

# **Diagnostic and Therapeutic Antibodies**

## METHODS IN MOLECULAR MEDICINE™

---

*John M. Walker*, SERIES EDITOR

- Gene Therapy Protocols**, edited by *Paul D. Robbins*, 1997
- Herpes Simplex Virus Protocols**, edited by *Moira S. Brown*  
and *Alisdair MacLean*, 1997
- Helicobacter pylori* Protocols**, edited by *Christopher L. Clayton*  
and *Harry T. Mobley*, 1997
- Lectins in Medical Research**, edited by *Jonathan M. Rhodes*  
and *Jeremy D. Milton*, 1997
- Human Cell Culture Protocols**, edited by *Gareth E. Jones*, 1996
- Antisense Therapeutics**, edited by *Sudhir Agrawal*, 1996
- Vaccine Protocols**, edited by *Andrew Robinson*, *Graham H. Farrar*,  
and *Christopher N. Wiblin*, 1996
- Prion Diseases**, edited by *Harry F. Baker* and *Rosalind M. Ridley*, 1996
- Molecular Diagnosis of Cancer**, edited by *Finbarr Cotter*, 1996
- Molecular Diagnosis of Genetic Diseases**, edited by *Rob Elles*, 1996

METHODS IN MOLECULAR MEDICINE™

# Diagnostic and Therapeutic Antibodies

Edited by

**Andrew J. T. George**

*Department of Immunology, Imperial College School of Medicine,  
Hammersmith Hospital, London, UK*

and

**Catherine E. Urch**

*Department of Pharmacology, University College, London, UK*

Humana Press



Totowa, New Jersey

© 2000 Humana Press Inc.  
999 Riverview Drive, Suite 208  
Totowa, New Jersey 07512

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

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

This publication is printed on acid-free paper.   
ANSI Z39.48-1984 (American Standards Institute) Permanence of Paper for Printed Library Materials.

The cover illustration shows a model of the entire human IgG<sub>1</sub> molecule. It was generated using the RasMol program (1) from a composite model generated by Dr. Eduardo Padlan of the National Institutes of Health, Bethesda, MD, using coordinates for F(ab')<sub>2</sub> and Fc fragments and theoretical modeling of the hinge region. The model is described in Padlan (2). The PDB file is available from <http://www.umass.edu/microbio/rasmol/padlan.htm>.

1. Sayle, R. A. and Milner, W.-E. J. (1995) RasMol: biomolecular graphics for all. *Trends Biochem. Sci.* **20**, 374.
2. Padlan, E. A. (1994) Anatomy of the antibody molecule. *Mol. Immunol.* **31**, 169–217.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: [humana@humanapr.com](mailto:humana@humanapr.com)

#### Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$10.00 per copy, plus US \$00.25 per page, is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [0-89603-798-3/00 \$10.00 + \$00.25].

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

Library of Congress Cataloging in Publication Data

Main entry under title:

Methods in molecular medicine™.

Diagnostic and therapeutic antibodies / edited by Andrew J. T. George and Catherine E. Urch.

p. cm. -- (Methods in molecular medicine™)

Includes index.

ISBN 0-89603-798-3 (alk. paper)

1. Monoclonal antibodies—Therapeutic use. 2. Immunoglobulins—Therapeutic use. 3. Immunotherapy. 4. Monoclonal antibodies—Diagnostic use. I. George, Andrew J. T. II. Urch, Catherine E. III. Series.

[DNLM: 1. Antibodies, Monoclonal—diagnostic use. 2. Antibodies, Monoclonal—Therapeutic use. 3. Immunotherapy—methods.

QW 575.5.A6 D536 2000]

RM282.M65D53

2000

---

## Preface

Soon after the first description of monoclonal antibodies in 1976, there was enormous interest in the clinical application of antibodies, especially in the context of cancer. Antibodies appeared to offer the “magic bullet” that would allow the specific destruction of neoplastic cells. However, many years’ effort resulted in very few cases of successful immunotherapy with antibodies. As a result there was a major backlash against antibody therapy, and the field lost a considerable amount of popularity.

