Hepatitis B and D Protocols

Volume 1

METHODS IN MOLECULAR MEDICINE™

John M. Walker, SERIES EDITOR

99. **Pain Research: Methods and Protocols**, edited by David Z. Luo, 2004
98. **Tumor Necrosis Factor: Methods and Protocols**, edited by Angelo Corti and Pietro Ghezzi, 2004
97. **Molecular Diagnosis of Cancer: Methods and Protocols, Second Edition**, edited by Joseph E. Roulston and John M. S. Bartlett, 2004
96. **Hepatitis B and D Protocols: Volume 2, Immunology, Model Systems, and Clinical Studies**, edited by Robert K. Hamatake and Johnson Y. N. Lau, 2004
95. **Hepatitis B and D Protocols: Volume 1, Detection, Genotypes, and Characterization**, edited by Robert K. Hamatake and Johnson Y. N. Lau, 2004
94. **Molecular Diagnosis of Infectious Diseases, Second Edition**, edited by Jochen Decker and Udo Reischl, 2004
93. **Anticoagulants, Antiplatelets, and Thrombolytics**, edited by Shaker A. Mousa, 2004
92. **Molecular Diagnosis of Genetic Diseases, Second Edition**, edited by Rob Elles and Roger Mountford, 2004
91. **Pediatric Hematology: Methods and Protocols**, edited by Nicholas J. Goulden and Colin G. Steward, 2003
90. **Suicide Gene Therapy: Methods and Reviews**, edited by Caroline J. Springer, 2004
89. **The Blood–Brain Barrier: Biology and Research Protocols**, edited by Sukriti Nag, 2003
88. **Cancer Cell Culture: Methods and Protocols**, edited by Simon P. Langdon, 2003
87. **Vaccine Protocols, Second Edition**, edited by Andrew Robinson, Michael J. Hudson, and Martin P. Cranage, 2003
86. **Renal Disease: Techniques and Protocols**, edited by Michael S. Goligorsky, 2003
85. **Novel Anticancer Drug Protocols**, edited by John K. Buolamwini and Alex A. Adjei, 2003
84. **Opioid Research: Methods and Protocols**, edited by Zhizhong Z. Pan, 2003
83. **Diabetes Mellitus: Methods and Protocols**, edited by Sabire Özcan, 2003
82. **Hemoglobin Disorders: Molecular Methods and Protocols**, edited by Ronald L. Nagel, 2003
81. **Prostate Cancer Methods and Protocols**, edited by Pamela J. Russell, Paul Jackson, and Elizabeth A. Kingsley, 2003
80. **Bone Research Protocols**, edited by Miep H. Helfrich and Stuart H. Ralston, 2003
79. **Drugs of Abuse: Neurological Reviews and Protocols**, edited by John Q. Wang, 2003
78. **Wound Healing: Methods and Protocols**, edited by Luisa A. DiPietro and Aime L. Burns, 2003
77. **Psychiatric Genetics: Methods and Reviews**, edited by Marion Leboyer and Frank Bellivier, 2003
76. **Viral Vectors for Gene Therapy: Methods and Protocols**, edited by Curtis A. Machida, 2003
75. **Lung Cancer: Volume 2, Diagnostic and Therapeutic Methods and Reviews**, edited by Barbara Driscoll, 2003
74. **Lung Cancer: Volume 1, Molecular Pathology Methods and Reviews**, edited by Barbara Driscoll, 2003
73. ***E. coli*: Shiga Toxin Methods and Protocols**, edited by Dana Philpott and Frank Ebel, 2003
72. **Malaria Methods and Protocols**, edited by Denise L. Doolan, 2002
71. ***Haemophilus influenzae* Protocols**, edited by Mark A. Herbert, Derek Hood, and E. Richard Moxon, 2002
70. **Cystic Fibrosis Methods and Protocols**, edited by William R. Skach, 2002
69. **Gene Therapy Protocols, Second Edition**, edited by Jeffrey R. Morgan, 2002
68. **Molecular Analysis of Cancer**, edited by Jacqueline Boultonwood and Carrie Fidler, 2002

METHODS IN MOLECULAR MEDICINE™

Hepatitis B and D Protocols

*Volume 1: Detection, Genotypes,
and Characterization*

Edited by

Robert K. Hamatake, PhD

Johnson Y. N. Lau, MD

Ribapharm Inc., Costa Mesa, CA

Forewords by

Timothy M. Block, PhD and Baruch S. Blumberg, MD, PhD
T. Jake Liang, MD

Humana Press

Totowa, New Jersey

© 2004 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 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.

