



HANDBOOK OF EXPERIMENTAL IMMUNOLOGY  
IN FOUR VOLUMES

**Volume 4: Applications of  
Immunological Methods in  
Biomedical Sciences**

EDITED BY

**D. M. WEIR MD, FRCP**

*Professor of Microbial Immunology,  
University of Edinburgh, Scotland*

CO-EDITORS

**L. A. Herzenberg PhD**

*Professor of Genetics,  
Stanford University, USA*

**Caroline Blackwell PhD**

*Lecturer, Department of Bacteriology,  
University of Edinburgh, Scotland*

**Leonore A. Herzenberg DSc**

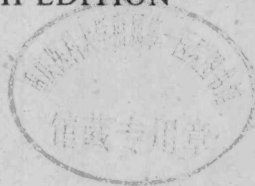
*Senior Research Associate, Department of Genetics,  
Stanford University, USA*

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# Contributors

- C. A. Allen, *Imperial Cancer Research Fund, London, UK*
- G. A. Andres, *Department of Pathology, State University of New York at Buffalo, NY, USA*
- J. D. Ansell, *Department of Zoology, University of Edinburgh, Edinburgh, UK*
- P. K. C. Austwick, *Department of Allergy and Clinical Immunology, Cardiothoracic Institute, London, UK*
- J. Austyn, *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- T. Bachi, *Institute for Immunology and Virology, University of Zurich, Zurich, Switzerland*
- J. A. Badwey, *Department of Biological Chemistry, Harvard Medical School, Boston, Mass, USA*
- D. Baltimore, *Whitehead Institute for Biomedical Research, Cambridge, Mass, USA*
- M. J. Banda, *Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, Ca, USA*
- A. N. Barclay, *MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- G. R. Barclay, *Blood Transfusion Centre, Royal Infirmary, Edinburgh, UK*
- B. Benacerraf, *Department of Pathology, Harvard Medical School, Boston, Mass, USA*
- P. Beverley, *ICRF Human Tumour Immunology Group, School of Medicine, University College London, London, UK*
- D. E. Bidwell, *Nuffield Laboratories of Comparative Medicine, Zoological Society of London, London, UK*
- C. Caroline Blackwell, *Department of Bacteriology, University of Edinburgh, Edinburgh, UK*
- A. E. Bolton, *Department of Biological Science, Sheffield City Polytechnic, Sheffield, UK*
- J. Braun, *Department of Pathology, University of California, Los Angeles, Ca, USA*
- P. H. Brodeur, *Department of Pathology, Tufts University, Boston, Mass, USA*
- C. J. Burrell, *Division of Virology, Institute of Medical and Veterinary Science, Adelaide, Australia*
- E. C. Butcher, *Department of Pathology, Stanford University Medical School, Stanford, Ca, USA*
- G. C. Butcher, *Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge, UK*
- H. Cantor, *Department of Pathology, Harvard Medical School, Boston, Mass, USA*
- P. Chandler, *Transplantation Biology Section, Clinical Research Centre, Harrow, UK*
- M. W. Clark, *Department of Biochemistry and Microbiology, University of Victoria, BC, Canada*
- J. E. Coligan, *National Institute of Allergy and Infectious Diseases, Bethesda, Md, USA*
- Suzanne Cory, *The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia*
- O. Cromwell, *Department of Allergy and Clinical Immunology, Cardiothoracic Institute, London, UK*
- M. R. Daha, *Department of Nephrology, University Hospital, Leiden, The Netherlands*
- M. M. Davis, *Department of Medical Microbiology, Stanford University Medical School, Stanford, Ca, USA*
- R. Dildrop, *Institute of Genetics, University of Cologne, Cologne, Federal Republic of Germany*
- G. J. Dougherty, *Imperial Cancer Research Fund, London, UK*
- S. Dreborg, *Allergy and Diagnostics Division, Pharmacia AB, Uppsala, Sweden*
- D. W. Dresser, *Division of Immunology, National Institute for Medical Research, Mill Hill, London, UK*
- S. R. Durham, *Department of Allergy and Clinical Immunology, Cardiothoracic Institute, London, UK*
- P. J. Edelson, *Department of Pediatrics, New York Hospital, Cornell Medical Centre, New York, NY, USA*
- A. B. Edmundson, *Department of Biology, University of Utah, Salt Lake City, Utah, USA*
- R. Einarsson, *Biochemistry Division, Pharmacia AB, University of Uppsala, Uppsala, Sweden*
- E. L. Elson, *Department of Biological Chemistry, Washington University Medical Centre, St. Louis, Mo, USA*
- R. A. Elton, *Medical Computing and Statistics Group, University of Edinburgh, Edinburgh, UK*
- Katherine R. Ely, *Department of Biology, University of Utah, Salt Lake City, Utah, USA*
- E. G. Engleman, *Department of Medicine, Stanford University, School of Medicine, Stanford, Ca, USA*
- R. A. B. Ezekowitz, *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- C. G. Fathman, *Department of Medicine, Stanford University School of Medicine, Stanford, Ca, USA*



