

Complement Methods and Protocols

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

151. **Matrix Metalloproteinase Protocols**, edited by *Ian M. Clark*, 2000
150. **Complement Methods and Protocols**, edited by *B. Paul Morgan*, 2000
149. **The ELISA Guidebook**, edited by *John R. Crowther*, 2000
148. **DNA-Protein Interactions: Principles and Protocols (2nd ed.)**, edited by *Tom Moss*, 2000
147. **Affinity Chromatography: Methods and Protocols**, edited by *Pascal Bailon, George K. Ehrlich, Wen-Jian Fung, and Wolfgang Berthold*, 2000
146. **Protein and Peptide Analysis: New Mass Spectrometric Applications**, edited by *John R. Chapman*, 2000
145. **Bacterial Toxins: Methods and Protocols**, edited by *Otto Holst*, 2000
144. **Calpain Methods and Protocols**, edited by *John S. Elce*, 2000
143. **Protein Structure Prediction: Methods and Protocols**, edited by *David Webster*, 2000
142. **Transforming Growth Factor-Beta Protocols**, edited by *Philip H. Howe*, 2000
141. **Plant Hormone Protocols**, edited by *Jeremy A. Roberts and Gregory A. Tucker*, 2000
140. **Chaperonin Protocols**, edited by *Christine Schneider*, 2000
139. **Extracellular Matrix Protocols**, edited by *Charles Streuli and Michael Grant*, 2000
138. **Chemokine Protocols**, edited by *Amanda E. I. Proudfoot, Timothy N. C. Wells, and Christine Power*, 2000
137. **Developmental Biology Protocols, Volume III**, edited by *Rocky S. Tuan and Cecilia W. Lo*, 2000
136. **Developmental Biology Protocols, Volume II**, edited by *Rocky S. Tuan and Cecilia W. Lo*, 2000
135. **Developmental Biology Protocols, Volume I**, edited by *Rocky S. Tuan and Cecilia W. Lo*, 2000
134. **T Cell Protocols: Development and Activation**, edited by *Kelly P. Kearse*, 2000
133. **Gene Targeting Protocols**, edited by *Eric B. Kniec*, 2000
132. **Bioinformatics Methods and Protocols**, edited by *Stephen Misener and Stephen A. Krawetz*, 2000
131. **Flavoprotein Protocols**, edited by *S. K. Chapman and G. A. Reid*, 1999
130. **Transcription Factor Protocols**, edited by *Martin J. Tymms*, 2000
129. **Integrin Protocols**, edited by *Anthony Howlett*, 1999
128. **NMDA Protocols**, edited by *Min Li*, 1999
127. **Molecular Methods in Developmental Biology: Xenopus and Zebrafish**, edited by *Matthew Guille*, 1999
126. **Adrenergic Receptor Protocols**, edited by *Curtis A. Machida*, 2000
125. **Glycoprotein Methods and Protocols: The Mucins**, edited by *Anthony P. Corfield*, 2000
124. **Protein Kinase Protocols**, edited by *Alastair D. Reith*, 2000
123. **In Situ Hybridization Protocols (2nd ed.)**, edited by *Ian A. Darby*, 2000
122. **Confocal Microscopy Methods and Protocols**, edited by *Stephen W. Paddock*, 1999
121. **Natural Killer Cell Protocols: Cellular and Molecular Methods**, edited by *Kerry S. Campbell and Marco Colonna*, 2000
120. **Eicosanoid Protocols**, edited by *Elias A. Lianos*, 1999
119. **Chromatin Protocols**, edited by *Peter B. Becker*, 1999
118. **RNA-Protein Interaction Protocols**, edited by *Susan R. Haynes*, 1999
117. **Electron Microscopy Methods and Protocols**, edited by *M. A. Nasser Hajibagheri*, 1999
116. **Protein Lipidation Protocols**, edited by *Michael H. Gelb*, 1999
115. **Immunocytochemical Methods and Protocols (2nd ed.)**, edited by *Lorette C. Javois*, 1999
114. **Calcium Signaling Protocols**, edited by *David G. Lambert*, 1999
113. **DNA Repair Protocols: Eukaryotic Systems**, edited by *Daryl S. Henderson*, 1999
112. **2-D Proteome Analysis Protocols**, edited by *Andrew J. Link*, 1999
111. **Plant Cell Culture Protocols**, edited by *Robert D. Hall*, 1999
110. **Lipoprotein Protocols**, edited by *Jose M. Ordovas*, 1998
109. **Lipase and Phospholipase Protocols**, edited by *Mark H. Doolittle and Karen Reue*, 1999
108. **Free Radical and Antioxidant Protocols**, edited by *Donald Armstrong*, 1998
107. **Cytochrome P450 Protocols**, edited by *Ian R. Phillips and Elizabeth A. Shephard*, 1998
106. **Receptor Binding Techniques**, edited by *Mary Keen*, 1999
105. **Phospholipid Signaling Protocols**, edited by *Ian M. Bird*, 1998
104. **Mycoplasma Protocols**, edited by *Roger J. Miles and Robin A. J. Nicholas*, 1998
103. **Pichia Protocols**, edited by *David R. Higgins and James M. Cregg*, 1998
102. **Bioluminescence Methods and Protocols**, edited by *Robert A. LaRossa*, 1998
101. **Mycobacteria Protocols**, edited by *Tanya Parish and Neil G. Stoker*, 1998
100. **Nitric Oxide Protocols**, edited by *Michael A. Titheradge*, 1998
99. **Stress Response: Methods and Protocols**, edited by *Stephen M. Keyse*, 2000
98. **Forensic DNA Profiling Protocols**, edited by *Patrick J. Lincoln and James M. Thomson*, 1998
97. **Molecular Embryology: Methods and Protocols**, edited by *Paul T. Sharpe and Ivor Mason*, 1999
96. **Adhesion Protein Protocols**, edited by *Elisabetta Dejana and Monica Corada*, 1999
95. **DNA Topoisomerases Protocols: II. Enzymology and Drugs**, edited by *Mary-Ann Bjornstii and Neil Osheroff*, 1999
94. **DNA Topoisomerases Protocols: I. DNA Topology and Enzymes**, edited by *Mary-Ann Bjornstii and Neil Osheroff*, 1999
93. **Protein Phosphatase Protocols**, edited by *John W. Ludlow*, 1998
92. **PCR in Bioanalysis**, edited by *Stephen J. Meltzer*, 1998
91. **Flow Cytometry Protocols**, edited by *Mark J. Jaroszeski, Richard Heller, and Richard Gilbert*, 1998
90. **Drug-DNA Interaction Protocols**, edited by *Keith R. Fox*, 1998
89. **Retinoid Protocols**, edited by *Christopher Redfern*, 1998
88. **Protein Targeting Protocols**, edited by *Roger A. Clegg*, 1998
87. **Combinatorial Peptide Library Protocols**, edited by *Shmuel Cabilly*, 1998
86. **RNA Isolation and Characterization Protocols**, edited by *Ralph Rapley and David L. Manning*, 1998