Cover design by Patricia F. Cleary.

Cover illustration: HBV genome. Artwork supplied by Robert Hamatake, PhD. Background: Figure 1, Chapter 13, K. Abid and F. Negro.

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 \$25.00 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: [1-58829-105-7/04 \$25.00].

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

ISSN 1543-1894

Library of Congress Cataloging in Publication Data

Hepatitis B and D protocols / edited by Robert K. Hamatake, Johnson Y. N. Lau
p. ; cm. —(methods in molecular medicine; 95-96)

Includes bibliographical references and indexes.

Contents: v. 1. Detection, genotypes, and characterization—v. 2. Immunology, model systems, and clinical studies.

ISBN 1-58829-105-7 (v. 1 : alk. paper) E-ISBN 1-59259-669-X (v. 1) — ISBN 1-58829-108-1 (v. 2 : alk. paper) E-ISBN 1-59259-670-3 (v. 2)

1. Hepatitis B—Laboratory manuals. 2. Hepatitis B virus—Laboratory manuals. 3. Delta-associated agent—Laboratory manuals. 4. Delta infection—Laboratory manuals. I. Hamatake, Robert Kiyoshi, 1953— II. Lau, Johnson Y. N. (Johnson Yiu-Nam). III. Series

[DNLM: 1. Hepatitis B. 2. Hepatitis D. WC 536 H53335 2004]

QR201.H46H43 2004

616.3'623-1—dc21

2003011624

*To our wives, April T.G. Hamatake and Jane W.S. Fang-Lau,
for their unfailing support, and our children, Kyle Hamatake,
David Hamatake, Grace (Ling-Ling) Lau, Gillian (Gi-Gi) Lau,
and Gabrielle (Ching-Ching) Lau, for keeping us rejuvenated.*

Foreword

Despite the availability of an effective vaccine, there are still 400 million people, worldwide who are chronically infected with hepatitis B virus (HBV). For them, the vaccine, as currently applied, has no value. Given the possible consequences of HBV infection, the number of those chronically infected with HBV presents an enormous public health challenge. For example, the major etiology of hepatocellular carcinoma (HCC) is chronic infection with HBV. Although fifth in cancer incidence, worldwide, HCC/liver cancer is the third leading cause of cancer death. The high mortality associated with HCC arises because the disease is often detected late and is unresponsive to treatment. The number of deaths caused by PHCC is expected to rise over the next 20 years. Those chronically infected with HBV have a life risk of death to HCC of between 10 and 25%. Even the limited efficacy of drugs for the treatment of chronic HBV helps underscore the point that this disease is responsive to therapy. Drugs that target the polymerase (e.g., hepsara and lamivudine) and interferon alpha represent two distinct strategies and show that both conventional antiviral and immunotherapeutic approaches can be used in management. However, the current inventory of therapeutics is inadequate. Interferon alpha is of limited value, only parenterally available, and fraught with adverse reactions. The polymerase inhibitors are undermined by either a high frequency of resistance or, in the case of hepsara, additional concerns about toxicity. Other areas of concern also remain. There is still no reliable means of predicting disease outcome for chronic carriers, making early detection of liver disease and decisions about drug efficacy, as well as who should be treated and for how long, difficult. Moreover, tests for HBV DNA levels (and other markers) are often of dubious quantitative value. Because HBV patients are becoming more sophisticated and more demanding about these markers and many follow their viremia (DNA) levels like investors follow the Dow Jones Industrial values, it is essential that meaningful lab values be provided. The current vaccines are effective, but they are expensive, and escape mutants may be a problem waiting to happen.