Fashion, in science as well as in other things, tends to be cyclical. Antibody-based therapy is once again attracting scientists and clinicians. There are several reasons for the renewed optimism; certainly the experience of the last two decades has provided a wealth of information about problems associated with antibody therapy, and possible solutions to these problems. Recombinant antibody engineering has rejuvenated the field, allowing both the modification of antibodies to improve their *in vivo* properties and the isolation of novel antibody molecules by such techniques as phage display. The results of recent clinical trials have demonstrated unequivocally the benefit of antibody therapy in a number of settings, and, finally, more careful consideration has been taken of the types of disease best treated using this approach.

The result is a more realistic climate of opinion, one in which antibody therapies are seen to have a role to play in the clinical management of patients, but are not seen as the panacea for all disease. *Diagnostic and Therapeutic Antibodies* is, therefore, especially timely, and is aimed at a new generation of clinicians and scientists who are entering the field and need to know both the background to the subject and also gain real competence in the basic techniques that they will be using. The book covers both theoretical and practical aspects of the clinical use of antibodies. It also looks at the *in vitro* diagnostic application of antibodies, an area where the impact of monoclonal antibodies has been enormous and con-

sistent.

To that end the book is divided into four sections. The first acts as a short introduction to the basic science of the antibody molecule, including its structure and how to generate antibodies. The second section is a series of reviews looking at different applications of antibodies in the clinic (including clinical laboratories). Such a section might seem unusual for a book in the *Methods in Molecular Medicine* series, but as every reader will have in mind a different *in vivo* application, this will allow them to get a picture of how antibodies can be used in varied clinical settings. It will also allow a certain degree of cross fertilization between different clinical disciplines. The third section covers the interaction between industry and the basic scientist. Such interaction is vital for the scientist to understand; if researchers have any ambitions to see their antibody in wide-scale clinical use, they will need to involve pharmaceutical and/or biotechnology companies. If they are to do this, then the nature of the intellectual property and the practicalities of its management need to be considered. This section consists of two chapters, one outlining the essentials of intellectual property and the second giving the case history of one antibody, CAMPATH-1, and the disasters and triumphs that accompanied its progress to the marketplace.

The final section contains a series of protocols that will be of use to people new to the field. The first set gives methods for producing and purifying antibodies, as well as the quality control procedures that are needed in preparing material for the clinic. The second set describes how to modify antibodies for clinical application, and how to measure the affinity and immunoreactivity of the molecules. The use of antibodies in a variety of *in vitro* assays and staining procedures is then given. Finally, a pair of chapters outline basic protocols for the early stages in antibody engineering.

The antibody is an extremely versatile molecule, with a myriad of potential applications. We trust that in *Diagnostic and Therapeutic Antibodies* we have collected together a series of chapters that will both inspire readers to explore some of the possibilities, and give them the basic theoretical and practical tools to start this task. We are extremely grateful to all the authors who have given their time, expertise, and energy to this project.

Finally, we would like to dedicate this book to Philippa, who was born as we started this project and without whom it would have been completed much sooner.

*Andrew J. T. George*  
*Catherine E. Urch*

---

# Contents

Preface .....	v
Contributors .....	xiii
PART I INTRODUCTION	
1 The Antibody Molecule <b>Andrew J. T. George</b> .....	1
2 Polyclonal and Monoclonal Antibodies <b>Mary A. Ritter</b> .....	23
3 Engineering Antibody Molecules <b>Rakesh Verma and Ekaterini Boleti</b> .....	35
4 Phage Display Technology <b>Michael Johns</b> .....	53
PART II ANTIBODIES IN MEDICINE	
5 Prospects for the Application of Antibodies in Medicine <b>Herman Waldmann</b> .....	63
6 Antibodies for Neoplastic Disease: <i>Solid Tumors</i> <b>Ian T. W. Matthews</b> .....	73
7 The Application of Monoclonal Antibodies in the Treatment of Lymphoma <b>Martin J. Glennie, Jamie Honeychurch, Ruth R. French, and Alison L. Tutt</b> .....	85
8 Antibodies for Inflammatory Disease: <i>Effector Cells</i> <b>Richard Smith</b> .....	99
9 Antibodies for Inflammatory Disease: <i>Cytokines</i> <b>Peter C. Taylor</b> .....	115
10 Antibodies for Transplantation <b>Denise L. Faustman</b> .....	141
11 Antibody-Based Therapies in Infectious Diseases <b>H. Barbaros Oral and Cezmi A. Akdis</b> .....	157



