

# **Allergy Methods and Protocols**

# METHODS IN MOLECULAR MEDICINE

*John M. Walker, SERIES EDITOR*

142. **New Antibiotic Targets**, edited by *W. Scott Champney*, 2008
141. **Clinical Bioinformatics**, edited by *Ronald J. A. Trent*, 2008
140. **Tissue Engineering**, edited by *Hansjörg Hauser and Martin M. Fussenegger*, 2008
139. **Vascular Biology Protocols**, edited by *Nair Sreejayan and Jun Ren*, 2008
138. **Allergy Methods and Protocols**, edited by *Meinir G. Jones and Penny Lympny*, 2008
137. **Microtubule Protocols**, edited by *Jun Zhou*, 2007
136. **Arthritis Research: Methods and Protocols**, Vol. 2, edited by *Andrew P. Cope*, 2007
135. **Arthritis Research: Methods and Protocols**, Vol. 1, edited by *Andrew P. Cope*, 2007
134. **Bone Marrow and Stem Cell Transplantation**, edited by *Meral Beksac*, 2007
133. **Cancer Radiotherapy**, edited by *Robert A. Huddart and Vedang Murthy*, 2007
132. **Single Cell Diagnostics: Methods and Protocols**, edited by *Alan Thornhill*, 2007
131. **Adenovirus Methods and Protocols**, Second Edition, Vol. 2: *Ad Proteins, RNA, Lifecycle, Host Interactions, and Phylogenetics*, edited by *William S. M. Wold and Ann E. Tollefson*, 2007
130. **Adenovirus Methods and Protocols**, Second Edition, Vol. 1: *Adenoviruses, Ad Vectors, Quantitation, and Animal Models*, edited by *William S. M. Wold and Ann E. Tollefson*, 2007
129. **Cardiovascular Disease: Methods and Protocols**, Volume 2: *Molecular Medicine*, edited by *Qing K. Wang*, 2006
128. **Cardiovascular Disease: Methods and Protocols**, Volume 1: *Genetics*, edited by *Qing K. Wang*, 2006
127. **DNA Vaccines: Methods and Protocols**, Second Edition, edited by *Mark W. Saltzman, Hong Shen, and Janet L. Brandsma*, 2006
126. **Congenital Heart Disease: Molecular Diagnostics**, edited by *Mary Kearns-Jonker*, 2006
125. **Myeloid Leukemia: Methods and Protocols**, edited by *Harry Iland, Mark Hertzberg, and Paula Marlton*, 2006
124. **Magnetic Resonance Imaging: Methods and Biologic Applications**, edited by *Pottumarthi V. Prasad*, 2006
123. **Marijuana and Cannabinoid Research: Methods and Protocols**, edited by *Emmanuel S. Onaivi*, 2006
122. **Placenta Research Methods and Protocols: Volume 2**, edited by *Michael J. Soares and Joan S. Hunt*, 2006
121. **Placenta Research Methods and Protocols: Volume 1**, edited by *Michael J. Soares and Joan S. Hunt*, 2006
120. **Breast Cancer Research Protocols**, edited by *Susan A. Brooks and Adrian Harris*, 2006
119. **Human Papillomaviruses: Methods and Protocols**, edited by *Clare Davy and John Doorbar*, 2005
118. **Antifungal Agents: Methods and Protocols**, edited by *Erika J. Ernst and P. David Rogers*, 2005
117. **Fibrosis Research: Methods and Protocols**, edited by *John Varga, David A. Brenner, and Sem H. Phan*, 2005
116. **Interferon Methods and Protocols**, edited by *Daniel J. J. Carr*, 2005
115. **Lymphoma: Methods and Protocols**, edited by *Timothy Illidge and Peter W. M. Johnson*, 2005
114. **Microarrays in Clinical Diagnostics**, edited by *Thomas O. Joos and Paolo Fortina*, 2005
113. **Multiple Myeloma: Methods and Protocols**, edited by *Ross D. Brown and P. Joy Ho*, 2005
112. **Molecular Cardiology: Methods and Protocols**, edited by *Zhongjie Sun*, 2005
111. **Chemosensitivity: Volume 2, In Vivo Models, Imaging, and Molecular Regulators**, edited by *Rosalyn D. Blumethal*, 2005
110. **Chemosensitivity: Volume 1, In Vitro Assays**, edited by *Rosalyn D. Blumethal*, 2005
109. **Adoptive Immunotherapy: Methods and Protocols**, edited by *Burkhard Ludewig and Matthias W. Hoffman*, 2005
108. **Hypertension: Methods and Protocols**, edited by *Jérôme P. Fennell and Andrew H. Baker*, 2005
107. **Human Cell Culture Protocols**, Second Edition, edited by *Joanna Picot*, 2005
106. **Antisense Therapeutics**, Second Edition, edited by *M. Ian Phillips*, 2005
105. **Developmental Hematopoiesis: Methods and Protocols**, edited by *Margaret H. Baron*, 2005
104. **Stroke Genomics: Methods and Reviews**, edited by *Simon J. Read and David Virley*, 2004
103. **Pancreatic Cancer: Methods and Protocols**, edited by *Gloria H. Su*, 2004
102. **Autoimmunity: Methods and Protocols**, edited by *Andras Perl*, 2004
101. **Cartilage and Osteoarthritis: Volume 2, Structure and In Vivo Analysis**, edited by *Frédéric De Ceuninck, Massimo Sabatini, and Philippe Pastoureau*, 2004
100. **Cartilage and Osteoarthritis: Volume 1, Cellular and Molecular Tools**, edited by *Massimo Sabatini, Philippe Pastoureau, and Frédéric De Ceuninck*, 2004
99. **Pain Research: Methods and Protocols**, edited by *David Z. Luo*, 2004
98. **Tumor Necrosis Factor: Methods and Protocols**, edited by *Angelo Corri and Pietro Ghezzi*, 2004

