HANDBOOK OF EXPERIMENTAL IMMUNOLOGY
IN FOUR VOLUMES

Volume 4: Applications of Immunological Methods in Biomedical Sciences

EDITED BY

D. M. WEIR MD, FRCP

CO-EDITORS

Leonore A. Herzenberg DSc

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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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Massachusetts 02108, 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 Reseach, 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*

V. 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, Berkelev, 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 immunology. 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.

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Chapter 107
Overview: monoclonal antibodies

C. MILSTEIN

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 spectrotype), 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

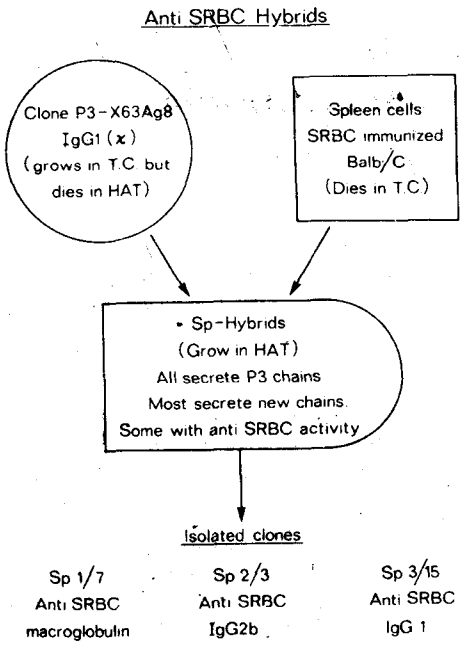

Fig. 107.1 Immortalization of cells producing specific antibody of predefined specificity. P3-X63 Ag 8 is an azaguanine resistant (HPGRT$^-$) myeloma cell line which actively secretes a P3 myeloma protein. After fusion with cells from an immunized mouse, hybrids are derived which grow in tissue culture (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$^-$). Such mutants are selected among cells resistant to azaguanine or thioguanine, either of which is a toxic analogue incorporated by HPGRT. Hybrids between HPGRT$^-$ mutant myelomas and normal lymphocytes can grow in HAT selective medium because they contain the HPGRT provided by the lymphocytes, while the HPGRT$^-$ 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 synkaryons, 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,