12	Antibodies in Nuclear Medicine <b>A. Michael Peters</b> .....	179
13	Animal Models for Tumor Localization <b>Gail Rowlinson-Busza</b> .....	193
14	Antibodies for Immunoassays <b>David J. Newman</b> .....	209
PART III ETHICS AND INDUSTRY		
15	Intellectual Property <b>William M. Brown</b> .....	227
16	From Laboratory to Clinic: <i>The Story of CAMPATH-1</i> <b>Geoff Hale and Herman Waldmann</b> .....	243
PART IV PRODUCTION AND PURIFICATION		
17	Production of Monoclonal Antibodies <b>Jóna Freysdóttir</b> .....	267
18	Purification of Monoclonal Antibodies Using Protein A/G <b>Bridget Heelan</b> .....	281
19	Preparation of Monoclonal Antibodies Using Ion Exchange Chromatography <b>Maureen Power</b> .....	289
20	Quality Control of Raw Materials <b>Patrick Harrison and Geoff Hale</b> .....	295
21	Cell Banks and Stability of Antibody Production <b>Pru Bird and Geoff Hale</b> .....	303
22	Measurement of Endotoxin <b>Jenny Phillips, Patrick Harrison, and Geoff Hale</b> .....	309
23	Aseptic Vial Filling <b>Kuldip Bhamra, Patrick Harrison, and Geoff Hale</b> .....	313
24	Measurement of Antibody Concentrations by Hemagglutination <b>Jenny Phillips and Geoff Hale</b> .....	317
PART V MODIFICATION OF ANTIBODIES		
25	Enzymatic Digestion of Monoclonal Antibodies <b>Sarah M. Andrew</b> .....	323
26	How to Make Bispecific Antibodies <b>Ruth R. French</b> .....	331

27	Radiolabeling Monoclonal Antibodies <b>Calvin S. R. Gooden</b> .....	339
28	Determination of the Immunoreactivity of Radiolabeled Monoclonal Antibodies <b>Gail Rowlinson-Busza</b> .....	349
29	Use of Biosensors to Measure the Kinetics of Antibody–Antigen Interactions <b>Andrew J. T. George</b> .....	361
PART VI APPLICATION OF ANTIBODIES IN VITRO		
30	How to Set Up an ELISA <b>Bill Jordan</b> .....	371
31	Measurement of HAMA and Anti-Idiotypic Antibodies <b>Steve Nicholson</b> .....	379
32	SDS-PAGE and Western Blotting <b>Abdulhamid A. Al-Tubuly</b> .....	389
33	Flow Cytometric Analysis <b>Paul F. McKay</b> .....	405
34	Immunocytochemistry <b>Susan Van Noorden</b> .....	413
35	Immunolabeling for Electron Microscopy <b>Catherine E. Sarraf</b> .....	437
PART VII ANTIBODY ENGINEERING		
36	PCR of the V-Region <b>Rakesh Verma</b> .....	451
37	Phage Display Technology: <i>Protocols</i> <b>Michael Johns and Donald B. Palmer</b> .....	459
	Index .....	469

---

## Contributors

CEZMI A. AKDIS • *Swiss Institute of Allergy and Asthma Research, Davos, Switzerland*

ABDULHAMID A. AL-TUBULY • *Department of Immunology and Medical Microbiology, Alfatah University for Medical Sciences, Tripoli, Libya*