Thus, without a doubt, more work needs to be done in all of the key areas of the management of HBV.

The good news is that there have been important advances. A clearer picture about the kinds of drugs that can be effective, their pathogenesis, and the development of research tools should facilitate the discovery process.

It is the matter of research tools that these volumes address. In an almost encyclopedic fashion, Hamatake and Lau have assembled a comprehensive, “how to” guide for the study of HBV. It is worth noting that many of the methods described will have value in the study of other viruses and liver diseases in general, despite being highly focused upon HBV.

This is a very practical resource, in every respect. In more than 40 chapters, leaders in the field who have “been there” have contributed step-by-step “programmed” approaches,

telling the readers how to reproduce their findings. It is something of a users guide for do-it-yourselfers.

It is not an overstatement to write that nearly every aspect of HBV research is covered, section by section, virus detection through vaccine and drug development. Thus, *Hepatitis B and D Protocols* is likely to become as essential a resource to those studying HBV as was the fabled Maniatis series (published by Cold Spring Harbor Press) for Molecular Biology. It is certainly going to get a great deal of attention in the Hepatitis B Foundation labs, and, because of its value, we hope it is used in labs throughout the world. The more it is used, the more likely HBV research will be advanced.

Timothy M. Block, PhD

*Professor and Director of the Jefferson Center
and Hepatitis B Foundation Laboratory
Thomas Jefferson University
and President, Hepatitis B Foundation
Doylestown, PA*

Baruch S. Blumberg, MD, PhD

*Nobel Laureate, 1976
Senior Adviser to the President of Fox Chase Cancer Center
and Professor of Medicine and Anthropology
University of Pennsylvania
Philadelphia, PA*

Foreword

The identification of the “Australian antigen” by Dr. Blumberg, which was subsequently recognized to be the hepatitis B virus surface antigen, marks the discovery of an important pathogen for liver diseases. The virus was the first hepatitis virus discovered almost four decades ago. Despite the magnitude of the infection and the significant efforts by the scientific community, hepatitis B continues to be a global health problem affecting more than 350 million people worldwide. Many scientific milestones in basic and clinical research on hepatitis B had profound impact on this viral infection in the history of medicine: hepatitis B virus vaccine was introduced two decades ago; interferon alpha was approved for the treatment of chronic hepatitis B and two other antiviral drugs have also been approved; liver transplantation under high dose hepatitis B immunoglobulin coverage can now be offered to patients with end-stage hepatitis B related liver disease. These advances have also introduced new paradigms in our understanding of the fundamental concepts of viral infection in general. Certainly, more work looms ahead of us in order to ultimately conquer this viral infection. To equip the basic and clinical investigators with all the available tools is of utmost importance in this quest. As illustrated in this book, the marriage between basic science and clinical medicine is critical.

In the last few decades, we have witnessed the groundbreaking progress and the introduction of new clinical paradigms in hepatitis B virus infection. The next decade must underscore further application of our knowledge to translational research, bridging basic science and clinical medicine, and to capitalize on our understandings to conquer this viral infection. Undoubtedly, with all the scientific efforts contributed by many dedicated scientists and with our willingness to share knowledge, the common goal of improving human health by eradicating this virus can be achieved in this millennium.

T. Jake Liang, MD
Chief, Liver Diseases Section
NIDDK National Institutes of Health
Bethesda, MD

Preface

The 36 years since the identification of hepatitis B virus (HBV) in 1967 has seen the establishment of HBV virology as a rich and extensive field of science and the recognition of HBV as one of the top 10 leading causes of death in the world. There are an estimated 400 million people chronically infected with HBV worldwide with 20–30% of these projected to die from complications of chronic liver disease, including cirrhosis and liver cancer. Hepatitis delta virus (HDV) was first identified in 1980 as a novel transmissible agent distinct from HBV that requires the HBV envelope protein for its infectivity. HDV co-infection in chronic HBV patients is associated with a more severe disease outcome than those not co-infected. HBV and HDV infections represent a significant health burden to the worldwide community.