METHODS IN MOLECULAR BIOLOGY™

Complement Methods and Protocols

Edited by

B. Paul Morgan

*Department of Medical Biochemistry
University of Wales College of Medicine
Cardiff, 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.

This publication is printed on acid-free paper. ∞

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

Cover design by Patricia F. Cleary.

Cover illustration:

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, or visit our Website at www.humanapress.com

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$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: [0-89603-654-5/00 \$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

Complement methods and protocols / edited by B. Paul Morgan
p. cm. -- (Methods in molecular biology; v. 150)

Includes bibliographical references and index.

ISBN 0-89603-654-5 (alk. paper)

1. Complement (Immunology)--Laboratory manuals. I. Morgan, B. Paul. II. Series

QR185.8.C6 C685 2000
616.07'997--dc21

99-058849

Preface

The complement system, first described more than a century ago, was for many years the ugly duckling of the immunology world, but no more. Complement in recent years has blossomed into a fascinating and fast moving field of immediate relevance to clinical scientists in fields as diverse as transplantation biology, virology, and inflammation. Despite its emergence from the shadows, complement retains an unwarranted reputation for being “difficult.” This impression derives in large part from the superficially complicated nomenclature, a relic of the long and tortuous process of unraveling the system, of naming components in order of discovery rather than in a systematic manner. Once the barrier of nomenclature has been surmounted, then the true simplicity of the system becomes apparent.

Complement comprises an activation system and a cytolytic system. The former has diverged to focus on complement to distinct targets—bacteria, immune complexes, and others—so that texts now describe three activation pathways, closely related to one another, but each with some unique features. The cytolytic pathway is the same regardless of the activation process and kills cells by creating pores in the membrane. Complement plays an important role in killing bacteria and is essential for the proper handling of immune complexes. Problems occur when complement is activated in an inappropriate manner—the potent inflammation-inducing products of the cascade then cause unwanted tissue damage and destruction.

Complement’s renaissance has been driven in large part by the discovery of the complement regulatory molecules and the realization that these molecules and other agents can provide effective anticomplement agents for use in therapy. As newer and better anticomplement agents become available, the requirement for laboratories to assess complement activation in clinical samples and to monitor the effects of anticomplement agents will grow.