- A. S. Fauci, *Laboratory of Immunoregulation, National Institute of Health, Bethesda, Md, USA*
- S. Felder, *Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Mo, USA*
- Lorraine Flaherty, *Centre for Laboratories and Research, New York State Department of Health, Albany, NY, USA*
- The late W. L. Ford, *Department of Immunology, University of Manchester, Manchester, UK*
- J. Forman, *Department of Microbiology, University of Texas Southwestern Medical School, Dallas, Texas, USA*
- Sara Fuchs, *Department of Clinical Immunology, The Weizmann Institute of Science, Rehovot, Israel*
- H. H. Fudenberg, *Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, SC, USA*
- W. Gerhard, *Wistar Institute of Anatomy and Biology, Philadelphia, Pa, USA*
- Elizabeth J. Glass, *Animal Breeding Research Organisation, Edinburgh, UK*
- W. Godfrey, *Department of Microbiology, University of California, San Francisco, Ca, USA*
- J. W. Goding, *Department of Pathology and Immunology, Monash Medical School, Victoria, Australia*
- S. Gordon, *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- D. R. Green, *Department of Immunology, University of Alberta, Edmonton, Canada*
- Margaret C. Green, *The Jackson Laboratory, Bar Harbor, Maine, USA*
- N. M. Gough, *Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Victoria, Australia*
- G. A. Gutman, *Department of Microbiology and Molecular Genetics, University of California, Irvine, Ca, USA*
- J. Guyden, *Department of Microbiology and Immunology, University of California, San Francisco, Ca, USA*
- S. Habu, *Department of Cell Biology, Tokai University, Japan*
- S. Hakomori, *Division of Biochemical Oncology, Fred Hutchinson Cancer Research Center, Seattle, Wash, USA*
- E. Handman, *Laboratory of Immunoparasitology, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia*
- R. R. Hardy, *Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan*
- R. A. Harrison, *Mechanisms in Tumour Immunity Unit, MRC Centre, Cambridge, UK*
- K. Hayakawa, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- W. J. Herbert, *Animal Services Unit, University of Dundee, Dundee, UK*
- L. A. Herzenberg, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- Leonore A. Herzenberg, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- Eadie Heyderman, *Department of Histopathology, St. Thomas's Hospital Medical School, London, UK*
- S. Hirsch, *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- Nancy M. Hogg, *Imperial Cancer Research Fund, London, UK*
- E. J. Holborow, *Bone and Joint Research Unit, London Hospital Medical College, London, UK*
- L. Hood, *Division of Biology, California Institute of Technology, Pasadena, Ca, USA*
- J. C. Howard, *Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge, UK*
- Maureen Howard, *National Institute of Allergy and Infectious Diseases, Bethesda, Md, USA*
- K. C. Hsu, *Department of Microbiology, College of Physicians and Surgeons of New York, NY, USA*
- D. A. Hume, *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- S. V. Hunt, *MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- W. M. Hunter, *Celltec Ltd, Slough, Berkshire, UK*
- G. M. Iverson, *Dept of Pathology, Howard Hughes Medical Institute, Yale University, New Haven, Conn, USA*
- M. K. Jenkins, *Department of Microbiology-Immunology, Northwestern University, Chicago, Ill, USA*
- G. D. Johnson, *Department of Immunology, University of Birmingham, Birmingham, UK*
- M. J. Johnston, *Department of Genetics, Stanford University Medical School, Stanford, Ca, USA*
- E. A. Kabat, *Department of Microbiology, College of Physicians and Surgeons of Columbia University, New York, NY, USA*
- M. E. Kamarck, *Department of Biology, Yale University, New Haven, Conn, USA*
- R. Kannagi, *Division of Biochemical Oncology, Fred Hutchinson Cancer, Seattle, Wash, USA*
- J. Kappler, *Department of Medicine, National Jewish Hospital and Research Center, Denver, Co, USA*

- M. J. Karnovsky, *Department of Biological Chemistry, Harvard Medical School, Boston, Mass, USA*
- M. L. Karnovsky, *Department of Pathology, Harvard Medical School, Boston, Mass, USA*
- R. A. Kautz, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- Paula Kavathas, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- A. B. Kay, *Department of Allergy and Clinical Immunology, Cardiothoracic Institute, London, UK*
- B. S. Kim, *Department of Microbiology-Immunology, Northwestern University, Chicago, Ill, USA*
- T. J. Kindt, *National Institute of Allergy and Infectious Disease, Bethesda, Md, USA*
- T. J. Kipps, *Scripps Clinic and Research Foundation, La Jolla, Ca, USA*
- M. Klein, *Department of Immunology, Toronto Western Hospital, Ontario, Canada*
- R. D. Kornberg, *Department of Cell Biology, Stanford University Medical School, Stanford, Ca, USA*
- P. J. Lachmann, *Mechanisms in Tumour Immunity Unit, MRC Centre, Cambridge, UK*
- H. C. Lane, *Laboratory of Immunoregulation, National Institute of Health, Bethesda, Maryland, USA*
- L. L. Lanier, *Becton Dickinson Monoclonal Center Inc, Mountain View, Ca, USA*
- P. C. J. Leijh, *Department of Infectious Diseases, University Hospital, Leiden, The Netherlands*
- Sheelagh Lloyd, *Department of Clinical Veterinary Medicine, University of Cambridge, Cambridge, UK*
- Joan L. Longbottom, *Department of Clinical Immunology, Cardiothoracic Institute, London, UK*
- Eva Lotta Larsen, *Department of Immunobiology, Karolinska Institute, Stockholm, Sweden*
- W. H. R. Lumsden, *19a Merchiston Crescent, Edinburgh, UK*
- W. Lutz, *Medical Computing and Statistics Group, University of Edinburgh, Edinburgh, UK*
- W. H. McBride, *Department of Radiation Oncology, University of California, Los Angeles, USA*
- M. C. McCann, *Blood Transfusion Centre, Royal Infirmary, Edinburgh, UK*
- J. A. McIntyre, *Department of Obstetrics and Gynaecology, Southern Illinois School of Medicine, Springfield, Ill, USA*
- Judith A. MacKay, *Department of Allergy and Clinical Immunology, Cardiothoracic Institute, London, UK*
- Rose A. Mage, *Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, Md, USA*
- O. Mäkelä, *Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland*
- Phillipa Marrack, *Department of Medicine, National Jewish Hospital and Research Center, Denver, Co, USA*
- Olivia Martinez, *Department of Microbiology and Immunology, University of California, Berkeley, Ca, USA*
- D. W. Mason, *MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- R. Melvold, *Dept of Microbiology and Immunology, Northwestern University Medical School, Chicago, Ill, USA*
- T. G. Merrett, *RAST Allergy Unit, Benenden Chest Hospital, Cranbrook, Kent, UK*
- H. S. Micklem, *Department of Zoology, University of Edinburgh, Edinburgh, UK*
- R. G. Miller, *Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada*
- S. D. Miller, *Department of Microbiology Immunology, The Medical and Dental Schools, Ill, Northwestern University, Chicago, USA*
- C. Milstein, *Laboratory of Molecular Biology, Medical Research Council The Medical School, Cambridge, UK*
- G. F. Mitchell, *Laboratory of Immunoparasitology, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia*
- W. A. Moore, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- Sherie L. Morrison, *Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY, USA*
- D. B. Murphy, *Department of Pathology, Yale University, New Haven, Conn, USA*
- R. M. Nakamura, *Department of Pathology, Scripps Clinic and Research Foundation, La Jolla, Ca, USA*
- Y. Nakao, *Department of Medicine, University of Kobe, Japan*
- C. J. Newby, *Department of Genetics, Stanford University Medical School, Stanford, Ca, USA*
- L. A. Nilsson, *Department of Medical Microbiology, University of Gothenburg, Gothenburg, Sweden*
- G. J. V. Nossal, *Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia*
- Y. Oi, *Becton Dickinson Monoclonal Center, Mountain View, Ca, USA*
- K. Okumura, *Department of Immunology, Juntendo University, Tokyo, Japan*