Despite the lack of an inflexible cell culture system, an enormous amount of information has been obtained on the virology and immunology of HBV and HDV infections. Modern molecular biological techniques and research with related viruses have enabled much of the HBV and HDV life cycle to be elucidated, leading to a number of therapeutic approaches for treating chronically infected patients. Innovative detection methods based on these findings and techniques have led to diagnostic and clinical monitoring assays of increasing sensitivity. However, our understanding of the viral life cycle and virus–host interactions is incomplete and will require much more research for a complete comprehension of the pathogenesis of disease caused by viral infection. Hepatitis B and D Protocols contains a collection of research techniques, divided into two volumes, used in the study of HBV and HDV. The authors represent a number of scientific disciplines, but share in common their interest in hepatitis research and their expertise in their respective areas. Although most of these techniques have been described in peer-reviewed journals, these chapters provide far more detail and are written so that investigators can use them as manuals. A few reviews are included in some specialized areas such as antiviral testing and the design of clinical trials. We hope that this compilation of techniques used in the different areas of HBV and HDV research will prove useful to scientists and encourage multidisciplinary approaches to their research, so that clinical investigators will find it beneficial for their understanding of the current HBV and HDV research.

We would like to take this opportunity to extend our gratitude to our teachers and friends: Dr. Zhi Hong, Dr. Maria Seifer, Dr. David Standring, Professor P. C. Wu, Professor C. L. Lai, Dr. H. J. Lin, Professor Roger Williams, and Dr. Graeme J. M. Alexander. Without their guidance and support, we would not have the opportunity of presenting this highly practical manual to you today.

*Robert K. Hamatake, PhD
Johnson Yiu-Nam Lau, MD*

Contents

Dedication	v
Foreword by Timothy M. Block and Baruch S. Blumberg.....	vii
Foreword by T. Jake Liang.....	ix
Preface	xi
Contributors	xvii
Contents for Volume 2	xxi

PART I. DETECTION OF VIRAL MARKERS

1 Overview of Commercial HBV Assay Systems Stefan Zeuzem	3
2 Detection of HBV DNA in Serum Using a PCR-Based Assay Hau Tim Chung	15
3 Detection of HBV DNA by Oligonucleotide Probing Hsiang Ju Lin	21
4 Detection of HBV RNA in Serum of Patients Wei Zhang, Hans Jörg Hacker, Maria Mildenberger, Qin Su, and Claus H. Schröder	29
5 Quantification of HBV Covalently Closed Circular DNA from Liver Tissue by Real-Time PCR Scott Bowden, Kathy Jackson, Margaret Littlejohn, and Stephen Locarnini	41
6 <i>In Situ</i> Hybridization for the Detection and Localization of HBV DNA in Liver Sections Vicky C. H. Lai and Johnson Y. N. Lau	51
7 Quantitative Assay of Hepatitis B Surface Antigen in Serum or Plasma Using Laurell Electrophoresis Wolfram H. Gerlich, Ulrike Wend, and Dieter Glebe	57
8 <i>In Situ</i> Detection of Hepatitis B Viral Antigens: <i>An Immunohistochemical Approach</i> Jane W. S. Fang and Johnson Y. N. Lau	65
9 Detection of Hepatitis B Virus X Antigen by Immunohistochemistry and Western Blotting Jie Liu and Mark A. Feitelson	71

10	Detection of Serum HDV RNA by RT-PCR <i>Antonina Smedile, Maria Grazia Niro, and Mario Rizzetto</i>	85
11	Nonradioisotopic <i>In Situ</i> Hybridization for HDV RNA <i>Francesco Negro</i>	95
12	Detection and Characterization of Small and Large HDV Antigens <i>Thomas B. Macnaughton and Michael M. C. Lai</i>	99
13	Immunohistochemical Detection of Hepatitis Delta Antigen <i>Karim Abid and Francesco Negro</i>	107

