Hepatitis C Virus: from Laboratory to Clinic

Hepatitis C infects 170 million people worldwide and has been labeled the 'silent epidemic' since it is asymptomatic for years after infection. This multidisciplinary overview covers basic concepts related to the discovery of the virus, development of serological and nucleic acid tests to detect infection, the structure of the virus genome, generation of virus gene products, and proposed replication scheme. It then goes on to introduce the epidemiology, transmission, pathogenesis of infection, the development of hepatocellular carcinoma associated with chronic virus infection, and current strategies for treatment. The book then discusses advances in cell culture systems, animal models of infection, and emerging treatments and vaccine development. Through its coverage of basic science, clinical consequences, and methods of research, this integrated and accessible account will be of immense value to biomedical scientists and clinicians alike, and a useful introduction for all those studying the virus and its effects.

- Combines basic science, clinical aspects and laboratory approaches to investigation
- Extensively cross-referenced for easy access to concepts
- An accessible introduction to a complex field.

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From Laboratory to Clinic

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This book is dedicated in the loving memory of my parents,

Ann and Seymour

Contents

Preface

Since the discovery of hepatitis C virus (HCV) in 1989, there has been an explosion of research and information on the virus. This is evidenced by the more than 20 000 papers on HCV (as of December, 2000), as well as the numerous reviews in journals and edited books. The purpose of this book is to present an overview of the different disciplines that have contributed to an understanding of the virus, its diseases, current and proposed treatments, and much more. Importantly, this book attempts to integrate the various disciplines to provide an overall picture of what has been accomplished in the field, and what major questions still remain.

This book is organized so that the reader will first understand how and why it was so difficult to identify HCV and then how the development of antibody and HCV RNA detection tests have nearly eliminated HCV-associated post-transfusion hepatitis. The book then explores the physical characteristics of the virus, the structure of the genome, polyprotein synthesis, and the proposed replication cycle of HCV. With a broad understanding of the virus, and the ability to screen for both viral antibodies and RNA in blood, considerable work has now been done to elucidate the epidemiology and transmission of HCV. For example, population-based viral antibody surveys have revealed the seroprevalence and burden of infection in different geographic regions of the world, which has been central to the identification of risk factors for transmission and the development of preventative measures to reduce risk. Further characterization of the virus genome from various sources has resulted in the discovery of virus genotypes, subtypes and quasispecies, which may contribute importantly to the outcome of infection. The genetic hererogeneity of HCV, and its implications to the natural history and pathogenesis of infection, is discussed in successive sections of the book.

The high rate of acute infections that become chronic, combined with a high frequency of liver diseases among chronically infected people, has placed considerable emphasis upon trying to understand natural history and pathogenesis. This book has likewise placed an emphasis upon these areas, since they are so important for understanding the outcome of natural infection and for therapeutic intervention. For example, it will be very important to know whether HCV triggers direct

cytopathic effects, or whether the bulk of chronic liver disease is immune mediated, since the answer to these questions will profoundly influence the types of therapy that are going to be developed in the future. The strong epidemiological association between chronic HCV infection and the development of hepatocellular carcinoma, which is one of the most frequent tumor types worldwide, calls for additional research into the mechanism(s) of hepatocarcinogenesis, which will also be essential for the development of therapeutics for people who develop this cancer. It is now recognized that HCV appears to infect lymphoid cells in addition to hepatocytes. This extrahepatic infection may contribute importantly to both the immune-mediated pathogenesis of chronic liver disease and the development of autoaggressive and autoimmune syndromes. In order to understand these host–virus relationships in more detail, this book presents a section on recent advances that includes attempts to propagate HCV in tissue culture cells, develop HCV animal models to study pathogenesis, and develop protective and/or therapeutic vaccines. A section on experimental approaches provides suggestions as to how some of these challenges will be met. For those who are interested in entering the field of HCV research, a discussion of several protocols and techniques that are uniquely used in the field are presented in the hope that they will provide guidelines for future work. Use of these approaches will promote advances in the field, which will be key to the future management of HCV infection.

The book closes with a series of questions that need to be addressed in the field, and some suggested approaches to solve them. The hope here is to stimulate both thinking and action, and to provide starting points for both. In this regard, it is anticipated that the fairly comprehensive treatment of HCV in this work will be readily accessible and understandable to both the inquisitive undergraduate student and the graduate student or postdoctoral fellow contemplating a project or career in the field of HCV. In addition, it is hoped that this book will be used by people who wish to broaden their education and gain an overall perspective in the field prior to a more detailed reading of the original publications. This includes people who are already working within a narrow area within the field of HCV research. The multi- and interdisciplinary presentation of information should also be useful for clinicians who wish to learn more about the basic biology of the virus and by basic scientists who wish to learn more about the clinical aspects of disease or therapeutic prospects for control. Finally, it is hoped that the contents of this book will provide the tools to help interested individuals with a basic background in biology to ask meaningful questions, and to obtain relevant answers to some of the outstanding problems that face the field today.