Complement Methods and Protocols aims to provide a comprehensive source of up-to-date protocols for the study of the complement system, both for the basic scientist interested in understanding the mechanisms of activation and the clinical scientist wishing to quantify complement activation. In the first

chapter, the complement system is briefly reviewed to set the stage for the methods chapters to follow. The next two chapters describe methods for purifying complement components, using classical chromatography and immunoaffinity approaches, respectively. Chapters 4 to 6 describe methods for the functional analysis of complement components, regulators, enzymes, and complexes, including a detailed description of the generation of the depleted sera essential for complement assays. Methods for measurement of complement activation fragments and complexes deposited on cells, in tissues, or in biological fluids are detailed in Chapters 7 to 10. Chapter 11 provides an overview of screening methods for identifying and assessing complement deficiency and Chapter 12 a detailed account of methods needed to assess deficiency of C1 inhibitor. Other clinically relevant protocols for analysis of complement autoantibodies, immune complexes, and complement allotypes are provided in Chapters 13 to 15. Chapter 16 departs from the main theme of the book to describe protocols for generating gene-deleted mice, included here because of the enormous influence such methods are now having on complement research. The final chapter reviews complement deficiencies in experimental animals, listing the different complement deficiencies defined in animals and the experimental models in which these deficient animals have been examined.

I am grateful to my friends and colleagues who have contributed to this volume for their willingness to make time in their busy schedules. In particular, I wish to thank the members of the Complement Biology Group in Cardiff, many of whom have contributed chapters to this volume and others who have reviewed parts of the manuscript or contributed to the tedious task of assembling the appendices. I promise I won't do it again in a while! Finally, thanks to The Wellcome Trust for their continued and generous support of complement research in Cardiff.

B. Paul Morgan

Contents

Preface	v
Contributors	ix
1 The Complement System: <i>An Overview</i>	1
B. Paul Morgan	
2 Purification of Complement Components, Regulators, and Receptors by Classical Methods	15
Carmen W. van den Berg	
3 Immunoaffinity Methods for Purification of Complement Components and Regulators	53
B. Paul Morgan	
4 Measurement of Complement Hemolytic Activity, Generation of Complement-Depleted Sera, and Production of Hemolytic Intermediates	61
B. Paul Morgan	
5 Measurement of Complement Lysis of Nucleated Cells	73
O. Brad Spiller	
6 Functional Assays for Complement Regulators	83
Claire L. Harris	
7 Immunochemical Measurement of Complement Components and Activation Products	103
Reinhard Würzner	
8 Complement Deposition in Tissues	113
Antti Väkevä and Seppo Meri	
9 Complement Regulators and Receptors in Tissues	123
Juha Hakulinen and Seppo Meri	
10 Measurement of C3 Fragment Deposition on Cells	131
O. Brad Spiller	
11 Screening for Complement Deficiency	139
Ann Orren	
12 C1-Inhibitor: <i>Antigenic and Functional Analysis</i>	159
C. Erik Hack	

13	Autoantibodies to Complement Components	173
	<i>Kevin A. Davies and Peter Norsworthy</i>	
14	Allotyping of Complement Components	193
	<i>Reinhard Würzner</i>	
15	Complement and Immune Complexes	203
	<i>Julian T. Nash and Kevin A. Davies</i>	
16	Knocking Out Complement Genes	215
	<i>Anne E. Bygrave and Marina Botto</i>	
17	Inherited Complement Deficiencies in Animals	229
	<i>Stuart Linton</i>	
Appendices		
	Suppliers	249
	Sources of C Components and Anti-C Antibodies	258
	cDNA Accession Numbers for C Components, Regulators, and Receptors	260
	Index	263

Contributors

- MARINA BOTTO • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*
- ANNE E. BYGRAVE • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*
- KEVIN A. DAVIES • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*
- C. ERIK HACK • *CLB and Department of Internal Medicine, Academic Hospital of the Free University Amsterdam, Amsterdam, The Netherlands*
- JUHA HAKULINEN • *Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland*
- CLAIRE L. HARRIS • *Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff, UK*
- STUART LINTON • *Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff, UK*
- SEPPO MERI • *Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland*
- B. PAUL MORGAN • *Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff*
- JULIAN T. NASH • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*
- PETER NORSWORTHY • *Rheumatology Section, Division of Medicine, Imperial College School of Medicine, Hammersmith Campus, London, UK*
- ANN ORREN • *Department of Microbiology, National University of Ireland, Galway, Galway, Ireland*
- O. BRAD SPILLER • *Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff, UK*
- ANTTI VÄKEVÄ • *Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland*
- CARMEN W. VAN DEN BERG • *Department of Pharmacology, University of Wales College of Medicine, Heath Park, Cardiff, UK*
- REINHARD WÜRZNER • *Institut für Hygiene, Innsbruck University, Innsbruck, Austria*