SARAH M. ANDREW • *Department of Biology, Chester College of Higher Education, Chester, UK*

KULDIP BHAMRA • *Therapeutic Antibody Centre, Oxford, UK*

PRU BIRD • *Therapeutic Antibody Centre, Oxford, UK*

EKATERINI BOLETI • *Department of Clinical Oncology, The Royal London Hospital, London, UK*

WILLIAM M. BROWN • *Taro Pharmaceuticals USA Inc., Hawthorne, NY*

DENISE L. FAUSTMAN • *Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts  
General Hospital, Charlestown, MA*

RUTH R. FRENCH • *Lymphoma Research Unit, Tenovus Research Laboratories, Southampton General Hospital, Southampton, UK*

JÓNA FREYSDÓTTIR • *Department of Oral Medicine, Leeds Dental Institute, Leeds, UK*

ANDREW J. T. GEORGE • *Department of Immunology, Imperial College School of Medicine, London, UK*

MARTIN J. GLENNIE • *Lymphoma Research Unit, Tenovus Research Laboratories, Southampton General Hospital, Southampton, UK*

CALVIN S. R. GOODEN • *SmithKline Beecham Pharmaceuticals, Essex, UK*

GEOFF HALE • *Sir William Dunn School of Pathology, Oxford University, Oxford, UK*

PATRICK HARRISON • *Therapeutic Antibody Centre, Oxford, UK*

BRIDGET HEELAN • *Department of Immunology, Imperial College School of Medicine, London, UK*

JAMIE HONEYCHURCH • *Lymphoma Research Unit, Tenovus Research Laboratories, Southampton General Hospital, Southampton, UK*

MICHAEL JOHNS • *Department of Immunology, Imperial College School of*

*Medicine, London, UK*

BILL JORDAN • *Department of Immunology, Imperial College School of Medicine, London, UK*

IAN T. W. MATTHEWS • *ChemOvation Ltd., Horsham, UK*

PAUL F. MCKAY • *Department of Viral Pathogenesis, Beth Israel Deaconess Medical Center and Department of Medicine, Harvard Medical School, Boston, MA*

DAVID J. NEWMAN • *South West Thames Institute for Renal Research, Surrey, UK*

STEVE NICOLSON • *Department of Oncology, St. George's Hospital, Tooting, UK*

H. BARBAROS ORAL • *Immunology Unit, Department of Microbiology, School of Medicine, Uludag University, Bursa, Turkey*

DONALD B. PALMER • *Department of Immunology, Imperial College School of Medicine, London, UK*

A. MICHAEL PETERS • *Department of Nuclear Medicine, New Addenbrookes Hospital, Cambridge, UK*

JENNY PHILLIPS • *Therapeutic Antibody Centre, Oxford, UK*

MAUREEN POWER • *Lymphoma Research Unit, Tenovus Research Laboratories, Southampton General Hospital, Southampton, UK*

MARY A. RITTER • *Department of Immunology, Imperial College School of Medicine, London, UK*

GAIL ROWLINSON-BUSZA • *Antisoma Research Laboratories, St. George's Hospital Medical School, London, UK*

CATHERINE E. SARRAF • *Department of Histology, Imperial College School of Medicine, London, UK*

RICHARD SMITH • *Academic Renal Unit, Southmead Hospital, Bristol, UK*

PETER C. TAYLOR • *The Mathilda and Terence Kennedy Institute of Rheumatology, Hammersmith Hospital, London, UK*

ALISON L. TUTT • *Lymphoma Research Unit, Tenovus Research Laboratories, Southampton General Hospital, Southampton, UK*

CATHERINE E. URCH • *Department of Pharmacology, University College, London, UK*

SUSAN VAN NOORDEN • *Department of Histochemistry, Division of Diagnostic Sciences, Imperial College School of Medicine, London, UK*

RAKESH VERMA • *Department of Immunology, Imperial College School of Medicine, London, UK*

HERMAN WALDMANN • *Sir William Dunn School of Pathology, Oxford University, Oxford, UK*