METHODS IN MOLECULAR MEDICINE

# Allergy Methods and Protocols

Edited by

**Meinir G. Jones**

*Department of Occupational and Environmental Medicine  
Imperial College  
London, United Kingdom*

**and**

**Penny Lympny**

*St. George's University of London  
London, United Kingdom*

 **Humana Press**

© 2008 Humana Press Inc.  
999 Riverview Drive, Suite 208  
Totowa, New Jersey 07512

**www.humanapress.com**

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 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.

Production Editor: Christina Thomas

Cover Design: Mark Buckley and Andrew F. Walls

Cover Illustration: From Ch. 24 Fig. 1.A. mast cells identified around the crypts in human colonic tissue, using an immunohistochemical procedure specific for tryptase.

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: orders@humanapr.com; or visit our Website: 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 \$30.00 per copy 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: [978-0-89603-896-7/08 \$30.00].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1  
eISBN: 978-1-59745-366-0

Library of Congress Control Number: 2007932488

---

## Preface

Allergy is a major problem in the 'Westernized' countries, and its prevalence continues to rise. It is therefore important to try and understand the reasons for the increase in allergy. Research in recent years has focused on the causes and mechanisms of allergy. In parallel, there is also an impetus to try to understand mechanisms of natural tolerance and immunotherapy where allergy is being dampened. This volume *Allergy Methods and Protocols* in the *Methods in Molecular Medicine* series aims to assist the researcher to gain an insight into the molecular mechanisms involved in allergy by featuring an array of protocols. These cover a range of disciplines including allergy, immunology, cell biology and histology and include methods to investigate the cellular response to allergens, cytokine profile, MHC restriction, T regulatory cells. The book is intended to be a useful bench tool for anyone embarking or continuing with their research in allergy. Techniques discussed include; B and T cell epitope mapping, characterisation of allergens, conjugation of haptens, preparation of monoclonal antibodies, collection and sampling of airborne allergens, IgG antibodies and facilitated antigen blocking assays, identification and purification of mast cells and *in situ* hybridisation. We thank all the authors who have shared their protocols with us and made this book possible.