- Ö. Ouchterlony, *Department of Medical Microbiology, University of Gothenborg, Gothenburg, Sweden*
- Z. Ovary, *Department of Pathology, New York University Medical Center, New York, NY, USA*
- W. Page Faulk, *Medi-Search AG, Meiringen, Switzerland*
- P. Parham, *Department of Cell Biology, Stanford University School of Medicine, Stanford, Ca, USA*
- D. R. Parks, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- M. Parson, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*
- T. W. Pearson, *Department of Biochemistry and Microbiology, University of Victoria, BC, Canada*
- N. O. Petersen, *Department of Chemistry, University of Western Ontario, London, Ontario, Canada*
- M. Potter, *Laboratory of Genetics, National Cancer Institute, Bethesda, Md, USA*
- I. R. Poxton, *Department of Bacteriology, University Medical School, Edinburgh, UK*
- A. Radbruch, *Institute of Genetics, University of Cologne, Cologne, Federal Republic of Germany*
- P. Ralph, *Department of Cell Biology, Cetus Corporation, Emeryville, Ca, USA*
- K. Rajewsky, *Institute of Genetics, University of Cologne, Cologne, Federal Republic of Germany*
- U. Ramstedt, *Department of Immunology, Karolinska Institute, Stockholm, Sweden*
- Elizabeth Raveche, *National Institute of Health, Bethesda, Md, USA*
- L. F. Reichardt, *Department of Neurology, University of California, San Francisco, Ca, USA*
- J. Reidler, *Department of Cell Biology, Stanford University School of Medicine, Stanford, Ca, USA*
- R. Riblet, *Department of Immunology, Medical Biology Institute, La Jolla, Ca, USA*
- J. M. Robinson, *Department of Pathology, Harvard Medical School, Boston, Mass., USA*
- F. S. Rosen, *Division of Immunology, Children's Hospital Medical Center, Boston, Mass, USA*
- R. V. Rouse, *Department of Pathology, Stanford University School of Medicine, Stanford, Ca, USA*
- F. A. Ruddle, *Department of Biology, Yale University, New Haven, Conn, USA*
- T. Sasazuki, *Department of Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan*
- M. S. Schanfield, *Genetic Testing Institute, Atlanta, Georgia, USA*
- Beatrice C. Seegal, *Department of Microbiology, College of Physicians and Surgeons of Columbia University, New York, NY, USA*
- M. Sela, *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel*
- I. J. T. Seppälä, *Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland*
- E. E. Sercarz, *Department of Microbiology, University of California, Los Angeles, Ca, USA*
- Eva Severinson, *Department of Immunobiology, Karolinska Institute, Stockholm, Sweden*
- N. Shastri, *Department of Microbiology, University of California, Los Angeles, Ca, USA*
- J. F. L. Shaw, *Department of Surgery, University of Cambridge, Cambridge, UK*
- S. C. Silverstein, *Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY, USA*
- R. J. Shaw, *Department of Allergy and Clinical Immunology, Cardiothoracic Institute, London, UK*
- K. Simonovitch, *Department of Immunology, Toronto Western Hospital, Ontario, Canada*
- Elizabeth Simpson, *Transplantation Biology Section, Clinical Research Centre, Harrow, UK*
- L. Smith, *Division of Biology, California Institute of Technology, Pasadena, Ca, USA*
- E. J. L. Soulsby, *Department of Clinical Veterinary Medicine, University of Cambridge, UK*
- D. M. Spalding, *Division of Clinical Immunology and Rheumatology, University of Alabama in Birmingham, Birmingham, Alabama, USA*
- T. A. Springer, *Dana Farber Cancer Institute, Harvard Medical School, Boston, Mass, USA*
- D. R. Stanworth, *Department of Immunology, University of Birmingham, Birmingham, UK*
- Phyllis M. Starkey, *Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- A. D. Steinberg, *National Institute of Health, Bethesda, Md, USA*
- R. M. Steinman, *Laboratory of Cellular Physiology, and Immunology, The Rockefeller University, New York, NY, USA*
- M. Steinmetz, *Basel Institute for Immunology, Basel, Switzerland*
- M. W. Steward, *Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London, UK*
- C. C. Stewart, *Experimental Pathology Group, Los*



