older children, but not for infants (Turnbaugh et al., 2009; Yatsunenko et al., 2012). The increased similarities between the child and maternal microbiota with age may imply the importance of shared diet and environmental factors on shaping the microbiota. Recent studies have shown that human milk, which has been traditionally considered sterile, represents a continuous supply of commensal or potentially probiotic bacteria to the infant gut (Fernández et al., 2013). In infants and children, an imbalanced or aberrant intestinal microbiota (dysbiosis) that induces defects in the immune system has been suggested to contribute to the rising incidence of allergic and autoimmune diseases (Huffnagle, 2010). Several studies have shown that maternal exposure to farm animals and ingestion of unpasteurized cow's milk resulted in increased microbial or microbial factor exposure, which is related to a reduced risk of allergic diseases in children (Von Mutius, 2012). A recent study using farming-related microbes in a mouse model of allergic airway inflammation showed that prenatal exposure to microbes protects from the development of an allergic inflammatory response in the next generation. The protection is induced by a low-level maternal innate immune response and transmission of the protection from mother to the fetus in Toll-like receptor (TLR)-dependent fashion (TLR2, 3, 4, 7, and/or 9; Conrad et al., 2009). Furthermore, studies that determine environmental exposures associated with the gene expression of innate immunity receptors during pregnancy and the first year of a child's life have revealed an association between farming-related exposures and a change in gene expression of innate immunity receptors in early life (Inman et al., 2012). It is clear that early-life events in utero, or fetal programming, can be influenced significantly by the maternal environment. The development of mucosal immunity is influenced by maternal immune status and maternal exposure to microbes and environmental and dietary antigens/allergens during pregnancy.

Early Development and Maturation of the Intestine

The development of gastrointestinal (GI) tract in utero involves extensive structural and functional changes in the intestinal epithelium. Early development of the intestine is important because it may potentially influence the responsiveness of the intestine to physiological and pathological challenges later in life. The GI tract develops from a simple tube to a complex specialized functional organ that is composed

of three germ layers: endoderm, mesoderm, and ectoderm. The endoderm germ layer forms the epithelial lining of the lumen, the mesoderm forms smooth muscle layers, and the ectoderm germ layer contributes to the most anterior and posterior luminal digestive structure and the enteric nervous system (De Santa Barbara et al., 2003). The development of the small intestine involves three developmental stages: morphogenesis and cell proliferation, cell differentiation, and functional maturation (Colony, 1983). The fetal development of the intestine may be regulated by various growth factors and transcription factors. Developmental defects in the GI tract have been detected in mice in which the transcription factor N-*myc* gene was knocked out (Stanton et al., 1992). The transcription factor Cdx-2 expression has been detected in the mouse intestinal morphogenesis process. This factor has also been shown to regulate small intestinal brush border membrane enzymes (Suh et al., 1994).

During intrauterine development, villus and microvillus formation results in a 10^5-fold increase in the intestinal surface area (Neu and Koldovsky, 1996). The development of intestinal crypts occurs in the human fetus. In contrast, crypts do not develop in rodents until after birth (Hirano and Kataoka, 1986). All four epithelial cell types of the intestinal mucosa are thought to be derived from one or more multipotent stem cells located in the intestinal crypt (Vidrich et al., 2009). The expansion of intestinal crypt epithelial stem cells is regulated by growth factors, such as fibroblast growth factor-3 through β-catenin/Tcf-4-dependent and -independent pathways (Vidrich et al., 2009). The β-catenin signaling pathway may interact with other growth factors, such as Wnt(s) and fibroblast growth factor-2 (Holnthoner et al., 2002). The balance in cellular proliferation that occurs in the crypts and differentiated cells in the villus is achieved by apoptosis of sencescent cells. Various growth and transcription factors may be involved in the complex regulation of GI development. Many of these factors are detected in the human fetal intestine, such as epidermal growth factor (EGF), TGFβ, insulin-like growth factor-2, hepatocyte growth factor, and glucagon-like peptide-2 (Podolsky, 1993; Lovshin et al., 2001).