PART II. VIRAL GENOTYPES AND VARIANTS

14	HBV Vaccine-Escape Variants <i>Ashraf A. Basuni and William F. Carman</i>	115
15	HBV Genotyping and Analysis for Unique Mutations <i>Anna Ayres, Stephen Locarnini, and Angeline Bartholomeusz</i>	125
16	A One-Filter–Three-Probe Assay for Defective Interference (DI) Effects of Naturally Occurring Core Internal Deletion (CID) Variants of Human Hepatitis B Virus <i>Chiaho Shih and Ta-Tung Thomas Yuan</i>	151
17	Detection of Hypermodified Middle-Envelope (M) Proteins Secreted from Naturally Occurring HBV Variants Containing a preS2 Internal Deletion <i>Chiaho Shih and Pei-Ching Tai</i>	165
18	Hepatitis B Viral Genotyping with the Research INNO-LiPA HBV Genotyping Line Probe Assay <i>Sija De Gendt, Fred Shapiro, Jelena Juras, Els Van Assche, Geert Maertens, and Erwin Sablon</i>	175

PART III. MOLECULAR BIOLOGICAL CHARACTERIZATION

19	Processing of Hepatitis B Virus Surface Proteins <i>Volker Bruss</i>	189
20	Binding of Duck Carboxypeptidase D to Duck Hepatitis B Virus <i>Stephan Urban</i>	199
21	Nucleoprotein Transport of HBV Capsid Particles <i>Michael Kann</i>	213
22	Phosphorylation Analysis of Hepatitis B Virus Core Protein in Mammalian Cells <i>Jie Li, Yanyan Zheng, Jinah Choi, and Jing-hsiung Ou</i>	227
23	Study of HBV Replication Capacity in Relation to Sequence Variation in the Precore and Core Promoter Regions <i>Fabien Zoulim, ShuPing Tong, and Christian Trépo</i>	235

24 A *cis/trans* Genetic Test for Pleiotropic Phenotypes
Associated with a Frequent Naturally Occurring Mutation
at Amino Acid 97 of HBV Core Protein
Chiaho Shih and Ta-Tung Thomas Yuan 247

25 Studying DHBV Polymerase by In Vitro Transcription
and Translation
Jianming Hu 259

26 Expression and Purification of Functional Hepatitis B Virus
Polymerase in the Baculovirus Insect Cell System
Lisa Lott, Lena Notvall, and Robert E. Lanford..... 271

27 Localization of Duck Hepatitis B Virus Polymerase Within Cells
Ermei Yao and John E. Tavis 281

28 Endogenous Polymerase Assay for the Analysis
of Hepatitis B Virus in Transgenic Mice
Zhenming Xu and Jing-hsiung Ou 295

29 Transcriptional Control of Hepatitis B Virus
Anneke K. Raney 303

30 In Vitro Reconstitution of ϵ -Dependent Duck Hepatitis B Virus
Replication Initiation
Jürgen Beck and Michael Nassal 315

31 Hepatitis B Viruses: A *Triple Threat* for Malignant Transformation
of Hepatocytes
Charles E. Rogler 327

Index 339

Contributors

- KARIM ABID • *Division of Gastroenterology and Hepatology, University Hospital, Geneva, Switzerland*
- ANNA AYRES • *Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia*
- ANGELINE BARTHOLOMEUSZ • *Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia*
- ASHRAF A. BASUNI • *West of Scotland Specialist Virology Centre, Gartnavel General Hospital, Glasgow, Scotland, UK*
- JÜRGEN BECK • *Department of Internal Medicine II / Molecular Biology, University Hospital Freiburg, Freiburg, Germany*
- SCOTT BOWDEN • *Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia*
- VOLKER BRUSS • *Department of Virology, University of Goettingen, Göttingen, Germany*
- W. F. CARMAN • *West of Scotland Specialist Virology Centre, Gartnavel General Hospital, Glasgow, Scotland, UK*
- JINAH CHOI • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
- HAU TIM CHUNG • *5/25 Anderson St., Chatswood NSW 2067 Australia*
- SJIA DE GENDT • *Hepatitis Diagnostics Program, Innogenetics N.V., Ghent, Belgium*
- JANE W. S. FANG • *Research and Development, Ribapharm, Inc, Costa Mesa, CA*
- MARK A. FEITELSON • *Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA*
- WOLFRAM H. GERLICH • *Institute of Medical Virology, Justus Liebig University, Giessen, Germany*
- DIETER GLEBE • *Institute of Medical Virology, Justus Liebig University, Giessen, Germany*
- HANS JÖRG HACKER • *Department of Virus Host Interactions, Deutsches Krebsforschungszentrum, Heidelberg, Germany*
- JIANMING HU • *Department of Microbiology, Boston University School of Medicine, Boston, MA*
- KATHY JACKSON • *Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia*
- JELENA JURAS • *Hepatitis Diagnostics Program, Innogenetics N.V., Ghent, Belgium*
- MICHAEL KANN • *Institute of Medical Virology, Justus Liebig University, Giessen, Germany*
- MICHAEL M.C. LAI • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
- VICKY C. H. LAI • *Research and Development, Ribapharm, Inc, Costa Mesa, CA*
- ROBERT E. LANFORD • *Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX*