- Alamos National Laboratory, Los Alamos, New Mexico, USA*
- J. Stewart, *Department of Bacteriology, University of Edinburgh, Edinburgh, UK*
- E. J. Stott, *Institute for Research on Animal Diseases, Newbury, Berkshire, UK*
- T. Tada, *Department of Immunology, University of Tokyo, Tokyo, Japan*
- N. Takemura, *Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan*
- R. Takemura, *Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, Ca, USA*
- M. Taniguchi, *Department of Immunology, Chiba University, Chiba, Japan*
- Glenys Thomson, *Department of Genetics, University of California, Berkeley, Ca, USA*
- P. W. Tucker, *Department of Microbiology, University of Texas, Southwestern Medical School, Dallas, Texas, USA*
- T. Tokuhisa, *Department of Immunology, Chiba University, Chiba, Japan*
- M. W. Turner, *Department of Immunology, Institute of Child Health, London, UK*
- D. A. J. Tyrrell, *MRC Common Cold Unit, Harvard Hospital, Salisbury, Wiltshire, UK*
- E. R. Unanue, *Department of Pathology, Harvard Medical School, Boston, Mass, USA*
- J. Unkeless, *The Rockefeller University, New York, NY, USA*
- R. J. Urbaniak, *Aberdeen and North East of Scotland Blood Transfusion Service, Aberdeen, UK*
- E. E. Uzgiris, *Department of Cell Biology, Stanford University School of Medicine, Stanford, Ca, USA*
- Karen L. Valentino, *Department of Physiology, University of California School of Medicine, San Francisco, Ca, USA*
- R. van Furth, *Department of Infectious Diseases, University Hospital, Leiden, The Netherlands*
- E. van Loghem, *The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands*
- W. C. van Voorhis, *Laboratory of Cellular Physiology, The Rockefeller University, New York, NY, USA*
- Theda L. van Zwet, *Department of Infectious Diseases, University Hospital, Leiden, The Netherlands*
- A. Voller, *Nuffield Laboratories of Comparative Medicine, Zoological Society of London, London, UK*
- C. Waltenbaugh, *Department of Microbiology-Immunology, Northwestern University, Chicago, Ill, USA*
- R. A. Warnke, *Department of Pathology, Stanford University Medical School, Stanford, Ca, USA*
- D. M. Weir, *Department of Bacteriology, University of Edinburgh, Edinburgh, UK*
- Zena Werb, *Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, Ca, USA*
- G. Whalen, *Laboratory of Immunoregulation, National Institute of Health, Md, USA*
- A. G. White, *Department of Surgery, Faculty of Medicine, Kuwait University, Kuwait*
- D. J. G. White, *Department of Surgery, University of Cambridge, Cambridge, UK*
- H. Wigzell, *Department of Immunology, Karolinska Institute, Stockholm, Sweden*
- P. C. Wilkinson, *Department of Bacteriology and Immunology, University of Glasgow, Glasgow, UK*
- A. F. Williams, *MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford, Oxford, UK*
- F. P. Winstanley, *Armed Services University, Washington, DC, USA*
- Janet Winter, *Department of Physiology, University of California, San Francisco, Ca, USA*
- L. Wofsy, *Department of Microbiology and Immunology, University of California, Berkeley, Ca, USA*
- S. D. Wright, *Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY, USA*
- M. Zauderer, *Department of Microbiology and Oncology, University of Rochester, Rochester, NY, USA*
- D. Zelaschi, *Department of Genetics, Stanford University School of Medicine, Stanford, Ca, USA*

## Preface

The pace of progress in immunology has not slackened since the last edition of this handbook. The subject now draws heavily on molecular biology and genetics and this has necessitated the inclusion of an additional volume on **Genetics and Molecular Immunology**. The explosion in the development of hybridoma technology and cell culture, since the last edition, can be seen from the many chapters in each volume that employ monoclonal reagents and cell lines. Some idea of the expansion of the field can be gained from the **Cellular Immunology** volume where contributions on phagocytes and lymphocytes now occupy 30 chapters compared to 12 in the previous edition. A new section on immunoregulation contains 14 chapters and there are now 6 chapters devoted to mammalian cell membrane antigens in the **Immunochemistry** volume.

It is now no longer possible for one editor to keep in touch with the enormous expansion in this field, and I am much indebted to my co-editors Len and Leonore Herzenberg who have joined me in the task of co-opting research workers in the wide range of disciplines now contributing to the field of immu-

nology. I am particularly grateful to my wife Dr Caroline Blackwell for her help with the massive editing task.

Amongst the many new features of this edition is the provision of overviews for many of the sections. I am most grateful to our contributors in the methodology sections for their efforts to achieve a consistent style of presentation of the procedures, and I hope that this will help in the accessibility of the descriptive material. A work of this size inevitably takes a number of years to put together but considerable effort has gone into introducing up to date material into the chapters. This has been achieved by enabling and encouraging contributors to introduce new material and references during the proof stages of their chapters.

I wish to thank Hilary Flenley for her careful and thorough index, and Nigel Palmer and his staff at Blackwell Scientific Publications Edinburgh office without whom production of the new edition would have been impossible. Per Saugman has as always, maintained a benevolent paternal interest in the project.

D.M.W.