Intestinal epithelial cells (IECs) also express a range of pattern recognition receptors (PRRs) sensing the presence of gut microbes, including TLRs and nucleotide oligomerization domain-like receptors (NLRs). These receptors play a critical role in pathogen recognition, activation of innate immunity, and induction of inflammation (Abreu, 2010; Wells et al., 2011). Recent evidence suggests that during embryonic development, fetal intestinal mucosa expresses an increase in surface TLR4, which can lead to the development of necrotizing enterocolitis (NEC) (Afrazi et al., 2011) and that amniotic fluid, by inducing EGF receptor activation, plays an inhibitory role in TLR4 signaling within the intestinal mucosa of the fetal and neonatal mice and in the attenuation of NEC (Good et al., 2012). Stimulation of the fetal intestinal cells with LPS resulted in higher levels of the nuclear factor-κB (NF-κB) activation and production of CXC-chemokine ligand (CXCL)-8 and CXCL2 compared with adult IECs (Lotz et al., 2006). Immature gut reacts to molecular patterns of colonizing bacteria and to endogenous inflammatory stimuli by mounting an excessive inflammatory (IL-8) response. This excessive inflammatory response of the immature intestine, a hallmark of NEC, is due to a developmental immaturity in innate immune response genes (Nanthakumar et al., 2011). Immature intestine is associated with an increased permeability, which decreases with the gut maturation process, a phenomenon, termed "gut closure". Intestinal growth, development, and maturation can be promoted significantly by various maternal factors, such as EGF and TGFβ, which is rich in colostrum and milk (Cummins and Thompson, 1997). Other environmental factors, such as initial colonization of the intestinal mucosa by commensal microorganisms and exposure to food either directly or indirectly, also play an important role in intestinal mucosa development and maturation.

THE DEVELOPMENT OF MUCOSAL IMMUNITY CONTINUES DURING THE POSTNATAL PERIOD

Postnatal Development of the Mucosal Immune System

The gut-associated lymphoid tissue consists of organized lymphoid tissues, such as mesenteric lymph node (MLN) and Peyer's patches (PPs), and more diffusely scattered lymphocytes in the intestinal LP and epithelium. At birth, because of the combined effects of hormones, the immaturity of antigen-presenting cells (APCs), and maternal derived immunosuppression, the mucosal immune system expresses a nonresponsiveness, but it is protected by passing mucosal immune factors in breast milk. However, the mucosal immune system is capable of a rapid response if challenged (Figure 1). There are active B cells in intestinal lymphoid follicles at birth. From birth to 12 weeks of age, maturation of B cells reach the peak period. After birth, germinal centers appear in the intestinal mucosa. In the intestine, the number of IgM-containing cells predominate up to 1 month of age, and then IgA-containing cells predominate and continue to increase until 2 years (Perkkiö and Savilahti, 1980; Knox, 1986). After birth, the number of intestinal IELs expands significantly, reaching adult levels by 2 years of age. The mucosal immune system is rapidly stimulated at birth by bacterial colonization of the intestinal mucosal surfaces (Figure 3). Ingestion of colostrum promotes mucosal maturation in the GI tract through regulatory factors and results in closure within 48 h of birth (Xanthou et al., 1995). Rapid closure of mucosal macromolecule transport is an important process in limiting systemic exposure to antigens.

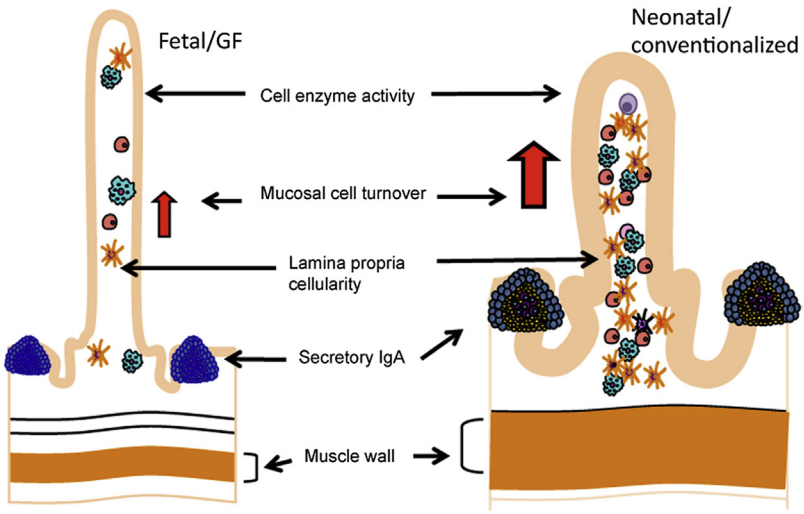

FIGURE 3 A cartoon of a cross-section of human fetal and neonatal small intestine (and/or a section of germ-free and conventionalized mouse intestine) showing proliferating epithelium and a paucity of lymphoid elements in contrast to the same cross section of a fully colonized infant (or mice reared under conventional condition) with actively proliferating, mature epithelium and an abundance of lymphoid elements. GF, germ-free.

Bacterial Colonization

During and soon after birth, infant mucosal surfaces are exposed to the mother's vaginal and fecal microbiota and other environmental exposures. The transition of the intestinal mucosa from a sterile fetal environment to one that has numerous microorganisms and is rich in food components takes place. Bacterial colonization and diet play significant part in the development and maturation of the neonatal mucosal immune system.

This colonization of the intestinal epithelium with many microorganisms establishes a microbial ecosystem in the gut. Recent studies have provided strong evidence that alterations of these gut microbial communities can result in inflammatory diseases not only of the intestine, but also of organs at distal sites (Littman and Pamer, 2011). Recent advances in culture-independent techniques have made it possible for a better understanding of the makeup and function of human gut microbiota (Fraher et al., 2012; Simrén et al., 2013), accelerating our knowledge of the complexity of this ecosystem. Commensal microbes, which are highly diverse, actively participate in the postnatal development of mucosal and systemic immunity. Using the new metagenomic technology (culture-independent 16s rRNA sequence analysis), approximately 15,000–36,000 species of bacteria have now been identified in the human GI tract (Frank et al., 2007; Frank and Pace, 2008). A total of 3.3 million nonredundant microbial genes in human fecal specimens have recently been identified from the metagenomics of the Human Intestinal Tract project (Qin et al., 2010). The diverse population of commensal bacteria plays an important role in the development, differentiation, expansion, and maintenance of immune cell populations and in the regulation of intestinal mucosal immunity. These immune populations include $CD4^+FoxP3^+$ Treg cells, Th17 cells, and γδT cells (Huang et al., 2005; Mazmanian et al., 2005; Ivanov et al., 2009; Atarashi et al., 2011; 2011; Duan et al., 2010; Atarashi et al., 2011). Mice reared under germ-free conditions in the absence of commensal microbiota exhibit numerous immunological defects. In these mice, the mucosal immune system is underdeveloped with poorly formed PPs, greatly reduced IgA-producing cells and $CD4^+$ T cells in the LP (Figure 3; Macpherson and Harris, 2004), and an altered gene-expression profile of IECs (Hooper et al., 2001). These defects in the mucosal immune system can be reversed by colonizing germ-free mice with commensal bacteria by co-housing these mice with specific pathogen free mice (Figure 3). These studies in germ-free animals have provided strong evidence to demonstrate a critical role of microbiota in the development, maturation, and function of several components of the mucosal immune system and of intestinal barrier function (Figure 3; Talham et al., 1999; Mazmanian et al., 2005; Hooper et al., 2001). The molecular mechanism responsible for this development may involve PRRs that are expressed in innate immune cells and are capable of detecting microorganism-associated molecular patterns (MAMPs), including TLRs, NLRs, and RIG-like receptors (Lavelle et al., 2010). These receptors play a critical role in pathogen recognition, activation of innate immunity, and induction of inflammation (Abreu, 2010; Wells et al., 2011).

Breast Milk/Food Intake

Milk and colostrum contain a broad array of oligosaccharides that seem to act as PRR agonists and may orchestrate gut colonization by commensal microbiota in the early phase of life and thus reduce the risks of pathogen invasion and inflammation. Breast milk contains cytokines, which may play a significant part in epithelial cell differentiation and maturation. For example, breast milk TGFβ2 is associated with healthy immune maturation and reduced risk of immune-mediated disease in infants (Rautava et al., 2012). A recent investigation showed that treatment of a primary human fetal IECs and a human fetal intestinal cell line with breast milk levels of TGFβ2 significantly attenuates the