
Preface

By any measure, hepatitis B is one of the world's most important infectious diseases, by which up to one third of the world's population may have been initially infected, with up to 400 million still suffering a chronic infection. The causative agent is hepatitis B virus (HBV), a virus that straddles the line between DNA and RNA viruses, with a DNA genome that replicates by reverse transcription. HBV and its relations in the family *hepadnaviridae* are solely liver tropic viruses and infect and replicate only in hepatocytes. The infectious virion particles contain a partially double-stranded, polymerase-linked circular DNA (termed relaxed circular, or rcDNA) molecule that is converted to an episomal covalently closed circular (ccc) DNA in the nucleus of the infected cell. This cccDNA genome is the "real," persistent virus genetic material, existing in multiple copies as extrachromosomal DNA that is continually transcribed during active infection into five mRNA species for the viral gene products; the longest form, namely pregenomic RNA (pgRNA), is a greater-than-genome length transcript that is encapsidated in the cytoplasm and then reverse transcribed into rcDNA by a complex process that includes the viral polymerase acting as a primer, the core protein, and host heat shock proteins. This "nucleocapsid" can then be enveloped by the three viral glycoproteins and secreted from the cell, or can be recycled to the nucleus to amplify the pool of cccDNA. This greatly simplified description of the intracellular life cycle does not capture many interesting aspects of HBV biology that appear to be important for the maintenance and propagation of the infection in a host, including mechanisms for modulating the host immune response. For example, the three envelope proteins, collectively known as hepatitis B surface antigen (HBsAg), are present in the serum to very high levels, a state that is thought to induce immunotolerance by HBsAg's possible effect in T-cell exhaustion, the titering out of antibodies, and so on. A secreted variant of the core protein, e antigen (HBeAg), which is detectable in many patients and correlates with a poorer prognosis, is also implicated in immune modulation. Even the core protein, X protein, and the polymerase have been reported to have activity in regulating innate immune signaling pathways and antigen. On the treatment front, the currently approved options for patients are limited to reverse transcription inhibitors (specifically, nucleoside/nucleotide analogues) and two forms of alpha interferon. There is much room for novel drug development and improvement of treatments.

Technically, the study of HBV has presented challenges that endure since its discovery in the 1960s. Even as the biology of many other viral species has systemically been unraveled, in some cases leading to effective therapies and even cures, the *hepadnaviridae* have stubbornly hung on to many of their secrets. Interspersed with many breakthroughs that have given us a good understanding of a complex life cycle, the details on many aspects of its life cycle and the disease it causes await elucidation. We still have an incomplete understanding of how the immune system of the host is affected to permit a chronic infection; the specifics of how the virus enters cells even after the discovery of the viral receptor; and most intriguingly, how the partially double-stranded relaxed circular genome is converted to cccDNA. The efforts to answer these questions have been hampered by the technical difficulties of studying this virus and the lack of truly robust, tractable systems that reproduce the full infection cycle in vitro and the most important immunological features of the disease process in vivo. Not surprisingly, the pace of discovery of new drugs and therapies has also suffered.

Nevertheless, recent technical progress in the field has been considerable, and this volume will hopefully serve as a reference for the dissemination of these advances. The authors' contributions span the gamut of the field, detailing protocols and techniques ranging from cell culture studies to in vivo and clinical immunology. Laboratory techniques for classical virology and genetic studies include thorough treatments of in vitro infection systems from the Li, Glebe, and Urban groups; analysis and quantification of cccDNA and its mutations from the Arbuthnot, Protzer, and Zhang groups; in vitro polymerase activity assays from the Hu and Tavis groups; the study of cellular trafficking of core protein from the Kann and Shih groups; effects on intracellular calcium metabolism by the Bouchard lab; detection, cloning, and sequencing of HBV markers in laboratory-generated and clinical samples by Dandri, Huang, Jilbert, Weiland, and Tong groups; new strategies aimed at exploiting novel mechanisms for drug discovery by Tavis and Arbuthnot groups; novel and already established animal and in vivo-derived models detailed by the groups of Chen, Lu, Menne, Ou, and Su; and methods contributed by the Robek lab for the study of T-cells in HBV mouse models. Finally the editors have also submitted chapters on the classic method for resolution of extracellular viral particles by native gel electrophoresis (Guo) and on the microtiter assay methods for detection of HBV antigens in drug discovery and other applications (Cuconati).

This project was made possible primarily by the very kind and patient cooperation of the chapter authors, and we thank them in earnest. We want to especially thank the senior series editor Dr. John Walker for the invitation to assemble this volume and his constructive guidance and support. A special thanks also goes out to Mr. David Casey for his excellent technical support. We believe the effort was very worthwhile and important to the advancement of this field, and we hope the readers will agree.

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