# Contents

List of contributors xi

Preface xvii

## VOLUME 4 APPLICATIONS OF IMMUNOLOGICAL METHODS IN BIOMEDICAL SCIENCES

### Monoclonal antibodies

- 17 Overview: Monoclonal antibodies  
C. MILSTEIN
- 8 Schemata for the production of monoclonal antibody-producing hybridomas  
T. J. KIPPS AND L. A. HERZENBERG
- 9 Hybridoma immunoglobulin isotype switch variant selection with the fluorescence activated cell sorter  
T. J. KIPPS AND L. A. HERZENBERG
- 10 Isotype switch variants  
A. RADBRUCH
- 11 Growing hybridoma and producing monoclonal antibodies *in vivo*  
K. OKUMURA AND S. HABU
- Applications:  
Monoclonal Antibodies**
- 12 Neurobiology  
JANET WINTER, KAREN L. VALENTINO AND L. F. REICHARDT
- 3 Monoclonal antibodies in the study of parasites and host-parasite relationships  
E. HANDMAN AND G. F. MITCHELL
- 1 Application of monoclonal antibodies in bacteriology  
C. CAROLINE BLACKWELL AND F. P. WINSTANLEY
- 115 Applications of monoclonal antibodies in virology  
W. GERHARDT AND T. BACHI

- 116 Special applications of tissue section immunologic staining in the characterization of monoclonal antibodies and in the study of normal and neoplastic tissues  
R. V. ROUSE AND R. A. WARNKE
- 117 Monoclonal antibodies directed to carbohydrate antigens  
R. KANNAGI AND S. HAKOMORI
- 118 Macrophages  
J. C. UNKELESS AND T. A. SPRINGER

### Applications: Immunological methods

- 119 Application of immunological techniques in bacteriology  
F. P. WINSTANLEY AND C. CAROLINE BLACKWELL
- 120 Application of immunological methods in virology  
E. J. STOTT AND D. A. J. TYRRELL
- 121 Applications of immunological methods in mycology  
JOAN L. LONGBOTTOM
- 122 Applications of immunological methods in protozoology  
E. H. R. LUMSDEN
- 123 Applications of immunological methods in helminthology  
W. J. L. SOULSBY AND SHEELAGH LLOYD
- 124 Immunological aspects of human pregnancy  
J. A. MACINTYRE AND W. PAGE FAULK
- 125 Applications of immunological techniques to the study of the tumour-host relationship  
G. J. DOUGHERTY, C. A. ALLEN AND NANCY M. HOGG



# Additional clinical applications of immunological methods

- 126 Tests of immune function  
S. J. URBANIAK, M. C. MCCANN, A. G. WHITE, G. R. BARCLAY AND A. B. KAY
- 127 Provocation tests and measurements of mediators from mast cells and basophils in asthma and allergic rhinitis  
O. CROMWELL, S. R. DURHAM, R. J. CHAW, JUDITH A. MACKAY AND A. B. KAY
- 128 Methods for detecting immune complexes in biological fluids  
M. H. KLEIN AND K. SIMINOVITCH
- 129 Immunohistochemistry in pathology  
EADIE HEYDERMAN, I. STRUDLEY AND T. C. RICHARDSON
- 130 Quantification of IgE and IgG4 both as total immunoglobulins and as allergic-specific antibodies  
T. G. MERRETT

# General methods for immunologic studies

- 131 Statistical aspects of planning and design of immunological experiments  
R. A. ELTON AND W. H. MCBRIDE
- 132 Guidelines to statistical analysis  
W. LUTZ
- 133 Laboratory animal techniques for immunology  
W. J. HERBERT, F. KRISTENSEN, with RUTH M. AITKEN, M. B. ESLAMI, ANNE FERGUSON, KATHLEEN G. GRAY, W. J. PENH/LE

## VOLUME 1 IMMUNOCHEMISTRY

### Antigens

- 1 Overview: Antigens  
M. SELA
- 2 Preparation of synthetic antigens  
M. SELA AND SARA FUCHS
- 3 Haptens and carriers  
O. MÄKELÄ AND I. J. T. SEPPÄLÄ

- 4 Isolation and identification of bacterial antigens  
I. R. POXTON AND C. CAROLINE BLACKWELL
- 5 Preparation of viral antigens  
C. J. BURRELL
- 6 Antigens of parasites  
T. W. PEARSON AND M. W. CLARK
- 7 Fungal antigens  
JOAN L. LONGBOTTOM AND P. K. C. AUSTWICK
- 8 Immunization of experimental animals  
D. W. DRESSER
- 9 Carbohydrate antigens in higher animals  
S. HAKOMORI AND R. KANNAGI
- 10 The chemistry and standardization of allergens  
S. DREBORG, R. EINARSSON, JOAN L. LONGBOTTOM

### Immunoglobulins: Purification and characterization

- 11 Overview: The quest for antibody homogeneity: the *sine qua non* for structural and genetic insights into antibody complementarity  
E. A. KABAT
- 12 Immunochemical analysis of human and rabbit immunoglobulins and their subunits  
D. R. STANWORTH AND M. W. TURNER
- 13 Purification and characterization of monoclonal antibodies  
R. R. HARDY
- 14 Preparation and purification of active fragments from mouse monoclonal antibodies  
P. PARHAM
- 15 Determination of the three-dimensional structures of immunoglobulins  
A. B. EDMUNDSON AND KATHERYN R. ELY
- 16 Immunoabsorbents  
SARA FUCHS AND M. SELA
- 17 Two-dimensional crystals of immunoglobulins  
J. REIDLER, E. E. UZGIRIS AND R. D. KORNBERG

- 18 Sources of myeloma proteins, and M-components  
M. POTTER

### Mammalian cell membrane antigens

- 19 Overview: Mammalian cell surface antigens  
R. D. KORNBERG
- 20 Membrane and secretory immunoglobulins: structure, biosynthesis and assembly  
J. W. GODING
- 21 Class I MHC antigens of mouse and humans  
J. E. COLIGAN AND T. J. KINDT
- 22 Glycoprotein antigens of the lymphocyte surface and their purification by antibody affinity chromatography  
A. F. WILLIAMS AND A. N. BARCLAY
- 23 Immunofluorescence methods to study cell surface and cytoplasmic proteins, and their dynamics  
E. R. UNANUE AND J. BRAUN
- 24 Measurement of lateral diffusion by fluorescence photobleaching recovery  
N. O. PETERSEN, S. FELDER AND E. L. ELSON