- JOHNSON Y. N. LAU • *Former Chief Executive Officer, Ribapharm Inc., Costa Mesa, CA*
- JIE LI • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
- HSIANG JU LIN • *Falls Church, VA*
- MARGARET LITTLEJOHN • *Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia*
- JIE LIU • *Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA*
- STEPHEN LOCARNINI • *Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia*
- LISA LOTT • *Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX*
- GEERT MAERTENS • *Hepatitis Diagnostics Program, Innogenetics N.V., Ghent, Belgium*
- THOMAS B. MACNAUGHTON • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
- MARIA MILDENBERGER • *Department of Virus Host Interactions, Deutsches Krebsforschungszentrum, Heidelberg, Germany*
- MICHAEL NASSAL • *Department of Internal Medicine II / Molecular Biology, University Hospital Freiburg, Freiburg, Germany*
- FRANCESCO NEGRO • *Division of Clinical Pathology, University Hospital, Geneva, Switzerland*
- MARIA GRAZI NIRO • *Department of Gastroenterology, Casa Sollievo della Sofferenza, San Giovanni Rotondo, FG, Italy*
- LENA NOTVALL • *Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, TX*
- JING-HSIUNG OU • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*
- ANNEKE K. RANEY • *Research and Development, Ribapharm, Inc, Costa Mesa, CA*
- MARIO RIZZETTO • *Department of Gastroenterology, University of Torino and Azienda Ospedaliera S. Giovanni, Battista, Torino, Italy*
- CHARLES E. ROGLER • *Marion Bessin Liver Research Center, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY*
- ERWIN SABLON • *Hepatitis Diagnostics Program, Innogenetics N.V., Ghent, Belgium*
- CLAUS H. SCHRÖDER • *Department of Virus Host Interactions, Deutsches Krebsforschungszentrum, Heidelberg, Germany*
- FRED SHAPIRO • *Department of Medical Affairs, Innogenetics N.V., Ghent, Belgium*
- CHIAHO SHIH • *Departments of Pathology, and Microbiology & Immunology, WHO Collaborating Center for Tropical Diseases and Sealy Center for Vaccine Developments, University of Texas Medical Branch, Galveston, TX*
- ANTONINA SMEDILE • *Department of Gastroenterology, University of Torino and Azienda Ospedaliera S. Giovanni, Battista, Torino, Italy*
- QIN SU • *Department of Pathology, Tangdu Hospital, The Fourth Military Medical University, Xi'an, China*

PEI-CHING TAI • *Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, TX*

JOHN E. TAVIS • *Department of Molecular Microbiology and Immunology, St. Louis University School of Medicine, St. Louis, MO*

SHUPING TONG • *The Liver Research Center, Rhode Island Hospital and Brown University School of Medicine, Providence, RI*

CHRISTIAN TRÉPO • *INSERM U271, 151 Cours Albert Thomas, 69003, Lyon, France*

STEPHAN URBAN • *Department of Molecular Virology, University of Heidelberg, Heidelberg, Germany*

ELS VAN ASSCHE • *Hepatitis Diagnostics Program, Innogenetics N.V., Ghent, Belgium*