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Foreword

Infection with the RNA-containing hepatitis C virus, which was isolated little more than 10 years ago, causes damage to the liver resulting in cirrhosis, liver failure and cancer of the liver. In the majority of individuals, hepatitis C infection progresses slowly over many years and approximately 85% of those who contract the disease remain chronically infected, with the virus replicating throughout their lifetime. Lifelong measures are, therefore, required to limit the spread of infection to others. The importance of the disease is clear from the fact that 170 million people are estimated to be chronically infected worldwide. In the USA, where liver failure from chronic hepatitis C infection is one of the most common reasons for liver transplants, some 4 million people are infected by the virus. Hepatitis C is probably also the most common cause of primary liver cancer in the developed world.

There is no doubt that important advances have been made in recent years. As detailed in this book, the development and deployment of assays that specifically detect anti-HCV antibodies and HCV RNA in infected patients have reduced the risk of acquiring HCV from contaminated blood to almost zero. However, there is currently no cure for liver disease caused by hepatitis C, and interferon therapy is of limited efficacy and has significant side-effects. Recently, it has been commented that not only is current therapy for HCV infection woefully inadequate but also some studies estimate that annual deaths from hepatitis C could triple over the first two decades of the twenty-first century unless new, more effective interventions are developed. To this end, genome analysis, virology, immunology, cell and tissue biology, pathogenesis, animal studies, and clinical investigations are being pursued in numerous multidisciplinary scientific and clinical investigations of acute and chronic infections caused by the virus. In the final section of this book, it is pointed out that perhaps one of the biggest challenges to stem the spread of hepatitis C in the future is the development of a safe and effective protective vaccine, and lacking that, a therapeutic vaccine.

The book is designed to appeal to a wide range of readers. Its contents include the pathogenesis of the disease, animal models, molecular approaches, the

propagation of the virus in culture systems, screening assays, and the development of effective and safe antiviral agents, therapeutic regimens, and vaccines. In addition, the book covers essential experimental protocols and techniques employed in studies on the virus. At one level, the book aims to convey the essential information needed for a well-informed entry into the subject. It will, therefore, be of value to anyone wishing to obtain an insight into hepatitis C, including postgraduate research students in the medical sciences, clinical investigators registered for a research degree, medical students pursuing research projects, researchers in universities or industry, and university teachers and clinical practitioners looking for an introduction to, or an integrated, "update". On another level, since thousands of papers are published each year on HCV, the book will provide an essential overview and well-documented distillation of work in the field for scientists and clinicians who are investigating specific aspects of the virus and/or its clinical manifestations.

There are many unsolved questions in research on hepatitis C, such as why the virus causes disease immediately in some people but takes years or decades to progress in others; why African-Americans respond so poorly to the current standard of care, and how the disease can persist virtually unnoticed in the body for decades. Understanding such issues will aid in the development of new ways to halt the virus before it causes disease or is passed on to others by people who are unaware that they are infected. Since better treatment and prevention strategies will come from carefully designed, innovative and interdisciplinary research, another important feature of this book is the section on outstanding scientific and clinical questions.

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Abbreviations

AAV	adeno-associated virus
ALT	alanine aminotransferase
Anti-HBc	antibodies to hepatitis B core antigen
AP-1	activation protein 1
AUG	translation initiation start codon
bDNA	branched chain signal amplification
CLD	chronic liver disease
core	nucleocapsid of hepatitis C virus
CTL	cytotoxic T lymphocytes
E1	envelope glycoprotein 1 of hepatitis C virus
E2	envelope glycoprotein 2 of hepatitis C virus
elF2, elF3	eucaryotic initiation factors 2 and 3 (for translation)
ELISA	enzyme-linked immunosorbant assay
EM	electron microscopy
EMC	essential mixed cryoglobulinemia
ER	endoplasmic reticulum
FasL	Fas ligand
GBV	GB virus
GM-CSF	granulocyte-macrophage colony-stimulating factor
HAV	hepatitis A virus
HBcAg	hepatitis B core antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBxAg	hepatitis B X antigen
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HVR	hypervariable region
IFN	interferon
IκB	inhibitor of nuclear factor kappa B

IL	interleukin
IRES	internal ribosome entry site
ISDR	interferon sensitivity-determining region
JNK	Jun amino-terminal kinase
LDL	low density lipoprotein
LOH	loss of heterozygosity
MHC	major histocompatibility complex
NANBH	non-A, non-B hepatitis
NF-κB	nuclear factor kappa B
NK cells	natural killer cells
NKT cells	natural killer T cells
NS3	nonstructural protein 3
NS5B	nonstructural protein 5B
NTP	nucleoside trisphosphate
ORF	open reading frame
PBMC	peripheral blood mononuclear cells
PKR	double-stranded RNA-induced protein kinase
PTB	polypyrimidine-tract-binding protein
PTH	post-transfusion hepatitis
RAG-2	recombinant activating gene 2
Rb	retinoblastoma tumor suppressor gene
RdRp	RNA-dependent RNA polymerase
RIBA	recombinant immunoblot assay
RT	reverse transcriptase
RT/PCR	reverse transcriptase/polymerase chain reaction
SCID	severe combined immunodeficient
SH3	Src homology domain 3
SV40	simian virus 40
TCR	T cell receptor
TGF	transforming growth factor
Th1, Th2	T helper cells types 1 and 2
TNF	tumor necrosis factor
UTR	untranslated region