### Antibody interaction with soluble and cellular antigens

- 25 Overview: Introduction to methods used to study the affinity and kinetics of antibody-antigen reactions  
M. W. STEWARD
- 26 Radioimmunoassays and related methods  
A. E. BOLTON AND W. M. HUNTER
- 27 Enzyme immunoassays: heterogenous and homogeneous systems  
R. M. NAKAMURA, A. VOLLER AND D. E. BIDWELL
- 28 Preparation and use of fluorochrome conjugates  
G. D. JOHNSON AND E. J. HOLBOROW
- 29 Flow cytometry and fluorescence activated cell sorting (FACS)  
D. R. PARKS, L. L. LANIER AND L. A. HERZENBERG

- 30 Data analysis in flow cytometry  
W. A. MOORE AND R. A. KAUTZ
- 31 Purification and coupling of fluorescent proteins for use in flow cytometry  
R. R. HARDY
- 32 Immunodiffusion and immunoelectrophoresis  
Ö. OUCHTERLONY AND L.-Å. NILSSON
- 33 Passive cutaneous anaphylaxis  
Z. OVARY
- 34 Solid-phase radioimmune assays  
C. J. NEWBY, K. HAYAKAWA AND LEONORE A. HERZENBERG
- 35 Immunological techniques for the identification of antigens and antibodies by electron microscopy  
G. A. ANDRES, K. C. HSU AND BEATRICE C. SEGAL
- 36 Affinity targeting and fusion of vesicles to cells  
W. GODFREY, J. GUYDEN AND L. WOFSY
- 37 Immunotoxins  
OLIVIA MARTINEZ AND L. WOFSY
- 38 Kinetics of antibody reaction and the analysis of cell surface antigens  
D. W. MASON AND A. F. WILLIAMS

### Complement

- 39 Complement technology  
E. A. HARRISON AND P. J. LACHMANN
- 40 Complement fixation by monoclonal antigen-antibody complexes  
R. R. HARDY

## VOLUME 2 CELLULAR IMMUNOLOGY

### Phagocytes

- 41 Overview: The function of receptors in phagocytosis  
S. D. WRIGHT AND S. C. SILVERSTEIN

- 42 Overview: The mononuclear phagocyte system  
R. VAN FURTH
  - 43 Plasma membrane markers to study differentiation, activation and localization of murine macrophages. Ag F4/80 and the mannosyl, fucosyl receptor  
S. GORDON, PHYLLIS M. STARKEY, D. HUME, R. A. B. EZEKOWITZ, S. HIRSCH AND J. AUSTYN
  - 44 Methods for studying the ontogeny of mononuclear phagocytes  
C. C. STEWART
  - 45 Macrophage cell lines  
P. RALPH
  - 46 *In vitro* determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes  
P. C. J. LEIJH, R. VAN FURTH AND THEDA L. VAN ZWET
  - 47 Secreted proteins of resting and activated macrophages  
ZENA WERB, M. J. BANDA, R. TAKEMURA AND S. GORDON
  - 48 Macrophage membrane receptors  
J. STEWART, ELIZABETH J. GLASS, D. M. WEIR AND M. R. DAHA
  - 49 Dendritic cells  
R. M. STEINMAN, W. C. VAN VOORHIS AND D. M. SPALDING
  - 50 Reduction and excitation of oxygen by phagocytic leukocytes: biochemical and cytochemical techniques  
J. A. BADWEY, J. M. ROBINSON, M. J. KARNOVSKY AND M. L. KARNOVSKY
  - 51 Locomotion and chemotaxis of leukocytes  
P. C. WILKINSON
  - 52 Assays for phagocyte ecto-enzymes  
P. J. EDELSON
  - 53 Overview: Lymphocytes and their relations  
H. S. MICKLEM
  - 54 Physical methods for separation of lymphocyte subpopulations  
R. G. MILLER
  - 55 Preparative immunoselection of lymphocyte populations  
S. V. HUNT
  - 56 Genetic markers for following cell populations  
J. D. ANSELL AND H. S. MICKLEM
  - 57 Following cellular traffic: methods of labelling lymphocytes and other cells to trace their migration *in vivo*  
E. C. BUTCHER AND W. L. FORD
  - 58 Human leukocyte subpopulations  
P. BEVERLEY
  - 59 Lymphokines  
J. KAPPLER AND PHILLIPA MARRACK
  - 60 Natural killer cells  
H. WIGZELL AND U. RAMSTEDT
  - 61 Genetics and cell distributions of mouse cell surface alloantigens  
LORRAINE FLAHERTY AND J. FORMAN
- Lymphocyte responses**
- 62 Overview: Lymphocyte responses  
G. J. V. NOSSAL
  - 63 Lymphocyte responses to polyclonal B and T cell activators  
EVA SEVERINSON AND EVA-LOTTA LARSSON
  - 64 Assays for immunoglobulin-secreting cells  
D. W. DRESSER
  - 65 Limiting dilution analysis of effector cells and their precursors *in vitro*  
M. ZAUDERER
  - 66 *In vitro* evaluation of human lymphocyte function  
H. C. LANE, GAIL WHALEN AND A. S. FAUCI
  - 67 Assay for *in vivo* adoptive immune response  
G. M. IVERSON
  - 68 Analysis of cytotoxic T cell responses  
ELIZABETH SIMPSON AND P. CHANDLER
- The lymphoid system**
- 53 Overview: Lymphocytes and their relations  
H. S. MICKLEM



- 69 T cell lines and hybrids in mouse and man  
C. G. FATHMAN AND E. G. ENGLEMAN

- 70 B cell growth factors  
MAUREEN HOWARD

### Immunoregulation

- 71 Overview: Helper and suppressor T cells  
K. OKUMARA AND T. TADA
- 72 Overview: Ir genes  
B. BENACERRAF
- 73 Overview: Idiotypic regulation  
K. RAJEWSKY
- 74 Overview: Epitope-specific regulation  
LEONORE A. HERZENBERG
- 75 Overview: T cell clones  
H. CANTOR
- 76 Subtractive cDNA hybridization and the T-cell receptor genes  
M. M. DAVIS
- 77 Detection of suppressor cells and suppressor factors for delayed-type hypersensitivity responses  
S. D. MILLER AND M. K. JENKINS
- 78 Detection of suppressor cells and suppressor factors for antibody responses  
C. WALTENBAUGH AND B. S. KIM
- 79 Contrasuppressor T cells: a practical guide to the identification of contrasuppressive effects in immunoregulatory systems  
D. R. GREEN
- 80 Antigen-specific suppressor molecules produced by T cells  
M. TANIGUCHI AND T. TOKUHISA
- 81 Immunosuppressive agents  
D. J. G. WHITE AND J. F. L. SHAW
- 82 Studies of autoimmune diseases  
ELIZABETH S. RAVECHE AND A. D. STEINBERG
- 83 Primary immunodeficiencies: definitions and diagnosis  
F. S. ROSEN

- 84 Studying immune regulation with protein and peptide antigens  
N. SHASTRI AND E. E. SERCARZ

## VOLUME 3 GENETICS AND MOLECULAR IMMUNOLOGY

### Molecular approaches to immunology

- 85 Overview: Introductory comments on molecular immunology  
D. BALTIMORE
- 86 Construction and screening of recombinant DNA libraries in bacteriophage lambda  
P. W. TUCKER
- 87 The major histocompatibility complex of the mouse  
L. SMITH, M. STEINMETZ AND L. HOOD
- 88 The murine immunoglobulin heavy chain constant region gene locus  
N. M. GOUGH AND SUZANNE CORY
- 89 The Igh-V genes of the mouse  
R. RIBLET AND P. H. BRODEUR
- 90 Classification of mouse V<sub>H</sub> sequences  
RENATE DILDROP
- 91 Transfection for lymphocyte cell surface antigens  
PAULA KAVATHAS AND L. A. HERZENBERG
- 92 Lymphoid cell gene transfer  
SHERIE L. MORRISON AND V. T. OI

### Immunoglobulin genetics

- 93 Overview: Allotypes  
H. H. FUDENBERG
- 94 Human immunoglobulin allotypes  
M. S. SCHANFIELD AND ERNA VAN LOGHEN
- 95 New methods for human allotyping  
D. ZELASCHI, M. J. JOHNSTON, C. J. NEWBY AND LEONORE A. HERZENBERG
- 96 Gm and disease  
Y. NAKAO AND T. SASAZUKI

- 97 Mouse immunoglobulin allotypes  
MARILYN PARSON, LEONORE A. HERZENBERG, A. M. STALL AND L. A. HERZENBERG
- 98 Rat immunoglobulin allotypes  
G. A. GUTMAN
- 99 Rabbit immunoglobulin allotypes  
ROSE G. MAGE

# Genetics of the major histocompatibility complex

- 100 Overview: The murine MHC  
D. B. MURPHY
- 101 The MHC of the laboratory rat, *Rattus norvegicus*  
G. W. BUTCHER AND J. C. HOWARD
- 102 Human HLA genetics and disease associations  
GLENYS THOMSON

- 103 Common assays for identifying immune response genes  
C. WALTENBAUGH AND S. D. MILLER

## Resources

- 104 The mouse linkage map  
MARGARET C. GREEN
- 105 Somatic cell genetics and the human gene map  
M. E. KAMARCK AND F. H. RUDDLE
- 106 Inbred, congenic, recombinant-inbred and mutant mouse strains  
R. W. MELYOLD

Index to Volume 4 xix

# Chapter 107

## Overview: monoclonal antibodies

C. MILSTEIN

Conventional antisera and  
monoclonal antibodies, 107.3

Which fusing partners? 107.7

Final comments, 107.9

Although it is almost a hundred years since the discovery of antibodies and fifty after the publication of the seminal book by Landsteiner describing their amazing diversity and specificity [1], the potential power of antibodies in all branches of biology, and even in clinical medicine and therapy, is only now beginning to be seriously exploited. The introduction of radioimmunoassays not only meant the development of saturation analysis in analytical biochemistry, but perhaps more important, the introduction of antibodies as its basic tool. Since it is theoretically possible to make antibodies to all sorts of biological substances and other chemicals, they are ideally suited as general specific recognition elements to be used for analytical, cytological, functional, therapeutic and biochemical purposes. We now know that this astonishing capability is the result of an extremely flexible utilization of complex genetic elements ranging from a combinatorial use of a considerable number of germ-line genes and gene fragments to still incompletely defined somatic diversification events. Paradoxically, one of the major drawbacks of antibodies has been precisely this flexibility.

Confronted by an antigenic stimulus, an animal responds by producing a large variety of antibody structures directed against the immunogen. They will, for example, recognize different proteins, polysaccharides and other structures of a bacterium, as well as different determinants within each of those structures. Even a single antigenic determinant is likely to be recognized by a variety of antibody structures. This heterogeneous mixture of antibody structures is itself continuously changing so that the antiserum from the same animal is different, when bleeds taken at different times are compared. As a consequence, it is impossible to produce antisera of sufficient purity and reproducibility as is required by a true chemical reagent. The different molecular species present in the antiserum cannot be separated from each other.