ULRIKE WEND • *Institute of Medical Virology, Justus Liebig University, Giessen, Germany*

ZHENMING XU • *Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA*

ERMEI YAO • *Department of Molecular Microbiology and Immunology, St. Louis University School of Medicine, St. Louis, MO*

TA-TUNG THOMAS YUAN • *Departments of Pathology, and Microbiology & Immunology, WHO Collaborating Center for Tropical Diseases and Sealy Center for Vaccine Developments, University of Texas Medical Branch, Galveston, TX*

STEFAN ZEUZEM • *Professor of Medicine, Department of Internal Medicine II, University Hospital, Homburg/Saar, Germany*

WEI ZHANG • *Department of Virus Host Interactions, Deutsches Krebsforschungszentrum, Heidelberg, Germany*

YANYAN ZHENG • *Department of Molecular Microbiology and Immunology, University of Southern California, Keck School of Medicine, Los Angeles, CA*

FABIEN ZOULIM • *INSERM U271, 151 Cours Albert Thomas, 69003, Lyon, France*

Contents for Volume 2

Dedication	v
Foreword by Timothy M. Block and Baruch S. Blumberg.....	vii
Foreword by T. Jake Liang.....	ix
Preface	xi
Contributors	xvii
Contents for Volume 1	xxi

PART I. VIRAL-SPECIFIC IMMUNOLOGICAL AND OTHER HOST RESPONSES

1 Studying Host Immune Responses Against Duck Hepatitis B Virus Infection <i>Darren S. Miller, Edward M. Bertram, Catherine A. Scougall, Ieva Kotlarski, and Allison R. Jilbert</i>	3
2 Measurement of Cell-Mediated Immune Response in Woodchucks <i>Stephan Menne and Paul J. Cote</i>	27
3 Study of Liver-Specific Expression of Cytokines During Woodchuck Hepatitis Virus Infection <i>Mengji Lu and Michael Roggendorf</i>	37
4 Induction of Anti-Hepatitis B Virus Immune Responses Through DNA Immunization <i>Michael Geissler, Robert Weth, Christian F. Grimm, Dörte Ortman, and Hubert E. Blum</i>	43
5 Monitoring Gene Expression Using DNA Microarrays During Hepatitis B Virus Infection <i>Michael R. Beard and Stephen Locarnini</i>	55
6 Determination of Hepatitis B Virus-Specific CD8 ⁺ T-Cell Activity in the Liver <i>Michelina Nascimbeni and Barbara Rehmann</i>	65
7 Determining the Precursor Frequency of HBV Nucleocapsid Antigen-Specific T Cells <i>Jennifer A. Waters and Howard C. Thomas</i>	85
8 Detection and Characterization of Virus-Specific CD8 ⁺ T Cells Using the Tetramer Approach <i>Xiao-Song He and Harry B. Greenberg</i>	89
9 In Vitro Analysis of Hepatitis B Virus Specific CD4 ⁺ T Cells <i>Shilpa Chokshi and Nikolai V. Naoumov</i>	97

- 10 Induction of Humoral and Cellular Immune Responses to Hepatitis Delta Virus Through DNA Immunization in BALB/c Mice
Ren-Shiang Lee, Shih-Jer Hsu, Li-Rung Huang, Hui-Lin Wu, Shiou-Lin Lin, Ding-Shinn Chen, and Pei-Jer Chen 111