*Meinir G. Jones*

---

# Contents

Preface .....	v
Contributors .....	ix
1. Understanding of the Molecular Mechanisms of Allergy .....	1
<i>Meinir Jones</i>	
2. T Cell — Primary Culture from Peripheral Blood .....	17
<i>Monika Raulf-Heimsoth</i>	
3. Production of T-Cell Lines .....	31
<i>Helga Kahlert</i>	
4. Production of Human T-Cell Clones .....	43
<i>Adrienne Verhoef</i>	
5. Mapping of Human T-Cell Epitopes of Allergens .....	51
<i>Thomas Zeiler and Tuomas Virtanen</i>	
6. Determining MHC Restriction of T-cell Responses .....	57
<i>Mark Larchè</i>	
7. Short-Term Culture of CD8 Cells and Intracellular Cytokine Staining .....	73
<i>Beejal Vyas and Alistair Noble</i>	
8. Isolation, Flow Cytometric Analysis, and Suppression Assay of CD4+ CD25+ T-Regulatory Cells .....	85
<i>Hayley Jeal</i>	
9. Monocyte-Derived Dendritic Cells as Antigen-Presenting Cells in T-Cell Proliferation and Cytokine Production .....	97
<i>Sun-sang J. Sung</i>	
10. Ultrasensitive ELISA for Measurement of Human Cytokine Responses in Primary Culture .....	107
<i>William P. Stefura, J. Darren Campbell, Renée Douville,     Monique J. Stinson, F. Estelle Simons, Allan B. Becker,     and Kent T. HayGlass</i>	
11. Quantification of Human Chemokine Production in TLR-Stimulated and Antigen-Specific Recall Responses ...	121
<i>Monique Stinson, Renee Douville, Yuriy Lissitsyn, Melanie     Blanchard, William Stefura, Estelle Simons, Allan Becker,     Peter Nickerson, Kevin Coombs, and Kent HayGlass</i>	

12.	Standardization of Allergen Extracts . . . . .	133
	<i>Jørgen Nedergaard Larsen and Sten Dreborg</i>	
13.	Immuno-electrophoresis for the Characterization of Allergen Extracts . . . . .	147
	<i>Gitte Nordskov Hansen and Jørgen Nedergaard Larsen</i>	
14.	Conjugation of Haptens . . . . .	167
	<i>Ranulfo Lemus and Meryl H. Karol</i>	
15.	Monoclonal Antibodies . . . . .	183
	<i>Helga Kahlert and Oliver Cromwell</i>	
16.	Purification of Antibodies . . . . .	197
	<i>Per H. Larsson</i>	
17.	Collection of Air Samples to Quantify Exposure to Airborne Allergens . . . . .	209
	<i>Susan Gordon</i>	
18.	Assay of Air Sample Eluates . . . . .	217
	<i>Anne Renström and Susan Gordon</i>	
19.	The Halogen Assay – A New Technique for Measuring Airborne Allergen . . . . .	227
	<i>Euan Tovey, Sandra De Lucca, Leanne Poulos and Tim O'Meara</i>	
20.	Measurement of Specific IgG Anti-Fel d 1 Antibodies . . . . .	247
	<i>Meinir G. Jones</i>	
21.	The Facilitated Antigen Binding (FAB) Assay – A Protocol to Measure Allergen-Specific Inhibitory Antibody Activity . . .	255
	<i>James N. Francis</i>	
22.	Microscopic Identification and Purity Determination of Pollen Grains . . . . .	263
	<i>Magdalena Rahl</i>	
23.	Biopanning for the Characterization of Allergen Mimotopes .	271
	<i>Isabella Pali-Schöll and Erika Jensen-Jarolim</i>	
24.	Identification of Mast Cells and Mast Cell Subpopulations . .	285
	<i>Mark Buckley and Andrew F. Walls</i>	
25.	Purification and Characterization of Mast Cell Tryptase and Chymase from Human Tissues . . . . .	299
	<i>Alan R. McEuen and Andrew F. Walls</i>	
26.	Experimental Activation of Mast Cells and Their Pharmacological Modulation . . . . .	319
	<i>Shaoheng He and Andrew F. Walls</i>	
27.	In situ Hybridization . . . . .	331
	<i>Kayhan T. Nouri-Aria</i>	
	Index . . . . .	349