The clonal selection theory of Burnet [2] states that each cell makes only one antibody structure. The

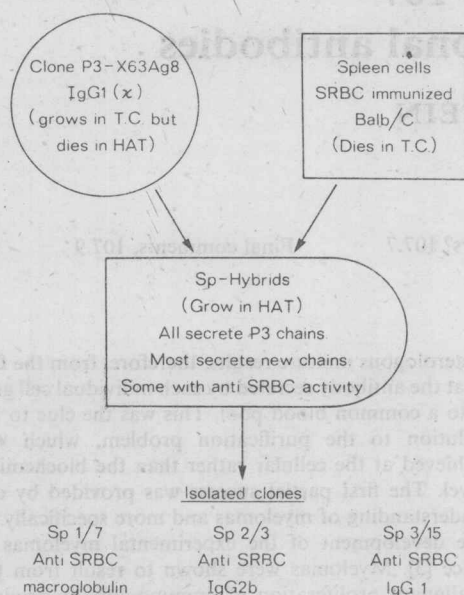
heterologous mixture results, therefore, from the fact that the antibody secreted by each individual cell goes into a common blood pool. This was the clue to the solution to the purification problem, which was achieved at the cellular rather than the biochemical level. The first partial success was provided by our understanding of myelomas and more specifically by the development of the experimental myelomas in mice [3]. Myelomas were shown to result from the malignant proliferation of immunoglobulin-producing cells. Since malignant transformation had a clonal origin, one myeloma produced one immunoglobulin. The experimental plasmacytomas demonstrated that it was possible not only to transplant the tumours, but also to adapt them to continuous culture and grow them *in vitro* [4]. Unfortunately, in spite of many efforts, the induction of those myelomas was not antigen dependent. In other words, it was not possible to derive and purify cell lines capable of producing monoclonal antibodies of any desired specificity.

Another approach for the immortalization and cloning of antibody-producing cells consisted of an *in vivo* proliferation of fragments of spleens taken from an immunized donor [5]. The fragments, containing on average only one specific antibody-producing clone (one spectrophore), were injected together with antigen into a recipient mouse which was itself rendered immunoincompetent by irradiation. The recipient mouse was in effect acting as a tissue culture flask permitting the proliferation of the otherwise short-lived spleen cells. Further passages, using spleen fragments from the recipient animal and antigen, allowed proliferation and hopefully purification of the original clone. In this way an essentially monoclonal antibody preparation of a predefined specificity was possible, although no formal purification or immortality was achieved.

The derivation of truly immortal cell lines, which had all the advantages of myelomas, and the genetic elements of the antibody-producing cells, was achieved by cell fusion (Fig. 107.1). A myeloma cell



## Anti SRBC Hybrids



**Fig. 107.1** Immortalization of cells producing specific antibody of predefined specificity. P3-X63 Ag 8 is an azaguanine resistant (HPGRT<sup>-</sup>) myeloma cell line which actively secretes a P3 myeloma protein. After fusion with cells from an immunized mouse, hybrids are derived which grow in tissue culture (T.C.) as permanent cell lines. Such hybrids co-dominantly express the ability to synthesize the immunoglobulin of both parents.

line was fused with spleen cells from an immunized donor [6]. The resulting hybrids acquired the essential properties of both parents, namely the permanent growth and malignancy as well as the high capacity for the synthesis and secretion characteristic of plasmacytomas, together with the genetic elements defining a specific antibody.

### Hybrid myelomas

The fusion process, even when optimized, is inefficient and the resulting hybrids are very few in comparison with the two parental cells [7]. These must be eliminated in order to grow the hybrids. Spleen cells do not survive for long under tissue culture conditions, but the myeloma parental cells must be killed by an efficient procedure. This is based on the use of selective HAT medium [8,9]. This medium contains hypoxanthine and thymidine together with aminopterin, a folic acid analogue which blocks *de novo* synthesis of

purines and pyrimidines. Cell survival in the presence of aminopterin requires the activity of a salvage pathway capable of utilizing the hypoxanthine and thymidine from the medium. The use of this salvage pathway can be prevented if the myeloma line is deficient in key enzymes, e.g. hypoxanthine-guanine phosphoribosyl transferase (HPGRT<sup>-</sup>). Such mutants are selected among cells resistant to azaguanine or thioguanine, either of which is a toxic analogue incorporated by HPGRT. Hybrids between HPGRT<sup>-</sup> mutant myelomas and normal lymphocytes can grow in HAT selective medium because they contain the HPGRT provided by the lymphocytes, while the HPGRT<sup>-</sup> myeloma cells die within a couple of days.

In essence, therefore, lymphoid cells—usually splenocytes from an immunized mouse or rat—are fused with a myeloma cell line using a fusing reagent, usually polyethylene glycol (PEG). The fusion gives rise to a large number of cells containing multiple nuclei (heterokaryons) [7]. Some of these merge into sinkaryons, which are cells containing a single nucleus with the chromosome content of the heterokaryons. Among them, viable cells emerge which divide and grow. This growth is accompanied by a considerable amount of chromosomal loss and better adaptation to culture conditions. Many factors are involved and the final outcome is a cell line, well adapted to growth, which arose by equilibrium of an unknown number of factors. Each cell line, and even subclones taken in early stages after fusion, are therefore likely to have different growth and stability properties. Loss of chromosomes also gives rise to loss of antibody expression. Clonal competition between variously adapted cells and loss of antibody activity play overlapping roles [10,11]. This process is faster in the early stages and 'older' clones tend towards stable characteristics. Therefore much depends on correct choice of subclones at early and subsequent stages, so that the chosen final clones have the desired characteristics.

The primary purpose of cloning is the fractionation and purification of the cells producing the desired monoclonal antibody (McAb). The identification of those few clones which express a desired antibody is one of the most important aspects of the hybridoma technology. Strategies and methods for the whole procedure have been critically discussed elsewhere (e.g. [12] and Chapter 13).

The hybrid myeloma procedure permits, therefore, the dissection of the immune response by immortalization and cloning of the antibody-producing cells (Fig. 107.2). The derived monoclonal antibodies in turn allow the dissection of the antigenic determinants which triggered the immune response. For instance,