PART II. IN VITRO AND IN VIVO MODELS

- 11 Infection of Primary Chimpanzee Hepatocytes with Recombinant Hepatitis D Virus Particles: A Surrogate Model for Hepatitis B Virus
Azeneth Barrera and Robert E. Lanford 131
- 12 Study of the Endocytosis and Intracellular Localization of Subviral Particles of Hepatitis B Virus in Primary Hepatocytes
Dieter Glebe and Wolfram H. Gerlich 143
- 13 The Tupaia Model for the Study of Hepatitis B Virus: Direct Infection and HBV Genome Transduction of Primary Tupaia Hepatocytes
Fritz von Weizsäcker, Josef Köck, Sabine MacNelly, Shaotang Ren, Hubert E. Blum, and Michael Nassal 153
- 14 Delivery of Hepatitis B Virus Therapeutic Agents Using Asialoglycoprotein Receptor-Based Liver-Specific Targeting
Masayoshi Konishi, Catherine H. Wu, and George Y. Wu 163
- 15 Woodchuck Hepatitis Virus Hepatocyte Culture Models
Norma D. Churchill and Tomasz I. Michalak 175
- 16 Duck Hepatitis B Virus Primary Hepatocyte Culture Model
Olivier Hantz and Fabien Zoulim 189
- 17 Enhancement of Infection of HepG2 Cells in Culture by Predigestion of Hepadnavirus with V8 Protease
Xuanyong Lu and Timothy M. Block 199
- 18 Construction of Recombinant Adenoviruses that Produce Infectious Hepatitis B Virus
Martin Sprinzl, Jérôme Dumortier, and Ulrike Protzer 209
- 19 Baculovirus-Mediated Gene Transfer for the Study of Hepatitis B Virus
Harriet C. Isom, Ayman M. Abdelhamed, John P. Bilello, and Thomas G. Miller 219
- 20 Transgenic Hepatitis B Virus Mouse Model in the Study of Chemotherapy
John D. Morrey 239

- 21 Transplantation of Human Hepatocytes in Immunodeficient
UPA Mice: *A Model for the Study of Hepatitis B Virus*
**Jörg Petersen, Martin R. Burda, Maura Dandri,
and Charles E. Rogler 253**
- 22 Duck Hepatitis B Virus Model in the Study of Hepatitis B Virus
Lucyna Cova and Fabien Zoulim 261
- 23 Hepatitis B Virus Transgenic Severe Combined Immunodeficient
Mouse Model of Acute and Chronic Liver Disease
Mark A. Feitelson, Jonathan D. Larkin, and Raymond F. Schinazi... 269
- 24 The Chimpanzee Model: *Contributions and Considerations
for Studies of Hepatitis B Virus*
Pascal Gagneux and Elaine A. Muchmore 289
- 25 Hepatitis B in Liver Transplant Recipients as a Special Model
of Antiviral Drug Development
Robert G. Gish and Adil Wakil 319
- 26 Hepatitis Delta Virus Transfection for the Mouse In Vivo Model
Jinhong Chang, Anthony Lerro, and John M. Taylor 343
- 27 Hepatitis Delta Virus RNA Transfection for the Cell Culture Model
Thomas B. Macnaughton and Michael M. C. Lai..... 351

PART III. ANTIVIRAL TESTING AND CLINICAL STUDIES

- 28 Analysis of Hepatitis B Virus Dynamics and Its Impact
on Antiviral Development
Manuel Tsiang and Craig S. Gibbs 361
- 29 Genotyping Anti-Hepatitis B Virus Drug Resistance
**Eric J. Bourne, Josée Gauthier, Vincent A. Lopez,
and Lynn D. Condeay 379**
- 30 A Parsimonious Method for Screening Drug Combinations for
Antihepadnaviral Activity Using a Parametric Dose–Response
Surface Approach
Tim Shaw and Stephen Locarnini 387
- 31 Hepatitis B Virus: *Where Are We and Where Are We Going?*
**Michael P. Manns, Johannes Hadem,
and Heiner Wedemeyer 415**
- 32 Designing Studies to Evaluate Anti-Hepatitis B Virus Therapies:
*From the Perspective of Studies for the Registration
of Pharmaceutical Products*
Alison B. Murray 445

33 Specific Considerations in the Design of Hepatitis B Virus
Clinical Studies in the Far East
Man-Fung Yuen and Ching-Lung Lai 457

34 Studying the Treatment of Chronic Hepatitis B Viral Infection
in Special Populations
Robert G. Gish and Stephen Locarnini 465

35 Designing Clinical Development Programs for
Anti-Hepatitis B Virus Drugs
Nathaniel A. Brown 499

36 Testing Antivirals Against Hepatitis Delta Virus:
Farnesyl Transferase Inhibitors
Bruno B. Bordier and Jeffrey S. Glenn 539

Index 555