---

## Contributors

- ALLAN B. BECKER • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- MELANIE BLANCHARD • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- MICHAEL BUCKLEY • *Immunopharmacology Group, Southampton General Hospital, Southampton*
- DARREN CAMPBELL • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- KEVIN COOMBS • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- OLIVER CROMWELL • *Allergopharma Joachim Ganzer, Reinbek, Germany*
- SANDRA DE LUCCA • *Department of Medicine, University of Sydney, Australia*
- STEN DREBORG • *Lerum, Sweden*
- RENÉE DOUVILLE • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- JAMES N. FRANCIS • *Department of Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College, South Kensington, London*
- SUSAN GORDON • *Institute of Occupational Medicine, Research Park North, Riccarton, Edinburgh, Scotland*
- GITTE NORDSKOV HANSEN • *ALK-Abello, Denmark*
- KENT T. HAYGLASS • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- SHAOHENG HE • *Immunopharmacology Group, Southampton General Hospital, Southampton*
- HAYLEY JEAL • *Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Imperial College School of Medicine, London, UK*
- ERIKA JENSEN-JAROLIM • *Medical University of Vienna, Vienna, Austria*
- MEINIR JONES • *Department of Occupational and Environmental Medicine, National Heart and Lung Institute, Imperial College School of Medicine, London, UK*
- HELGA KAHLERT • *Allergopharma Joachim Ganzer KG, Germany*
- MERYL KAROL • *Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA*



- MARK LARCHE • *Department of Allergy and Clinical Immunology, Imperial College London, National Heart and Lung Institute, London, UK*
- JØRGEN NEDERGAARD LARSEN • *ALK-Abello, Horsholm, Denmark*
- PER H. LARSSON • *Mabtech AB, Nacka Strand, Sweden*
- RANULFO LEMUS • *Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA*
- YURIY LISSITSYN • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- ALAN R. MCEUEN • *Immunopharmacology Group, Southampton General Hospital, Southampton, UK*
- PETER NICKERSON • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- ALISTAIR NOBLE • *Department of Asthma, Allergy and Respiratory Science, King's College London, London, UK*
- KAYHAN T. NOURI-ARIA • *Department of Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College, South Kensington, London, UK*
- TIM O'MEARA • *Department of Medicine, University of Sydney, Australia*
- LEANNE POULOS • *Department of Medicine, University of Sydney, Australia*
- MAGDALENA RAHL • *ALLERGON AB, Ångelholm, Sweden*
- MONIKA RAULF-HEIMSOTH • *Institut der Ruhr-Universität Bochum, Bereich Allergologie/Immunologie, Germany*
- ANNE RENSTRÖM • *Lung-och allergiforskning, Karolinska Institutet, Stockholm, Sweden*
- ISABELLA SCHÖLL • *Centre of Physiology and Pathophysiology, Medical University of Vienna, Vienna, Austria*
- F ESTELLE SIMONS • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- WILLIAM P. STEFURA • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- MONIQUE J. STINSON • *Department of Immunology, University of Manitoba, Winnipeg, Canada*
- SUN-SANG J. SUNG • *Division of Rheumatology, University of Virginia Health Sciences Center, Charlottesville, VA*
- EUAN TOVEY • *Department of Medicine, University of Sydney, Australia*
- ADRIENNE VERHOEF • *Department of Allergy and Clinical Immunology, National Heart and Lung Institute, Imperial College School of Medicine, South Kensington, London, UK*
- TUOMAS VIRTANEN • *Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland*

BEEJAL VYAS • *Department of Asthma, Allergy and Respiratory Science, King's College London, London, UK*

ANDREW F. WALLS • *Immunopharmacology Group, Southampton General Hospital, Southampton, UK*

THOMAS ZEILER • *Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland*